Project Title: Genes, isotopes, and ecosystem biogeochemistry: dissecting methane flux at the leading edge of global change

Applicant/Institution: Scott Saleska, University of Arizona
Street Address/City/State/Zip: University of Arizona, 1041 E. Lowell St., BioSciences West, Room 510, Tucson, AZ 85721
Principal Investigator: Scott Saleska
Postal Address: University of Arizona, 1041 E. Lowell St., BioSciences West, Room 510, Tucson, AZ 85721
Telephone Number: (520) 626-1500
Email: saleska@email.arizona.edu
Funding Opportunity Announcement Number: DE-PS02-09ER09-25
DOE/Office of Science Program Office: Office of Biological and Environmental Research
DOE/Office of Science Program Office Technical Contact: Dr. Joseph Graber

Collaborating Institutions/PIs:

University of Arizona, Scott Saleska: Project Coordinator, point of contact, and Biogeochemistry Research Coordinator
University of Queensland, Gene Tyson: Molecular Microbial Ecology Research Coordinator
University of New Hampshire, Changsheng Li: Modeling Coordinator
Stockholm University, Patrick Crill, biogeochemistry
University of Arizona, Virginia Rich, molecular microbial ecology
University of New Hampshire, Steve Frolking, modeling
Florida State University, Jeff Chanton, biogeochemistry and modeling

Project Title: **Genes, isotopes, and ecosystem biogeochemistry: dissecting methane flux at the leading edge of global change**

Applicant and Principal Director: Scott Saleska, University of Arizona
Collaborators: Molecular Microbial Ecology: Gene Tyson, University of Queensland and Virginia Rich, University of Arizona; *Biogeochemistry*: Patrick Crill, Stockholm University; *Modeling*: Changsheng Li and Steve Frolking, University of New Hampshire, and Jeff Chanton, Florida State University

Microbial communities in northern wetlands are central to understanding current and future global carbon cycling. Northern wetlands are both critical, contributing a tenth of global CH_4 emissions and containing one-quarter of global soil carbon, and vulnerable, with permafrost area expected to shrink 50% by 2050. As permafrost thaws, increasing CH_4 emissions from northern wetlands are likely to cause positive feedback to atmospheric warming. Wetland CH_4 cycling is mediated by microbes, but connecting ecosystem-scale fluxes to underlying microbial population dynamics and genomics has not been achieved. Recent transformative technical advances in both high-throughput investigations of microbial communities and high temporal-resolution biogeochemical isotope measurements together now permit a uniquely comprehensive approach to opening the microbial "black boxes" of wetland methane cycling that impact carbon cycling on global scales.

We propose to investigate how microbial community composition and function scale to ecosystem biogeochemistry of CH_4 and CO_2, and how such scaling is affected by climate change. To accomplish this, we propose a three-pronged interdisciplinary investigation of Sweden's Stordalen Mire, an established wetland field site at the thawing southern edge of the discontinuous permafrost zone:
(1) Molecular microbial ecology *to identify the genes and lineages that mediate CH_4 cycling through the soil column, across the major wetland habitats, and over the growing season, using:* (i) *pyrotagging*: profiling community diversity using the 16S rRNA gene, (ii) *metagenomics*: community metabolic potential by bulk sequencing of microbial DNA, (iii) *metatranscriptomics*: community expressed genes by sequencing microbial mRNA, and (iv) *metaproteomics*: mass spectrometry analysis of community proteins. Coupled to biogeochemistry and modeling, these methods link microbes, genes, transcripts and proteins with biogeochemical processes and ecosystem fluxes. *(Investigators: Tyson and Rich)*
(2) Continuous biogeochemical measurements *of CO_2 and CH_4 fluxes and isotopic compositions to quantify carbon characteristics and cycling* ***at three spatial scales****:* (i) ***ecosystem***, through an in-place eddy flux tower, (ii) ***site***, through an in-place system of autochambers, and (iii) ***soil profile***, through an in-development system of soil gas samplers. C isotopes of CH_4 and CO_2 at scales (ii) and (iii) will be automatically measured in the field using a recently developed tunable laser absorption spectrometer, and H isotopes of CH_4 and H_2O will be analyzed by traditional IRMS. Acetate and dissolved carbon species will also be quantified and isotopically characterized. The average age of the mineralized organic matter will be measured via the ^{14}C ratios of CO_2 and CH_4. *(Investigators: Saleska, Crill, and Chanton)*
(3) Modeling *soil processes and ecosystems, to characterize the details of CH_4 production, and to test the importance of microbial ecology to ecosystem biogeochemistry***.** (i) *Gas diffusion and fractionation modeling:* Stable isotope and flux data will be incorporated into a diffusion model to discriminate between methanogenesis pathways, and quantify CH_4 oxidation. This will identify the zones and times of maximum and minimum methanogenesis and methanotrophy, as well as transitions between types of methanogenesis. (ii) *Ecosystem process modeling using the Wetland-DNDC model,* which simulates wetland carbon gas fluxes. We propose to first test this model against basic flux data from the site; second, develop the model to include isotopes, followed by testing against isotope data; third, compare the model's separately simulated methane production and consumption processes with the corresponding observed microbial functional activity, as recorded in metatranscriptomic and -proteomic data; and fourth, use the refined Wetland-DNDC to project the impacts of continued permafrost thaw on wetland CH_4 cycling at this site. *(Investigators: Chanton, Li and Frolking).*

Project Narrative

1. Introduction & Research Goals

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 will respond to climate change (IPCC, 2007). These grand challenges intersect in the need to understand the global carbon cycle, which is both mediated by biological processes and a key driver of climate through the greenhouse gases carbon dioxide (CO_2) and methane (CH_4).

Here we focus on understanding the biological and earth science aspects of CO_2 and CH_4 cycling at "the leading edge of climate change" – a subarctic wetland system where climate change-induced permafrost melt is transforming methane sinks into sources. **Our research goals are: (1) to discover functional relations for scaling microbial community composition and metabolism to the ecosystem biogeochemistry of CH_4 and CO_2; (2) to learn how these relations are affected by shifting environmental variables, and (3) apply this knowledge to better understand and predict changing carbon budgets in subarctic ecosystems already experiencing substantial climate change.**

Methane cycling: Our understanding of controls on CH_4, a key greenhouse gas with the potential for strong feedbacks under climate change, is poor. For example, explaining the abrupt cessation in the mid-1990s of the century-long 1%-per-year increase in global atmospheric CH_4 (Dlugokencky et al., 2003; Dlugokencky et al., 2009) remains an outstanding challenge in biogeochemistry (Reeburgh, 2005).

Resolving this challenge – and, as important, understanding future trajectories – likely depends, in part, on understanding wetlands, the largest natural source of CH_4 to the atmosphere (Ehhalt et al., 2001; Denman et al., 2007). Northern wetlands account for 5-10% of annual CH_4 emissions (UNEP, 2003) and a quarter of global soil carbon (Gorham, 1991), and thus play a central role in altered C-cycling under global change as thawing permafrost releases CO_2 and increases CH_4 production (Denman et al., 2007; Schuur and et al, 2009). Over the next century, climate change is predicted to dramatically shrink permafrost (Lawrence and Slater, 2005) (Figure 1), causing potentially significant positive feedbacks to global warming (Zhuang et al., 2006; Denman et al., 2007; Schuur and et al, 2009). Yet the nature, magnitude, and dynamics of this CO_2 and CH_4 release are poorly understood due to technical limitations across disciplines.

Microbial Communities : The balance between CH_4 production in anaerobic zones hosting archaeal methanogens and aerobic CH_4 oxidation fueling methanotrophs is abiotically controlled primarily by hydrology (water table depth and soil saturation), the "on-off" switch for CH_4 production (Christensen et al., 2003; Turetsky et al., 2008). Soil structure, plant root respiration, and diffusional transport factors are also important. Within anaerobic zones (Gauci et al., 2002; Gauci et al., 2004) two methanogenic pathways dominate: (1) *acetoclastic:* $CH_3COOH \rightarrow CH_4 + CO_2$, and (2) *$CO_2$-reductive:* $CO_2 + 4H_2 \rightarrow 2H_2O + CH_4$. Labile organic matter favors the acetoclastic pathway, while recalcitrant or low amounts of

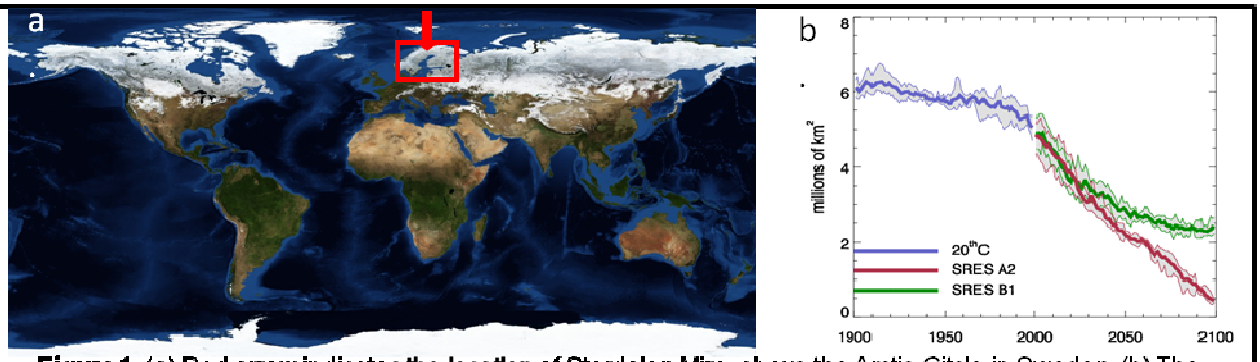


Figure 1. (a) Red arrow indicates the location of Stordalen Mire, above the Arctic Circle in Sweden. (b) The predicted permafrost area change by 2100, under 2 model scenarios, from Lawrence and Slater 2005.

organic matter favor CO_2-reduction (Schoell, 1980, 1988; Sugimoto and Wada, 1993). Characteristic isotopic fractionation of participating compounds along the methano -genic and -trophic pathways allows isotope measurements to quantify relative contributions of the different processes.

Despite this knowledge of abiotic controls on system-level CH_4 cycling, we lack fundamental knowledge of underlying microbial community dynamics, and how these shape system output. Net emissions arise from the small difference between the large gross fluxes of production and oxidation (Reeburgh, 2005), so small changes in this microbially-mediated cycle could result in large changes in net flux. Poor understanding of microbial dynamics leads these biological drivers of CH_4 cycling to be treated as a "black box" in biogeochemical models. **Critically needed are studies that elucidate microbial community dynamics, connect these to methane biogeochemistry, and observe how both are jointly affected by environmental/climatic changes.**

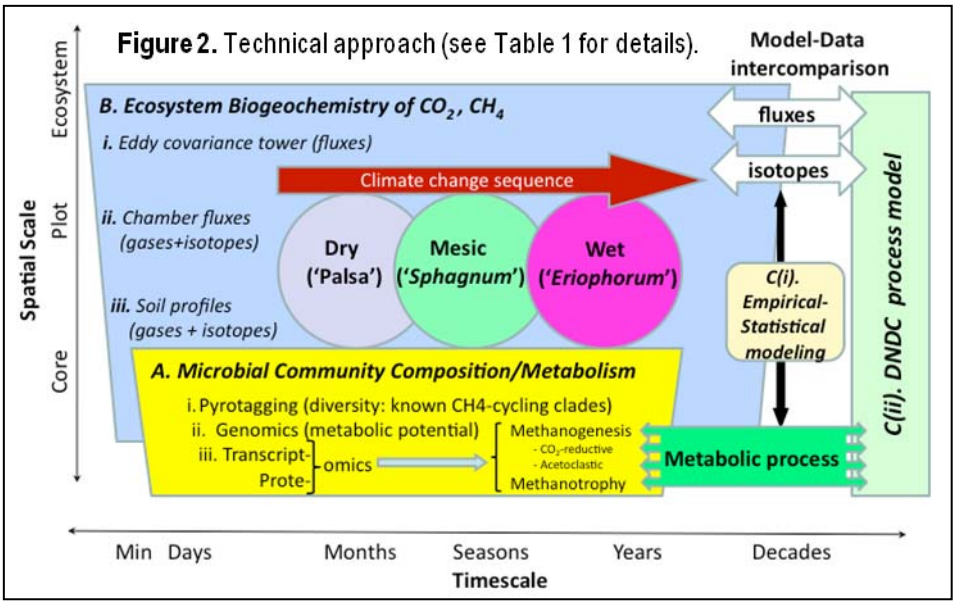
Despite numerous investigations of gas flux rates and isotopic compositions in freshwater wetlands (Happell et al., 1993; Walter et al., 2008), and independent studies of microbial ecophysiology (Zeikus, 1977; Updegraff et al., 1995), few, if any, studies have combined detailed investigations of microbes with high-resolution biogeochemical measurements of both fluxes and isotopes. **Recent technical advances in both high-temporal-resolution biogeochemical isotope measurements and high-throughput nucleic-acid sequencing now permit a uniquely detailed combined approach that promises to reveal biogeochemical consequences of microbial community dynamics, and to improve our understanding of wetland carbon cycling and CH_4 emissions on a changing planet.**

2. Proposed research objectives and relevance to DOE goals

We propose to achieve overall research goals (outlined on the previous page) through the following objectives, pursued on each of 3 wetland habitats that encompass a range of expected climate change-induced permafrost degradation (**Figure 2** and **Table 1**):

(A) *Characterize microbial community composition and metabolic function associated with* in situ *methano -genic and -trophic pathways,* using ***(i)*** pyrotagging (to identify the phylogenetic diversity of microorganisms); ***(ii)*** community genomics (to characterize community metabolic potential), and ***(iii)*** meta -transcriptomics and ***(iv)*** -proteomics to identify each community's expressed function (metabolic activity). This will be the first terrestrial environmental study crossing all three levels of biological information from DNA through mRNA to proteins.

(B) *Characterize ecosystem biogeochemical cycling of CH_4 and CO_2, using isotopes to partition contributions from separate methano -genic and -trophic metabolisms,* at three spatial scales: ***(i)*** ecosystem, through an in-place eddy flux tower, ***(ii)*** plot, through an in-place system of automated flux chambers, and ***(iii)*** soil profile, through an in-development system for sampling profiles of soil gas and pore-waters. We will achieve substantial advances over previous biogeochemical studies of this type by leveraging newly developed



technology to automatically measure the ^{13}C isotopic composition of CH_4 and CO_2 *in situ* at scales (ii) and (iii), supplementing these novel measurements with manual sampling of gas to obtain deuterium and ^{14}C by conventional methods, and of key soil carbon substrates (acetate and DOM), and soil properties (pH, redox potential).

(C) <u>*Link molecular microbial community datasets (from A) to biogeochemical observations (from B)*</u>, by:

(i) Analyzing how dynamics of methane production/consumption profiles, as inferred from modeled transport and expected isotopic fractionation along methano -genic and -trophic pathways, correspond to microbial metabolic potential and activity levels;

(ii) Testing a cutting-edge process model (DNDC) to investigate whether microbial ecology matters to biogeochemical cycling. Specifically, we will: (a) drive DNDC with climatic/hydrological inputs; (b) test model outputs with multi-scale flux and isotopic data, and with a novel comparison of observed microbial metabolism-specific activity to the corresponding modeled processes (acetoclastic and CO_2-reductive methanogenesis, CH_4 oxidation); and (c) use the results to make new predictions of the effect of climate change-induced permafrost melt on CH_4 cycling in northern wetlands.

These objectives directly respond to *goal two* of the DOE FOA: *"Development of metatranscriptomic, metaproteomic, and other genome-enabled approaches to understand how shifts in environmental*

Table 1 . Technical approach, across three habitat types spanning a climate-change analog sequence: dry (with permafrost, "Palsa"), mesic ("*Sphagnum*"), and wet (permafrost absent, "*Eriophorum*"). Represented in Figure 2.

A. Molecular Microbial Ecology *(Proposal section 4.A., Figures 3 and 4)*		
Approach	*Methods*	*Product/Purpose*
1. Pyrotagging	Detailed sequencing of community 16S rRNAs from all samples	Microbial community phylogenetic diversity
2. Metagenomics	Sequencing of community DNA from targeted samples	Community metabolic potential
3. Meta-transcriptomics and proteomics	Sequencing of community cDNA and protein from paired samples with metagenomics.	Realized metabolism-specific activity levels (for comparison to redox-driven modeled process)
B. Ecosystem Biogeochemistry of CH_4 and CO_2 *(Proposal section 4.B., and Figures 5 and 6)*		
Measurement	*Sensor / method*	*Status / sampling*
1 . Eddy covariance measurements of CO2 and CH4: fluxes and environmental drivers		
• Net fluxes of CO_2, H_2O, CH_4	• sonic anemometer (Gill) + IRGA + CH_4 Tunable Diode (TDL) Laser (ARI) *(a)*	existing equipment to be upgraded with separate funding *(continuous measurements)*
• Environmental variables	• Radiometers (PAR, net Rad.), rain gauge, Vaisala temperature sensors	
2. Plot-scale chamber fluxes of gases and their isotopic composition		
• <u>Net fluxes:</u> CO_2, CH_4	• Automatic chamber system *(b)*	• Existing infrastructure, funding
+ ^{14}C, D isotopes	• Manual sampling via IRMS and AMS *(c)*	• Proposed (with microbial cores)
+ ^{13}C, ^{18}O isotopic composition	• 2 new QC laser systems for isotopologues of CO_2 (incl. ^{13}C, ^{18}O) *(d)* and CH_4 (^{13}C) *(e)*	• Proposed, *continuous sampling*
• Soil/air temp, water table depth	• Thermocouples (*ref:* CSI T_107)	• Existing infrastructure, funding
• Soil pH, O_2, redox potential	• Manual: Orion pH probe; Yellow Springs Instr. oxygen probe; custom platinum probe	
3. Soil gas and Pore water profiles of gases and their isotopic composition		
• <u>Manual</u> samples (CH_4, CO_2, H_2) including isotopes of D, ^{14}C, ^{13}C	• 5-level depth profile (to 0.5 m) of soil equilibrators	• Existing infrastructure for gas, *proposed funding for isotopes*
• <u>Automatic</u> samples (CH_4,, CO_2) including isotopes of ^{13}C, ^{18}O	• Modified equilibrators for automated sampling by QC laser systems (as in 2) *(f)*	• *Proposed continuous sampling of isotopes*
C. Modeling and Hypothesis testing *(Proposal section 4.C., and Figures 7 and 8)*		
Approach	*Methods*	*Product/Purpose*
1. (a) derive methane production/ consumption profiles	• production/transport modeling constrained by profiles of isotopes and concentrations *(a)*	• partition fluxes into production/ consumption processes
(b) observe CH_4 profiles v. microbial activity correlations	• Statistical modeling of isotope-microbial data	• connect biogeochemistry to microbial ecology
2. DNDC carbon-cycle process Model *(b)*	• Process model simulations (soil redox conditions and microclimate drive biogeochemical process)	• make process-specific predictions, testable by fluxes/ isotopes (B), & by relative microbial metabolic activity (A.3).
<u>References:</u> *B. Biogeochem: (a)* Jackowicz-Korczynski (2009); *Christensen et al. (2004); (b) Backstrand et al (2008); (c) Chanton; (d) Saleska et al (2006); Nelson et al. (2008); Tuszon et al (2008); (e) Zahniser et al., (2009); Santoni et al (in prep); (f) Hirsch et al (2003); Mastepenov & Christensen (2008);* *C. Analysis/Modeling: (a)* Chanton et al. (2005); Blodeau et al (2007); (b) Li et al. (2007)		

ivariables impact microbially-mediated carbon cycling processes in terrestrial ecosystems." In particular, we seek to open a window on the so-called "microbial black box" through "innovative approaches aimed at linking structural and functional characterization of microbial communities with quantitative measurement of carbon cycle processes." (DOE FOA, p.3)

3. Study Location: Stordalen Mire in Abisko, Sweden

3a. Abisko, a century of Arctic climate monitoring. Abisko Scientific Research Station (in operation since 1913) sits at the southern fringe of the discontinuous permafrost zone in northern Sweden (Figure 1). The Station provides a unique long-term record of Arctic climate change (Holmgren and Tjus, 1996; Kohler et al., 2006), and permafrost thawing (Johansson et al., 2006a; Åkerman and Johansson, 2008; Kokfelt et al., 2009), with a continuous record of permafrost depth since 1978. Abisko's **Stordalen Mire** was chosen for the International Biological Programme (IBP) in the 1970's and has been a key site in numerous international polar monitoring efforts. Some of the world's first wetland methane flux measurements were conducted at the Mire (Svensson, 1980), and a number of studies have examined the effects of thawing permafrost on the Mire's carbon cycling (Öquist and Svensson, 2002; Bäckstrand et al., 2008b; Bäckstrand et al., 2008a), 30-year changes in vegetation (Malmer et al., 2005; Johansson et al., 2006a), hydrology (Johansson et al., 2006a; Johansson, 2006), and greenhouse gas production (Johansson et al., 2006a; Bäckstrand et al., 2009).

Stordalen Mire is thoroughly instrumented with automated gas sampling chambers, an eddy covariance tower, and diverse analytical instruments for gas flux quantification, hydrology, and meteorology. It therefore provides an excellent field location for layering in new technology to enable advanced study of the relation between microbial community function and biogeochemistry.

The mire complex, 10km east of the Research Station, has the rare combined advantage of line power and nearby passenger rail line and road system for easy access and inexpensive transport of supplies and equipment. No comparable permafrost wetland field site in North America provides the combination of scientific context, infrastructure, and logistics needed to implement our proposed study.

3b. Three habitat types with distinct carbon cycle profiles and fates under climate change. Stordalen Mire contains three distinct subhabitats (Table 2, and in Figure 3a), common to northern wetlands and together covering ~98% of the Mire surface: (i) permafrost-dominated, well-drained palsas occupied by feather mosses and ericaceous and woody plants, covering 49% of the mire (ii) intermediate permafrost sites with variable water table depth, dominated by *Sphagnum* spp., covering 37% of the mire, and (iii) full summer-thaw, wet sites with *Eriophorum angustifolium,* covering 12% of the mire. Between 1970 and 2000, as permafrost melted and palsas collapsed, *Sphagnum* sites and *Eriophorum* sites expanded by 3% and 54%, respectively (Table 2, and (Johansson et al., 2006a)).

The three habitat types generate different greenhouse gas footprints, with the wetter sites drawing down more CO_2 during the growing season but also producing more CH_4. By accounting for CH_4 in CO_2-equivalents (1:25 using a 100-year time horizon, (Whiting and Chanton, 2001; Johansson et al., 2006; Forster et al., 2007) the balance shows that **the 30-**

Table 2: Characteristics of the three Mire habitats.

	I. Palsa	II. *"Sphagnum"*	III. *"Eriophorum"*
Percent of mire area[a]	49%	37%	12%
Net carbon gas emission (CO_2 equivalent gC/m²/yr)[b]	**30 (=1 X)**	**82 (=2.7 X)**	**646 (=21.5 X)**
Change in area 1970-2000 due to permafrost thaw[a]	**-10%**	**+3%**	**+54%**
Watertable depth[c]	N/A[e]	5-25cm	+/- 5cm
Permafrosttable depth[c]	50-55cm	60->100cm	>100cm

a. From Johansson et al., 2006. b. From Bäckstrand et al., in press.. c. Depth below ground surface range of water table depths, and permafrost table depths by August 30th, for 2003-2005, from Bäckstrand *et al.*, 2008, Tellus

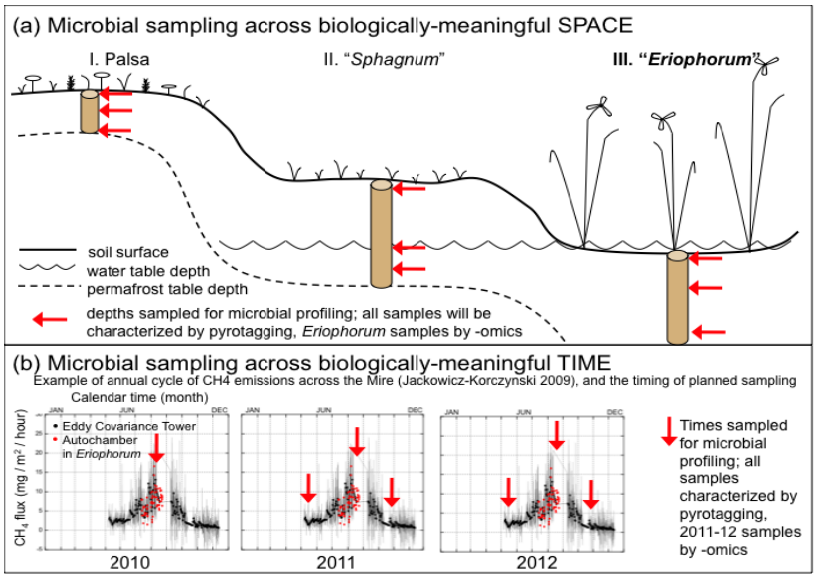


Figure 3: Microbial sampling strategy (red arrows) across (a) biologically meaningful space, spanning the Mire's three habitats, and biogeochemically distinct regions of the soil column, and (b) biologically meaningful time, spanning the early, peak, and late growing season corresponding to changing methane production within the Mire. Example of annual Mire (eddy covariance tower) and *Eriophorum* (autochamber) CH_4 production from Jackowicz-Korczynski 2009.

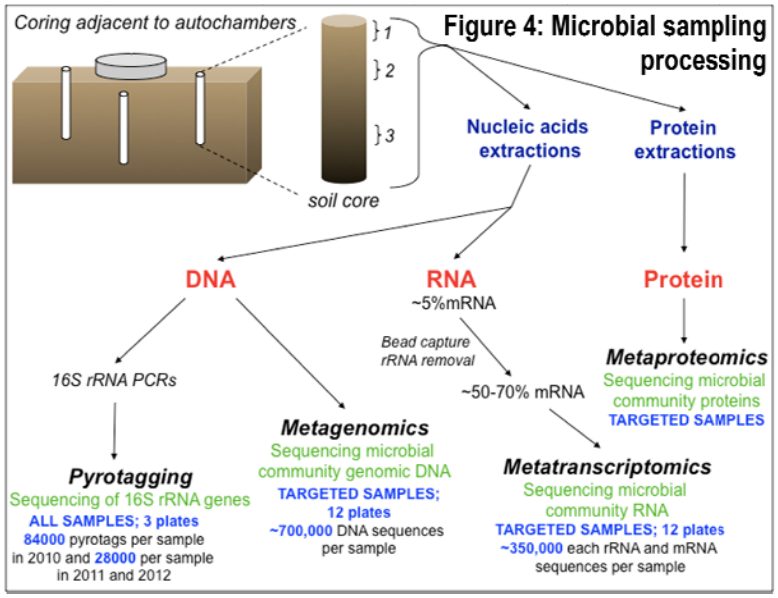
year thawing-induced change from palsas to wetter sites increased the Mire's greenhouse gas footprint 27% from 1970 to 2000, due to increased methane production (Bäckstrand *et al.* in press). Similar results have been reported in discontinuous permafrost in Alberta, Canada (Prater et al., 2007).

4. Technical Approach and Methods for Achieving Objectives

4a. Microbial ecology: profiling Stordalen's biogeochemical drivers

The Mire's habitat differences in CH_4 production are driven by their microbial communities. We will sample these communities in each habitat type through vertical space in the soil column, and across time in the growing seasons of several years (Figure 3). Four strategies will be used to characterize the Mire's microbial communities (Figure 4): all samples will be pyrotagged, to identify community composition and diversity; a subset of samples will be profiled by community genomics, transcriptomics, and proteomics to describe each community's metabolic potential and its *in situ* activity.

<u>i. Sample collection.</u> **In August 2010, we will take an initial set of microbial cores from each habitat. These samples will be used to: (1) optimize extraction methods (see section ii), (2) provide baseline information on the dominant microbes in each habitat, and (3) describe the variability among replicate cores. In 2011 and 2012 we will sample each habitat at the beginning, peak, and end of the growing seasons (Figure 3).**



Specifically, at each sampling date we will take triplicate soil cores (8cm diameter, to a maximum depth of 0.5m based on thaw depth) surrounding 1 autochamber and adjacent soil gas sampling manifolds in each of the Mire's three habitats. For each core, three 3cm subsections will be removed at several depths targeting distinct soil conditions (Figures 3 and 4). These depths will

vary between Mire habitats due to differences in water table and active layer depth. In all habitats we will examine (i) the surface community (1-4cm), which has been suggested to play a significant but poorly-explored role in methane flux. In the wet *Eriophorum* habitat we will also target: (ii) 5-8cm below the surface water table, targeting peak methanogenesis, and (iii) the average rooting depth. In the intermediate *Sphagnum* habitat we will target (ii) the average depth of the water table, and (iii) 5-8cm below, representing the zone of peak methanogenesis. In the dry, permafrost Palsa site we will target (ii) the middle and (iii) base of the active layer (the thawed portion of the soil column), as it moves through the growing season. In each habitat, the precise depths targeted will be empirically determined based on adjacent soil column gas sampling for gas flux and isotope composition, outlined in the introduction and described in greater detail in section 4.B below.

The remaining core material (~80% of original, with increased volume due to disturbance) will be used in its original orientation to backfill core holes. This is critical to minimize both the physical disturbance of this hydrologically delicate environment and the biological disturbance of the native microbial community. Core subsections will be immediately frozen and stored in liquid nitrogen and transferred to Stockholm University for storage in -80°C freezers. Samples will be shipped on dry ice to co-I Tyson for initial nucleic acid extraction.

This sampling plan generates 189 subsamples: 7 sampling dates, 3 Mire habitat types, 3 soil column depths, with each site sampled in spatial triplicate. All of these will be pyrotagged, while a subset will be used for community genomic, transcriptomic and proteomic characterization (Figure 4).

ii. Nucleic acid and protein extractions. Each sample will be split to extract total nucleic acids and proteins. Subsamples for proteins will be sent on to co-PI Rich, and extractions will be performed at Oak Ridge National Labs as in VerBerkmoes (2009) (see VerkBerkmoes Letter of Support, Field 12). Nucleic acid extracts will be divided into DNA and RNA, and sequenced as described below and diagrammed in Figure 4.

In order to identify the best nucleic acid extraction method for this environment and to maximize microbial:eukaryotic yields and minimize biases, we will use three nucleic acid extraction strategies on representative first-year samples from the surface Palsa and *Eriophorum* sites. Specifically, we will use one "direct" extraction method in which DNA is extracted from the bulk soil/peat matrix, and two "indirect" methods which first separate cells from the sediment matrix and then extract them (one variant will include a pre-extraction DNAse treatment of the separated cells). Soil DNA extractions from a variety of soil habitats range in eukaryotic DNA from ~5% to 95%, with indirect methods generally producing much less eukaryotic DNA though potentially creating bias against cells that adhere tightly to the soil matrix (e.g. (Gabor et al., 2003; Treusch et al., 2004; Daniel, 2005)). Extracted DNA and RNA will be quantified and characterized spectroscopically, DNA will be pyrotagged (see next section) to compare community composition, and shallow metagenomic sequencing will be performed to assess the relative representation of bacteria, archaea, and eukarya. The optimized extraction method will be used for all subsequent extractions.

iii. Overall community diversity: "pyrotagging". We will examine the microbial community compositional changes over space and time (Figure 4), by sequencing a region of the 16S rRNA gene as a fingerprint of microbial phylogenetic diversity. We will target the hypervariable V6-V8 region of this gene, using the Joint Genome Institute's Standard Operating Procedure primer set (926f 5'-AACGCGAAGAACCTTAC-3' and 1392r 5'-ACGGGCGGTGTGTRC-3'), and the archaeal forward primer ARCH915f (5'-AGGAATTGGCGGGGGAGCAC-3'; (Yu et al., 2008)), which produce amplicons of ~460-490bp suitable for GS FLX Titanium sequencing. Amplicons will be sequenced at a ~1:1 bacterial:archaeal ratio; although these microbial communities likely have a ratio of bacteria:archaea from 3-90:1 (Wagner, 2008), we are interested in deeper sequencing of the archaeal portion since it contains the methanogens. Each sample

will be tagged with a unique DNA barcode (e.g. MID tags) prior to sequencing, to allow samples to be combined on a single plate.

For the samples collected in 2010 (27 samples), we will perform deep pyrotag sequencing to (a) obtain a high degree of initial community resolution, and (b) compare results among triplicate cores. We will use 1 full plate for pyrotags, resulting in **~28,000 tags per subsample replicate, thus ~84,000 per habitat and depth**. Depending on analyses of this data, we anticipate using 1 plate per year to sequence 2011 and 2012 samples (81 samples each year), to produce ~28,000 tags per habitat and sampling date and depth (when triplicates are pooled *in silico*). Based on the limited pyrotag surveys of other soil environments, this sequencing effort will provide a comprehensive survey of the dominant members of the community as well as an appreciable glimpse of rarer members, likely saturating diversity observations at all but 0% divergence OTU clusters (see next section) (Roesch et al., 2007).

Pyrotags will be assigned phylogeny (using e.g. the Joint Genome Institute's pipeline, PyroTagger) and analyzed to describe community composition, diversity, and correlation to environmental parameters. Sequences will be clustered into OTUs of defined sequence variation that range from unique sequences (no variation) to 10% clusters (spanning 10% divergence) by using DOTUR (Schloss and Handelsman, 2005). The total diversity in each sample will be estimated using several standard methods from microbial diversity studies (reviewed in (Bohannan and Hughes, 2003; Shaw et al., 2008), including Chao1 (Chao, 1987) and ACE (Chao and Lee, 1992). These estimates will allow us to test the relationship between diversity and ecosystem function, as quantified by our paired biogeochemical measurements. In addition, correlations between particular lineages and ecosystem properties will be analyzed by applying tailored statistical tests (e.g., Unifrac (Lozupone et al., 2006) that accounts for branch lengths and tree structure) to phylogenetic trees of each sample to evaluate clustering with respect to sample, depth zone, sampling season, measured biogeochemistry, and CH_4 production or consumption. This will facilitate linking clades with intermediate versus surficial methanotrophy, for example, or acetoclastic versus CO_2-reductive methanogenesis.

iv. Community metabolism: genomic, transcriptomic and proteomic profiling. In conjunction with this investigation of phylogenetic diversity, we will profile community genomic repertoires through metagenomic sampling, and place these into the context of community activity by paired metatranscriptomics and -proteomics. Of the 189 collected and pyrotagged samples, we will target 72 (24 sampling dates and habitats, each with triplicate spatial sampling) for complete "-omic" profiling. Specifically, we will target the endpoint habitat of the thawing climate change process, the wet *Eriophorum* habitat, which is already responsible for the bulk of the Mire's methanogenesis and is rapidly expanding under thawing permafrost. Within the *Eriophorum* soil column, we will focus sequencing at the depths of peak methanogenesis and methanotrophy, the latter housing the oxidative methanotrophic filter controlling the methane released from the underlying soil column. These depths will again be identified by the soil gas sampling, with the peak methanotrophic zone expected either at the surface or in the *Eriophorum* rooting zone (enabled by root-mediated O_2 transport and diffusion).

Total extracted RNA will be enriched (in the lab of co-I Tyson) for mRNA using the rRNA capture-bead method developed in the DeLong Lab (pers. comm.). This reduces rRNA from ~90-95% of total RNA to approximately 30-50%, greatly enriching the relative mRNA proportion (DeLong Lab pers. comm.). Therefore the metatranscriptomics data will supply *both* a deep assessment of the diversity, independent of the PCR-amplified pyrotag data, and a portrait of the relative activity of the microbial community. cDNA prepared in this way has been successfully sequenced using GS20 (Frias-Lopez et al., 2008) and GS FLX (Tyson, pers. comm.) technologies.

Prior to sequencing, the DNA and mRNA-enriched cDNA from the triplicate core extracts for each targeted sampling date and depth will be combined, after barcoding to retain their identities post-sequencing. Each sampling point will then be sequenced using two Titanium plates, one for DNA and

one for cDNA (one plate Titanium is equivalent to 2.5 runs of FLX Standard, with longer read lengths). For comparison, this is ~8x as many transcript sequences per sample as generated in the groundbreaking 2008 marine metatranscriptomics survey (Frias-Lopez et al., 2008), as appropriate for the more complex soil community. This will result in 24 plates of 454 sequencing; 6 sampling dates spanning 2011-12, targeting the peak methanogenic and –trophic depths of the *Eriophorum* habitat.

For both metatranscriptomic and metagenomic data, we will identify sequences by comparison to the NCBI non-redundant protein database, and assign preliminary taxonomy by MEGAN (Huson et al., 2007). Metatranscriptomic rRNA reads will be assigned taxonomy through tailored BLAST comparison to the Greengenes database. Metabolic pathways will be assessed using the COG, KEGG, and SEED databases. We will perform protein family rarefaction analysis in order to assess the relative coverage of the community metabolic potential and expression achieved. In addition, protein-coding sequences known to be involved in methane cycling will be examined for conservation of active sites, to clarify their likelihood of functionality, and placed phylogenetically. Observed novel environmental clades will be assessed for their distributions and possible roles.

Metaproteomics will be performed by Rich as a guest at the Oak Ridge National Labs (ORNL) proteomics facility (see VerBerkmoes Letter of Support, Field 12). We will use ORNL-developed protein extraction methods tailored to complex environmental samples including soils (VerBerkmoes et al., 2009a), (Chourey in prep). Extracted proteins will be prepared following standard protocols to produce peptides appropriate for shotgun proteomic analysis. Total sample complexity will be reduced by on-line 2-D liquid chromatography to separate peptides based on charge and hydrophobicity. Fractions from the separation will be sequentially ionized into the mass spectrometer, oscillating between full scan and "MS/MS" modes, the former acquiring data on intact peptides and the latter first fragmenting peptides and then acquiring data (thus providing sequence information). The 2-D LC-MS/MS process is completely automated (using an LTQ-Orbitrap (Verberkmoes et al., 2009b)). Raw MS/MS spectra will then be compared to genomic data using ORNL's automated proteome informatics pipeline.

The matched metagenomic sequencing from these samples provides a critical database for protein identification. In this proposal the sequenced metagenomes from all 24 samples (each comprised of 3 spatial triplicates) will be used to build a protein database to search the tandem mass spectra. Matching of MS/MS spectra to predicted peptides will be accomplished with the SEQUEST algorithm (Eng et al., 1994) (Verberkmoes et al., 2009b)). ORNL has website portals for data dissemination, so that as we process and analyze samples we can access them remotely and share them with all project collaborators and the general public.

These -omics surveys serve several purposes. The metagenomic survey accesses community diversity and metabolic potential. First, it provides an independent window into community diversity that does not rely upon single-gene amplifications and thus avoids their potential biases. Second, it represents the community's genomic metabolic potential. Third, it provides a normalization dataset for the metatranscriptomic data, so the abundance of each transcript can be normalized to its gene abundance to obtain an accurate index of its expression (Frias-Lopez et al., 2008; Shi et al., 2009). We anticipate the presence of known CH_4 cycling pathways (including mcrA and pmoA genes), differentially distributed among the surveyed habitats and depths.

In turn, the metatranscriptomic and -proteomic surveys will define which clades and genes present in each habitat are actually expressed, and therefore likely mediating the biogeochemical processes measured. These surveys will likely indicate the high expression of known CH_4 cycling genes at times and zones of peak methanogenesis and methanotrophy, while *also revealing other highly expressed genes that are potentially important in this system*.

This metatranscriptomic and proteomic aspect of the proposed sequencing is vital to (a) linking genome-encoded metabolic potential to the measured biogeochemical system outputs, and (b)

identifying potentially overlooked processes occurring in these soils during peak CH_4 production and consumption that may be interacting with C-cycling in this ecosystem.

4.B. Ecosystem Biogeochemistry: measuring CH_4, CO_2 concentrations, fluxes, and isotopes

We will measure CO_2 and CH_4 fluxes, and soil climate and chemistry parameters, in the Mire at three spatial scales: ecosystem, plot, and soil profile (Figure 5), covering the three habitats along the climate change sequence – palsa (dry), *Sphagnum (*mesic*)*, to *Eriophorum* (wet). **A key innovation of our proposed work is continuous *in situ* automated measurements of the ^{13}C isotopic composition of carbon gas concentrations and fluxes, made possible by new QC-laser spectrometer technology, developed by members of the proposal team and our collaborators (see 4.B.iv, below)**. The proposed isotope work is critical for connecting the biogeochemistry to the microbial ecology, because it enables partitioning of measured fluxes into component processes and organic substrate sources due to characteristic isotopic fractionation along different methano -genic and -trophic pathways (Figure 5).

i. Ecosystem C flux via eddy covariance. A 3-m eddy covariance (EC) tower has been in intermittent operation at Stordalen over the last 10 years, measuring fluxes of CO_2 and CH_4 at the Mire {Jackowicz-Korczynski, 2009 #373}. The CH_4 measurements are made by a fast-response tunable diode laser (TDL) system made by Aerodyne Inc {Zahniser, 1995 #130} (our collaborators on this project for the newly developed QC-laser system). This system will be upgraded and operated with separate funding obtained by our collaborators in Sweden, and the data made available to this project.

ii. Plot-level C flux via automated chambers. A system of 8 automatic gas-sampling chambers of transparent Lexan was installed on the three habitat types at Stordalen Mire in 2001 (n=3 each in the palsa and *Sphagnum* habitats, and n=2 in the *Eriophorum* habitat) ({Bäckstrand, 2008 #143}. Chambers cover 0.14m^2 to a height of 25-45cm, depending on the vegetation, and are inserted to a depth of 5-10cm. A transparent lid closes automatically every 3 hours for 5 minutes, during which chamber air flows through dekoron tubing to the gas analysis system in a nearby heated shed. Dried and filtered (0.4 µm) sample air is measured by a non-dispersive CO_2 analyzer (Li-cor) and a total hydrocarbon analyzer (California Instruments), and changes in gas concentration indicate fluxes (as in Figure 6). In the past, CH_4 and H_2 gases have been determined via manual syringe samples measured via GC FID (Shimadzu) within 10 hours of collection at Abisko Field Station.

We will substantially upgrade gas analysis by deploying two newly developed quantum cascade (QC) laser absorption spectrometers for analysis of isotopologues of carbon dioxide ($^{13}CO_2$, $C^{18}OO$, and

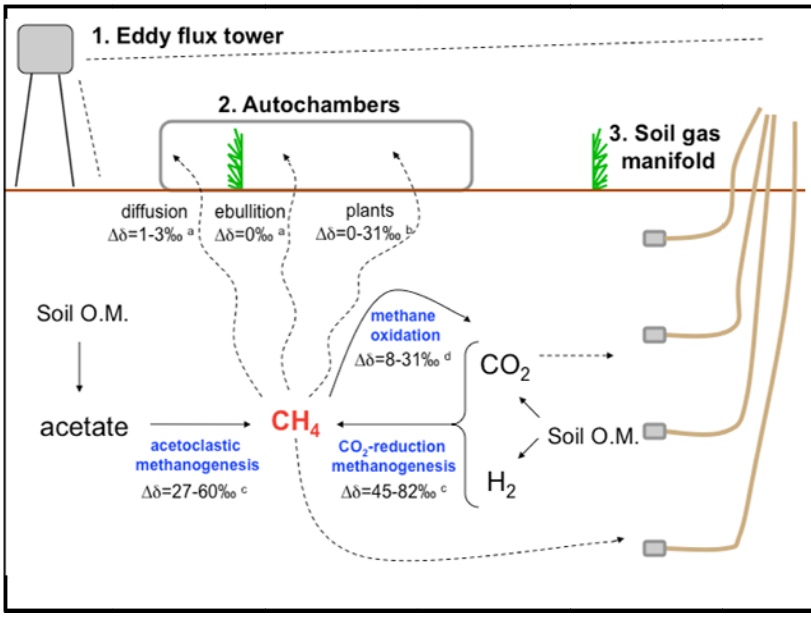


Figure 5: Investigating the methane cycle at three spatial scales, and the expected C isotopic fractionation associated with methane transformations and transport. Dotted black lines indicate transport, solid lines indicate transformations. Δδ = the isotopic fractionation associated with each process.
Notes: a. Chanton2005b. b. fractionation depends on mechanism which depends on plant species; effusion Δδ = up to 31 ‰, bulk flow Δδ = 0 ‰, diffusion Δδ = 1-16 ‰ ;Chanton 2005bc. note fractionation from acetate involved the methyl C; Conrad 2005 d. Chanton 2005.

CO_2), and methane ($^{13}CH_4$ and CH_4) (see **section 4.iv**, below, for instrumentation details).

iii. Soil gas profiles via automatic sampling. The soil gas sampling system (modified from Hirsch et al., 2002 and using components developed in Mastepanov and Christensen, 2008) will use 3m of thin-walled teflon tubing buried in the soils as a sampler, which will be connected to analyzers through a solenoid manifold. We use microporous Teflon because it excludes liquid water but allows rapid equilibration with dissolved gases. Wood depth guides spaced every 30 cm will allow for easy installation and depth maintenance (at 4 different depths) until the cuts in the peat heal after about 1 month. The large surface to volume ratio gives rapid equilibration, allowing at least daily measurements, during which each sampler tube is switched for 2 minutes into the sample line flowing at 1.5 slp (the second minute provides the measurement after initial flushing during the first minute).

Three manual equilibration samplers of 2 m Teflon tubes length are already installed in the mire, and demonstrate the feasibility of our approach (Figure 5).

iv. Isotope ratio measurements via cutting-edge instrumentation. It is widely recognized that isotopes provide a powerful means for partitioning net fluxes among production, consumption, and transport processes (Figure 5), but broader usage in field studies has been impeded by the time, labor and expense associated with standard methods based on manual sample collection (Bowling et al., 1999),(DOE, 2008). This limitation is beginning to be addressed by a range of new instruments using tunable laser absorption spectroscopy for obtaining isotope ratios – including tunable diode lasers, or TDLs (Bowling et al., 2005; Griffis et al., 2008), and a newer generation of technologies that, unlike diode lasers, do not require cryogenic cooling (from e.g. Aerodyne, Picarro, Los Gatos Research). However, the QC laser system we propose to deploy here is the only one that we are aware of that has achieved adequate precision for field measurements of CH_4 isotopes at ambient concentrations.

Co-PI Saleska has collaborated with Aerodyne Research on development of field-deployable laser spectrometers for measurement of isotopologues of CO_2 (McManus et al., 2005; Saleska et al., 2006; Nelson et al., 2008; Tuzson et al., 2008), and of CH_4 (Zahniser et al., 2009). Technical feasibility of these new systems has been demonstrated by field deployments: the CO_2 QCL system (SD on 1-minute air samples of $\delta^{13}C_{CO2}$ =0.06‰) is now being deployed to Harvard Forest for long-term eddy flux measurements under a separate DOE grant to Co-PI Saleska (Wehr et al, in prep); the more recently developed CH_4 QCL system (SD on 1-minute air samples of $\delta^{13}C_{CH4}$ =0.5‰) has sampled flux autochambers at Sallie's Fen natural wetlands in New Hampshire, giving estimates of the isotopic composition of the CH_4 source to within 0.9 ‰ (Figure 6; Santoni et al, in prep).

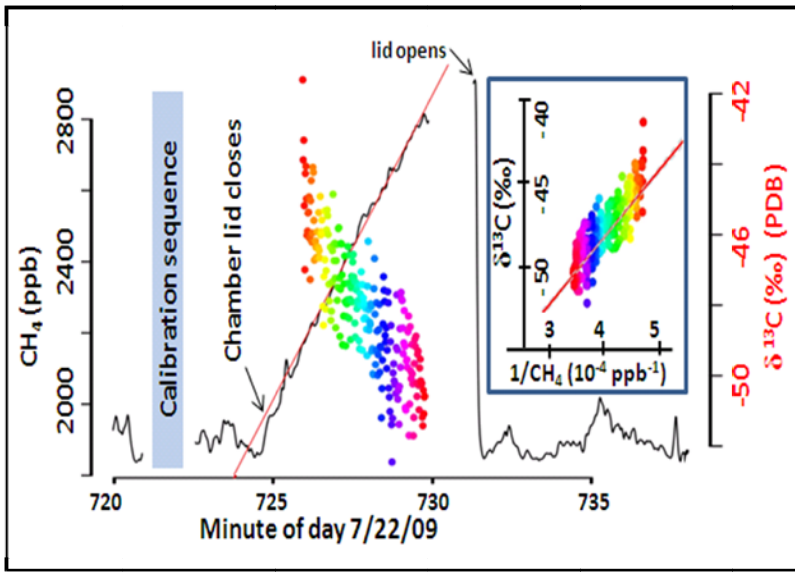


Figure 6. Field demonstration of ARI's prototype CH_4QCL system at Sallie's Fen, NH, July 2009: automatic chamber timeseries showing CH_4 rise (black line, with red regression line giving emission of 65.8 mgCH_4 m^{-2} d^{-1}) and associated decline in $\delta^{13}C$ (colored points, coded by time) following chamber closure at 725 min (around noon EST). **Inset:** Keeling plot fit (via geometric mean regression, Pataki et al., 2003) of $\delta^{13}C$ vs. ($1/CH_4$) gives isotope composition of methane source, -68.01 ± 0.88‰ (±1 SD). Data courtesy ARI, G. Santoni and S. Wofsy.

Measurement protocol includes a rigorous calibration schedule, similar to that employed by Bowling et al. (2005) and Griffis et al. (2008), using 3 traceable calibration gases spanning the anticipated range of sample air. We will also intercompare with IRMS flask sampling to test for biases or fractionation effects in the sampling system.

v. Soil chemistry and manual sampling of isotopes in soil gases and solids: We will collect soil gas and pore water samples manually, on the same timetable as for the microbial soil cores (beginning, middle, and end of growing season, as in Figure 3), to measure gases (H_2 concentrations, isotopes of C and H in CH_4), pore water constituents (DIC, DOC, and D/H ratios in H_2O, acetate), and solids (peat organic matter isotopes). Stable carbon and hydrogen isotope ratios will be measured using traditional IRMS methods, with samples split between Stockholm University (Co-PI Crill) and the isotope laboratory at Florida State University (PI Chanton). In addition to stable isotope data, we will measure natural abundance ^{14}C in emitted CH_4 and CO_2 (automatically collected via molecular sieve traps placed in the automatic sampling line, Hirsch et al., 2002), in pore water CH_4, DIC, DOC, and solid phase peat to estimate the average age of the source organic matter used to fuel methanogenesis (radiocarbon distinguishes the relative importance of decomposing permafrost peat in fueling methane production, as this peat will be of greater age) (Chasar et al., 2000b; Walter et al., 2006; Chanton et al., 2008; Walter et al., 2008). ^{14}C analyses will be conducted at NOSAMS (Woods Hole) following sample preparation and cryogenic purification at Co-PI Chanton's lab at FSU.

In addition, we will determine the isotope ratio of the methyl carbon of acetate, since it undergoes fractionation during the acetoclastic formation of CH_4 (Chanton et al., 2005)) and therefore can provide an additional end-member for inferring CH_4 source and production rates (Conrad, 2005). This entails measuring total acetate $\delta^{13}C$ by HPLC-coupled to IRMS by assuming a 20-24 ‰ fractionation between the methyl and carboxyl carbons of acetate (Conrad et al., 2007; Conrad et al., 2009).

To place the gas flux and isotope data into context, we will leverage ongoing measurements of soil hydrology and chemistry, including water table depth, pH, oxygen concentration, and surface water element concentrations (see Table 1). Soil redox potential is highly relevant for testing the DNDC process model (sect.4.C.ii, below), so we will measure soil redox potential via platinum probe (Rabenhorst et al., 2009), and also constraint it with soil gas concentrations of H_2 (Chapelle, 1997). (Chanton et al., 2005; Conrad, 2005; Conrad et al., 2007; Conrad et al., 2009).

These measurements, in addition to rigorously testing the more frequent laser spectrometer measurements, provide key additional constraints on methane production/consumption (see Figure 5, and section 4.C.i, below).

vi. Potential technical challenges and considerations.

The automated soil water and gas sampling system (sect 4B.iii) has been used successfully for automated acquisition of gas concentration data (Hirsch et al., 2002); we propose to further develop this method for isotopic retrieval, using the QC laser spectrometers. This will require rigorous testing under experimental conditions to adjust for isotopic fractionation between the sampled air and the soil air/water being measured. Before deployment to the field in Sweden, we will build and test probes in the artificial wetland of the University of Arizona's Biosphere 2 facility (Finn, 1996; Huxman et al., 2009), whose laboratories have a CO2 QCL system identical to that proposed for deployment to Sweden. This provides the realism of the field in an enclosed facility close to University resources.

4.C. Modeling and Hypothesis-testing

i. Modeling fluxes and isotopes to infer methane production pathways and associated spatial and temporal hotspots of methane production and consumption

The concentration, flux and isotope data, by constraining a flux-gradient transport model of soil gas, will allow us to map spatial patterns of production and consumption and different methanogenic pathways (Figure 5), as in (Chanton et al., 2005; Chanton, 2005; Conrad, 2005), and (Blodau et al., 2007).

The isotope data are critical to this mapping (Chanton et al., 1995; Popp et al., 1999; Chasar et al., 2000a; Chasar et al., 2000b; Chanton et al., 2005). For example, apparent fractionation factors (α) between carbon as DIC and as CH_4 -- where $\alpha_C = \left(\delta^{13}C_{DIC} + 1000\right) / \left(\delta^{13}C_{CH_4} + 1000\right)$, and an analogous α_D term calculated from δD_{H2O} and δD_{CH4} (e.g. (Hornibrook et al., 1997; Whiticar, 1999; Conrad, 2002)) – represent changes in production mechanisms: larger α_C (1.065 to 1.090) and smaller α_D values (1.20 to 1.35) are typical of CO_2 reduction while the opposite is typical of acetate fermentation (Whiticar et al., 1986; Sugimoto and Wada, 1993; Whiticar, 1999; Conrad, 2002). We refer to these factors as apparent, because while CO_2 and water are the precursors for CH_4 formed from CO_2 reduction, they are not the immediate precursors for CH_4 formed from acetate fermentation. They nonetheless consistently represent variations in CH_4 production mechanisms (Hornibrook et al., 1997; Chasar et al., 2000a; Hornibrook et al., 2000b, a; Conrad, 2002; Chanton et al., 2005; Chanton, 2005; Chanton et al., 2008). Similar analysis with α_D will distinguish oxidation from production effects (Chanton et al., 2006).

Residual ambiguities in partitioning will be resolved by: (a) incubations using the inhibitors methyl fluoride or BES to isolate specific pathways (Janssen and Frenzel, 1997; Hines et al., 2009); and (b) partitioning of methanogenesis between the acetoclastic and CO_2-reductive pathways, based on isotope composition of the methyl group of acetate (Conrad 2005).

We will address the climate change question: "is decomposing permafrost the source of increasing methane production?" by following the approach of Zimov 1997 (Walter et al., 2006; Walter et al., 2008) and (Chanton et al., 2008), and using the natural abundance ^{14}C (Chasar et al., 2000b; Chanton et al., 2008) to distinguish the relative importance of decomposing permafrost peat in fueling methane production, as this peat will be of greater age than modern production. (Prater et al., 2007)

Our transport-fractionation model of methane production/consumption will be updated as field data are generated to provide a rapid assessment of CH_4 cycling in the Mire, with visualized output available to collaborators via a central website. This analysis will provide the basis for an initial comparison, via statistical regression techniques, of the biogeochemical data (specifically variation in production-consumption fluxes) to the microbial transcriptomic and proteomic data (which gives relative microbial activity levels in different process pathways).

ii. System process modeling with DNDC: testing the importance of microbial ecology to ecosystem biogeochemistry

The **DNDC** (DeNitrification-DeComposition) model, originally developed for quantifying C sequestration and greenhouse gas emissions for agricultural lands (Li et al., 1992; Li et al., 1994), has been developed, tested and applied to a wide range of terrestrial ecosystems to predict C and N transport and transformation by simulating how external drivers (e.g., weather, soil, human activity) affect environmental factors (e.g., soil climate, redox potential, substrate concentrations), which then determine the rates of biochemical and geochemical reactions (Figure 7). Of particular relevance to the work proposed here, DNDC explicitly represents processes arising from specific soil microbial activities (e.g., decomposition, nitrification, denitrification, fermentation, H_2-based and acetoclastic methanogenesis, and aerobic methane oxidation), and has been evaluated against a number of field data sets (Cai et al., 2003; Jagadeesh Babu et al., 2006; Beheydt et al., 2007; Smith et al., 2008). We propose to use a wetland version of DNDC, driven by the soil hydro-climate conditions, including modeled or prescribed water table fluctuation and soil water fluxes (Figures 7 and 8; (Zhang et al., 2002; Li et al., 2003; Frolking et al., 2004; Cui et al., 2005; Sun et al., 2006; Li, 2007; Kurbatova et al., 2008)).

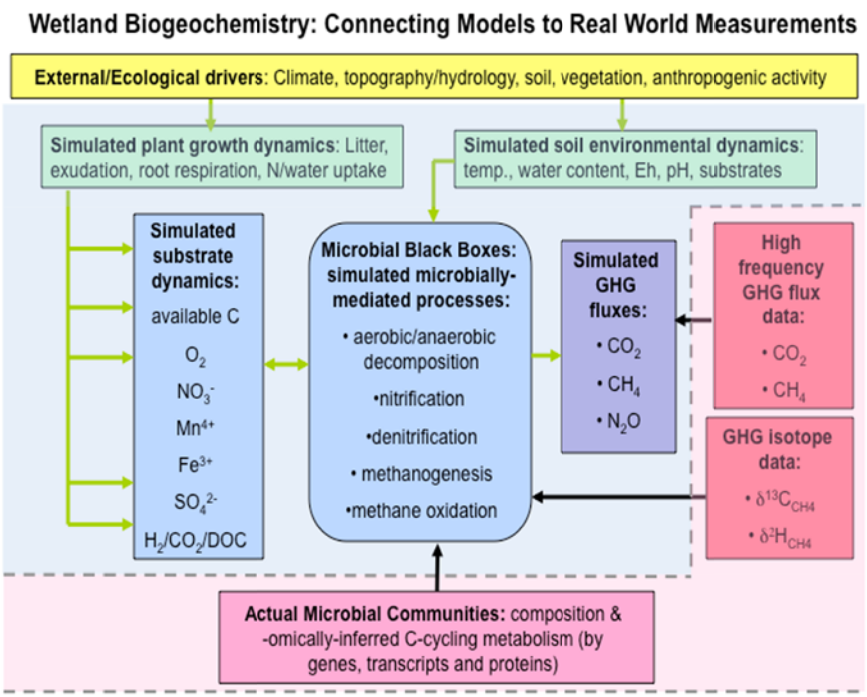


Figure 7 – Link between DNDC simulations, where microbial process rates are controlled by soil Eh (Nernst Equation) and substrate availability (Michaelis-Menten kinetics) (blue shading) that are controlled indirectly by ecological drivers (yellow shading) and more directly by soil environmental conditions (green shading), but actual microbial biomass and activity are only inferred. DNDC output (GHG fluxes, purple shading) can be tested (black arrows) against gas flux data (standard model evaluation) but also against two new data sets that probe deeper into model mechanisms related to microbially-mediated processes: isotope data and microbial community profiling (composition and –omically-inferred metagenomic) data.

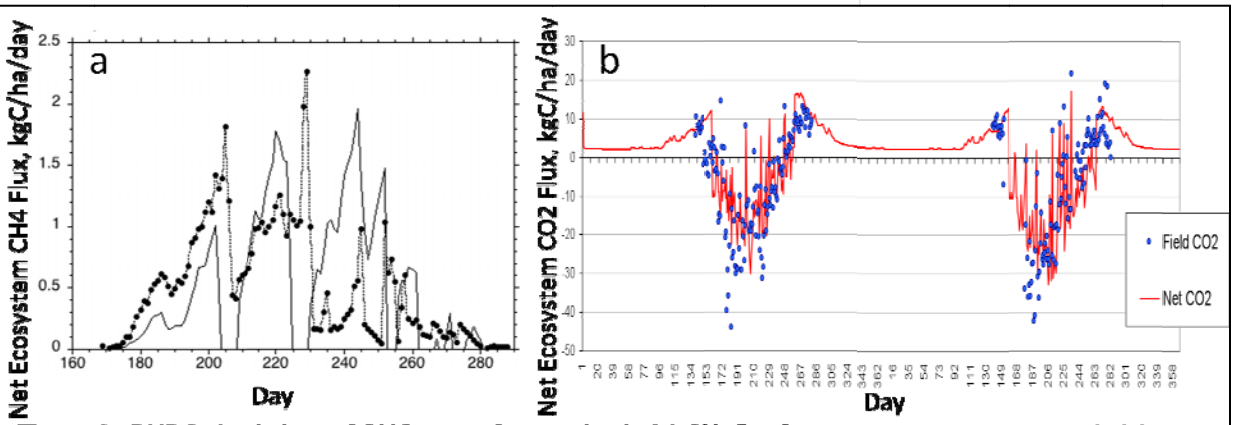


Figure 8. DNDC simulations of GHG gases from wetlands (a) CH_4 flux from paddy with mid-season draining, Jiangsu Province, China (data from Zheng et al. 1999), and (b) seasonal variation in net CO2 exchange fluxes for a fen in Saskachewan, Canada (data from Suyker et al. 1997).

DNDC Application/Role in this Project We propose 4 tasks for this project: **Task 1** will be to simulate net CO_2 and CH_4 fluxes for the Abisko sites for comparison with high temporal (hourly) resolution flux data (see Section 4.B.). **Task 2** will be to further develop DNDC to track the isotopic signature associated with the various modeled carbon pools and flows (using known isotopic fractionation factors, see Figure 5). This will subject the model to more rigorous testing of net gas fluxes against observed istotopic signatures as well as of intermediate processes such as the absolute rates of methane production and oxidation, not just their net sum. **Task 3** will be a novel comparison of simulated rates of microbially-mediated biogeochemical processes (e.g., methanogenesis and methanotrophy) directly against observations of the corresponding relative functional activity levels of the microbial community, as recorded in metatranscriptomic and -proteomic data (see Section 4.A.). We believe this will be the first study to directly use microbial population and expression data representing processes on the microscopic scale to test mechanisms in a process-based biogeochemical model that scales to the ecosystem and continental scales. Such a comparison is made possible by two developments: (i)

advances in the molecular biologist's toolkit to include methods (meta-transcriptomics and proteomics) which can quantify the relative activity level, within the whole *in situ* microbial community, of the expressed RNA and proteins which operate distinct biochemical pathways causing production and consumption of carbon gases, and (ii) models (such as DNDC), which, though they do not represent microbial populations directly, nonetheless use environmental conditions (particularly redox potential) and substrate availability to separately predict processes corresponding to the microbially mediated biochemical paths. This project will provide an important additional axis on which to test the model, but more importantly, should provide a specific example of how to tackle the fundamental problem of scaling from genes to ecosystems. Finally, **Task 4** is extrapolation and prognostic simulation. After adequate testing against field observations, the model will be used to project impacts on methane emissions of continuing permafrost thaw at Abisko, both scaling the measurements up to the full site level and projecting forward in time for a range of climate futures.

5. Scientific Deliverables

This study will deliver results in: *microbial ecology*, including **(a)** the first environmental dataset encompassing all 3 levels of biological information analysis from DNA (genomics) through mRNA (transcriptomics) to proteins (proteomics); **(b)** unprecedented resolution of microbial community dynamics involved in C-cycling across growing seasons, across three habitat types of thawing northern wetlands, and at distinct soil horizons; **(c)** specific microbial community expression data on known CH_4 cycling genes, and on competing and synergistic metabolic pathways for CH_4 transformations, which will likely open new windows on how microbial ecology controls CH_4 flux.

This study will also deliver results in: *biogeochemistry of wetland carbon cycling,* including **(a)** a novel high-resolution dataset of CO_2 / CH_4 isotopic compositions in fluxes and soil gas profiles, enabled by the new QC-laser absorption spectrometer; **(b)** quantification of the contribution of acetoclastic and CO_2-reductive methanogenesis and methanotrophy to CH_4 flux, in three habitat types along a climate-change sequence in northern wetland systems; and **(c)** a quantification of the relative role of newly thawing old peat vs. recent plant photosynthate as methanogenic substrates, and thereby the mechanism by which permafrost degradation may fuel enhanced methane emissions.

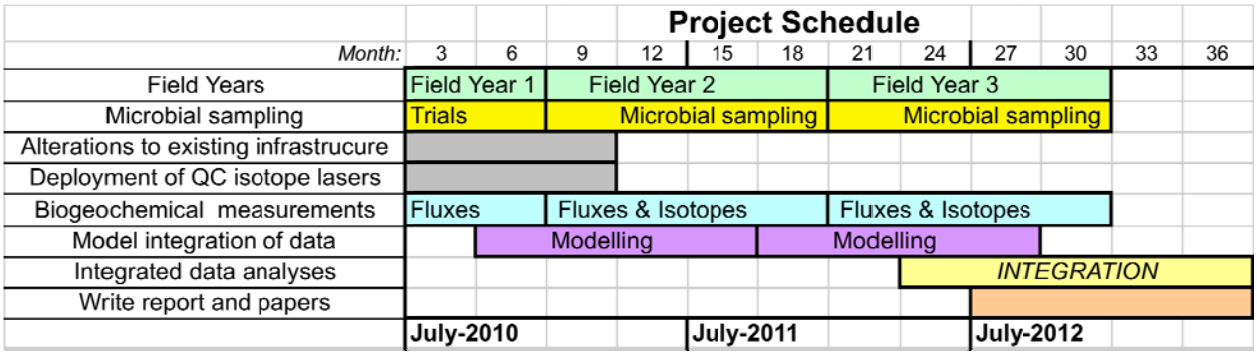
This study will deliver *new synthetic understanding* on scaling from microbial communities to ecosystem biogeochemistry, as embodied in **(a)** linkages between microbial community structure and metabolism and carbon gas production and consumption under environmental conditions; and **(b)** novel empirical tests of biogeochemical model (DNDC) processes with datasets of corresponding molecular microbial processes.

Finally, this study will deliver new insights into how both microbial communities and biogeochemical processes will likely change with climate, based on our observations across a three-point climate sequence. These new insights will be embodied in an improved DNDC process model, and in model simulations derived on these improvements, of the fate of northern wetland carbon and methane emissions in a globally changing climate.

6. Management Plan

6a. Project Timetable: We propose a 3-year schedule beginning July 1st 2010 (Figure 9) with biogeochemical flux measurements and a collection of trial microbial samples in August 2010 for initial community characterization and methods optimization. Deployment and testing of the QC-lasers will occur in winter 2010/11 (facilitated across frozen ground). The DNDC model will be tested initially in 2010/2011, against existing site flux data. The two field seasons of 2011 and 2012 will include: automated flux and isotope measurements, synoptic sampling of microbial communities in each habitat at the beginning, peak, and end of the growing season, DNDC model development to incorporate isotopic tracers (in year 1) followed by simulations and testing (in year 2). In the fall/winter of each of these years, we will sequence extracted DNA, cDNA and proteins using the methods optimized in year 1.

Figure 9. Project Timetable



In fall 2012-July 2013, we will write papers on the results from within each discipline as well as several papers synthesizing the broader scope of interdisciplinary work.

6b. Project Team and workplan: This is an interdisciplinary project with technical expertise required in diverse areas. We have assembled a team of domestic and international collaborators to fill this requirement. PI **Scott Saleska** (University of Arizona (UA)), an expert in carbon-cycle science with experience in international project management (as PI and Director of "Amazon-PIRE", a 5-year NSF-funded training and research program in the Amazon of Brazil), will coordinate the overall project, with project components allocated among investigators as follows (see CVs in Appendix 1):

1. *Molecular microbial ecology*: **Gene Tyson** (senior research fellow, University of Queensland, UQ), a pioneer of metagenomics and metatranscriptomic methods, will coordinate molecular microbial ecology work, optimize and perform nucleic acid extractions, and prepare mRNA-enriched cDNA. **Virginia Rich** (UA post-doc), experienced in cutting edge molecular microbial ecology methods, will collect microbial samples, perform protein extractions at ORNL, prepare extracted nucleic acids for sequencing (including pyrotagging), and analyze sequence data.
2. *Biogeochemistry and northern wetland C cycling:* **Patrick Crill** (Stockholm University (SU)), an expert in trace gas measurements from the tropics to the tundra, will supervise a field technician at Stordalen Mire, and, with Chanton, will analyze δD composition of manually collected soil gas samples. A **Saleska** lab post-doc (TBD) with expertise in instrumentation, will test, deploy, maintain, and analyze data from the QC laser systems used to acquire CO_2 and CH_4 isotopologues.
3. *Carbon gas transport/production modeling:* **Jeff Chanton** (Florida State University, FSU), an expert in obtaining and interpreting isotope measurements, will supervise a graduate student to obtain geochemical, δD and natural abundance ^{14}C data to constrain a model of spatial and temporal dynamics of methanogenesis and methanotrophy through the wetland soil column.
4. *Wetland ecosystem carbon cycle modeling:* **Changsheng Li** and **Steve Frolking** (University of New Hampshire), experts in carbon cycle modeling and developers of the Wetland-DNDC process model, will work with a research scientist (TBD) to conduct model development to add isotope tracers, and model simulations of carbon and methane cycling in Stordalen Mire to compare to microbial meta-omic data.

The project leverages significant benefits in cost from existing salary support for Dr. Rich, and from related projects already underway, including: Dr. Crill's support from European sources (covering his salary, research infrastructure and ongoing measurements at Stordalan Mire), and Dr. Li's recently funded NASA study of high-latitude wetlands using the Wetland-DNDC Model. Li's NASA project spans several established field sites, including Stordalen Mire, and will be leveraged here for travel costs to Sweden for field site visit(s), and for acquisition of key site data (e.g. vegetation and peat/soil type distributions, daily weather data, water table depth) to drive and evaluate DNDC model simulations.

Appendix 1: Biosketches

Appendix 1 includes biosketches for all project investigators:

Scott Saleska
Gene Tyson
Patrick Crill
Changsheng Li
Steve Frolking
Jeff Chanton
Virginia Rich

Scott R. Saleska
Dept. of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721
email: saleska@email.arizona.edu; web: http://www.eebweb.arizona.edu/faculty/saleska/

Education:

Massachusetts Institute of Technology (Physics) B.S. 1986
University of California, Berkeley (Energy and Resources Group) Ph.D., 1998
Harvard University (Dept. of Earth & Planetary Sciences), Post-Doctoral fellow 1999-2001

Appointments:

2005-present: *Assistant Professor*, University of Arizona, Ecology & Evolutionary Biology
2002-2004: *Research Associate*, Harvard University, Dept. of Earth & Planetary Sciences

Synergistic Activities

- Director, *Amazon-PIRE*, an NSF-funded 5-year (2007-2012) "Partnership for International Research and Education" (PIRE) focusing on Amazon forest-climate interactions. Partners include Harvard, University of São Paulo (Brazil), Federal University of Pará (in the Brazilian Amazon), and the Brazilian National Institute for Amazonian Research.
- Member, Science Steering Committee, UofA Biosphere 2 (2007 to present)
- Panel Reviewer for NASA (Carbon Cycle Science) and NSF (Ecosystems Panel)
- Associate Editor, *J. of Geophys Research – Biogeosciences*
- Reviewer for *Ecological Applications, Ecology Letters; Global Change Biology*; *Global Biogeochemical Cycles; Isotopes in Environmental and Health Studies; Nature; Oecologia; Philosophical Transactions of the Royal Society; Plant, Cell and Environment; Proceedings of the National Academy of Sciences; Science*

Selected publications (10 related)

Saleska, S., H.R. da Rocha, B. Kruijt, and A. Nobre. Ecosystem carbon fluxes and Amazon forest metabolism (in press). Invited peer-reviewed book chapter for *Amazonia and Global Change,* World Scientific Publishing.

Hutyra, L.R., J.W. Munger, E.-H. Pyle, S.R. ***Saleska***, N. Restrepo-Coupe, P.B. de Camargo, B.C. Daube, S.C. Wofsy. Resolving systematic errors in estimates of net ecosystem exchange of CO_2 and ecosystem respiration in a tall-stature forest: application to a tropical forest biome. 2008. *Agric. Forest Meteorology*. **148**: 1266-1279.

Saleska, S.R., K. Didan, A.R. Huete, and H.R. da Rocha. (2007). Amazon forests green-up during 2005 drought. *Science*, **318**: 612. (Published online 20 Sep 2007, doi: 10.1126/science.1146663).

Harte, J, S.R. ***Saleska***, T. Shih. 2006. Shifts in plant dominance control short and long-term carbon-cycle responses to widespread drought, *Env. Res. Lett*. **1**: 014001. (Online at stacks.iop.org/ERL/1/014001).

Huete, A.R., K. Didan, Y.E. Shimabukuro, P. Ratana, S.R. ***Saleska***, L.R. Hutyra, W. Yang, RR. Nemani, R. Myneni (2006). Amazon rainforests green-up with sunlight in dry season, *Geophys Res. Lett*, vol 33, L06405, doi:10.1029/2005GL025583.

Saleska, S; J. Shorter, S. Herndon, R. Jimenez, B. McManus, D. Nelson, M. Zahniser (2006). What are the instrumentation requirements for measuring the isotopic composition of net

ecosystem exchange of CO_2 using eddy covariance methods? *Isotopes Env. Health Studies,* **42** (2), 115-133.

McManus, J.B., D.D. Nelson, J.H. Shorter, R. Jiménez, S. Herndon, S. ***Saleska***, and M.S. Zahniser, (2005). A high precision pulsed QCL spectrometer for measurements of stable isotopes of carbon dioxide, *J. Modern Optics*, **52**, 2309-2321.

Dunne, J.A., S.R. ***Saleska***, M.L. Fischer, J. Harte. Integrating experimental and gradient methods in ecological climate change research. 2004. *Ecology.* 85: 904-916.

Saleska, S.R., S.D. Miller, D.M. Matross, M.L. Goulden, S.C. Wofsy, H. da Rocha, P.B. de Camargo, P.M. Crill, B.C. Daube, C. Freitas, L. Hutyra, M. Keller, V. Kirchhoff, M. Menton, J.W. Munger, E.H. Pyle, A.H. Rice, H. Silva (2003). Carbon in Amazon forests: unexpected seasonal fluxes and disturbance-induced losses. *Science*. 302: 1554-1557.

Saleska, S.R; M.R. Shaw, M. Fischer, J. Dunne, C.J. Still, M. Holman, and J. Harte (2002). Plant community composition mediates both large transient decline and predicted long-term recovery of soil carbon under climate warming. *Global Biogeochemical Cycles.* 16(4): 1055, doi:10.1029/2001GB001573.

Collaborators in the past 48 months: P.B. de Camargo, U-Sao Paulo, Brazil; P.M. Crill, Uppsala University, Sweden; B.C. Daube, Harvard University; K. Didan, U-Arizona, D. Fitzjarrald, SUNY-Albany; L.G. de Goncalves, NASA-Goddard; M.L. Goulden, U.C. Irvine; J. Harte, U.C. Berkeley; S. Herndon, Aerodyne Research, Inc; A. Huete, U-Arizona; L. Hutyra, University of Washington; Rodrigo Jimenez, Harvard University; M. Keller, NEON, Inc.; B. Kruit, Wageningen University, Netherlands; M.L. Lefsky, Colorado State University; Y. Malhi, Oxford University, U.K.; A. Manzi, INPA, Brazil; B. McManus, Aerodyne Research, Inc.; C.S. Martens, U-North Carolina, S.D. Miller, SUNY-Albany; P. Moorcroft, Harvard University; J.W. Munger, Harvard University; D. Nelson, Aerodyne Research, Inc.; A. Nobre, INPA, Brazil; H. da Rocha, U Sao Paulo Brazil; Y.E. Shimabukuro, INPE, Brazil; S.C. Wofsy, Harvard University; M. Zahniser, Aerodyne Research, Inc.

Graduate and Post-Doctoral Advisers
John Harte, U.C. Berkeley (PhD. advisor)
Steven Wofsy, Harvard University (post-doctoral advisor)
Graduate student thesis advisees: Joost van Haren (current), Bradley Christoffersen (current), Scott Stark (current), Jin Wu (current)
Post-doctoral advisees: Natalia Restrepo-Coupe (current), Richard Wehr (current), Kolby Jardine (current), Virginia Rich (current)

Dr Gene W. Tyson
Advanced Water Management Centre, University of Queensland, Australia 4072
Email: g.tyson@uq.edu.au; Web: http://www.awmc.uq.edu.au/index.html?page=108642

Education and Training

University of Queensland, Australia (Microbiology) B.Sc. (Hons), 1998

University of California, Berkeley, Dept. of Environ. Science Policy and Management Ph.D., 2006

Massachusetts Institute of Technology, Dept. of Civil and Environ. Engineering Post-Doctoral fellow, 2006-09

Research and Professional Experience

Queen Elizabeth II Fellow, University of Queensland, Advanced Water Management Centre & Australian Centre for Ecogenomics Research January 2010
Senior Research Fellow, University of Queensland, Advanced Water Management Centre April 2009

Publications

Shi, Y., **G.W. Tyson** and E. DeLong (2009). Microbial community transcriptomes provide new perspective on naturally occurring small RNAs. *Nature,* 459:266–269.

Hugenholtz, P., and **G.W. Tyson** (2008). Microbiology – Metagenomics. *Nature*, 455:481-483.

Frias-Lopez, J., Y. Shi, **G.W. Tyson**, M. Coleman, P. Chisholm, E. DeLong (2008). Microbial community gene expression in ocean surface waters. *Proceeding of the National Academy Sciences*, 105:3805-3810.

Tyson, G.W., and J.F. Banfield (2007). Rapidly evolving CRISPRs implicated in acquired resistance of microorganisms to viruses. *Environmental Microbiology*, 10:200-207.

Tyson, G.W., J.M. Eppley, W. Getz, J.F. Banfield (2007). Genetic exchange across an archaeal species boundary. *Genetics,* 177:407-416.

Allen E.E., **G.W. Tyson**, C. Detter, R. Whitaker, P. Richardson, J.F. Banfield. (2007) Genome evolution in a natural microbial strain population. *Proceeding of the National Academy Sciences*, 104:1883-1888.

Baker, B.J., **G.W. Tyson**, R.I. Webb, J. Flanagan, P. Hugenholtz, E.E. Allen, J.F. Banfield. (2006) Novel lineages of acidophilic, ultra-small Archaea revealed by community genome sequencing. *Science*, 314,1933-1935.

Tyson, G.W., I. Lo, B.J. Baker, E.E. Allen, P. Hugenholtz, J.F. Banfield (2005). Genome directed isolation of the key nitrogen fixer, *Leptospirillum ferrodiazotrophum* sp. nov., from an acidophilic microbial community. *Applied and Environmental Microbiology* 71, 6319-6324.

Ram, R.J., N.C.VerBerkmoes, M.P. Thelen, **G.W. Tyson,** B.J. Baker, R.C. Blake II, M. Shah, R.L. Hettich, J.F. Banfield (2005). Community proteomics reveals key roles for "hypothetical" proteins in a natural microbial biofilm. *Science* 308, 1915-1920.

Tyson, G.W., J. Chapman, P. Hugenholtz, E.E. Allen, R.J. Ram, P.M. Richardson, V.V. Solovyev, E.M. Rubin, D.S. Rokhsar, J.F. Banfield (2004). Community structure and metabolism through reconstruction of microbial genomes from the environment. *Nature* 428, 37-43.

Synergistic Activities

- Dr. Tyson has presented invited talks at 12 international meetings, including International Society for Microbial Ecology (ISME2008), Genomes, Medicine and the Environment (GME2007), Integrating Metabolism and Genomics (IMAGE2), Gordon Research Conference on Applied and Environmental Microbiology Conference, 12th International Conference on Microbial Genomes, NASA Astrobiology Institute, 3rd American Society of Microbiology (ASM) and The Institute for Genome Research (TIGR) Conference on Microbial Genomes.

- He is an editorial board member of the International Society for Microbial Ecology (ISME) journal.
- He is a reviewer for the following research funding authorities: *National Science Foundation (NSF), Australian Research Council*
- He is a reviewer for the following international journals: *Environmental Microbiology, Applied and Environmental Microbiology, The ISME Journal: Multidisciplinary Journal of Microbial Ecology, Proceedings of the National Academy of Sciences.*
- He was an invited instructor at the C-MORE international summer course on Microbial Oceanography: Genomes to Biomes, and the 2009 Winter School in Mathematical & Computational Biology at the University of Queensland, Australia.

Collaborators and Co-editors

Phil Bond, University of Queensland, Australia; Penny Chisholm, Massachusetts Institute of Technology, USA; Maureen Coleman, Massachusetts Institute of Technology, USA; Edward DeLong, Massachusetts Institute of Technology, USA; Jorge Frias-Lopez, Massachusetts Institute of Technology, USA; Philip Hugenholtz, Joint Genome Institute, USA; Asuncion Martinez, Massachusetts Institute of Technology, USA;Justin Seymour, Massachusetts Institute of Technology, USA; Yanmei Shi, Massachusetts Institute of Technology, USA; Matthew Sullivan, University of Arizona, USA; Rick Webb, University of Queensland, Australia; Zhiguo Yuan, University of Queensland, Australia

Graduate and Post-doctoral Advisers

Jillian F. Banfield, U.C. Berkeley (Ph.D advisor)
Edward F. DeLong, Massachusetts Institute of Technology (Post-doctoral advisor)

Graduate students and postdoctoral associates: Jeremy Barr (Ph.D., current), Lauren Bragg (Ph.D., current), Barry Cayford (Ph.D., current), Mohammed Fauzi Haroon (Honors, current), Hui Jie Lim (Honours, current), Hasina Pervina (Ph.D., current)

Patrick Michael Crill
Professor of Biogeochemistry
Department of Geology and Geochemistry
Stockholm University
106 91 Stockholm
Sweden
46 (0)8 16 4740 fax: 46 (0)8 674 7855
email: patrick.crill@geo.su.se

Education & Training:

1972: Marine Science Technician "A" School, U.S. Coast Guard, Governor's Island, NY
1978: **B.S. Biochemistry**, cum laude, University of Massachusetts Amherst, MA
1981: **M.S. Marine Sciences**, University of North Carolina, Chapel Hill, NC under the direction of Dr. C.S. Martens.
1984: **Ph.D. Marine Sciences**, *Methane Production and Sulfate Reduction in an Anoxic Marine Sediment*, University of North Carolina, Chapel Hill, NC under the direction of Dr. C.S. Martens.
1984- 1986: National Research Council Resident Post Doctoral Research Associate, NASA Langley Research Center, Hampton, VA. under the direction of Dr. R.C. Harriss

Research and Professional Experience:

October 2003 – present: **Professor of Biogeochemistry, Department of Geology and Geochemistry, Stockholm University, Stockholm, Sweden**. 80% research/ 20% teaching. Atmosphere/biosphere exchange particularly impacts of climate and landscape change and land use on processes of formation and exchange of biogenic gases, biogeochemistry of soils, peats, lacustrine and marine environments, urban sources of CH_4 and CO_2, urban metabolism, biogeochemistry of CH_3Br and other halogens on local to global scales.
1971- 1975: Marine science technician, U.S.Coast Guard, Boston, MA, VietNam Era Veteran
1975-1978: B.S. Biochemistry, University of Massachusetts Amherst, MA
1977- 1978: Research assistant, Biochemistry department University of MA.
1978-1981: M.S. Marine Sciences, University of North Carolina, Chapel Hill, NC under the direction of Dr. C.S. Martens.
1978- 1980: Research assistant, Univ. North Carolina Institute of Marine Sciences, Morehead City (summer) and Curriculum in Marine Sciences, Chapel Hill, NC.
1980- 1980: Microbial Ecology, Marine Biological Laboratory, Woods Hole, MA, Dr. Holger Jannasch.
1980- 1984: NASA graduate researcher, Univ. North Carolina, Chapel Hill, NC.
1981-1984: Ph.D. studies, Univ. North Carolina, Chapel Hill, NC.
1986- 1987: Senior Scientist, Bionetics Corporation, NASA Langley Research Center, Hampton, VA.
1987- 1988: Research Scientist, Department of Chemistry, College of William and Mary, Williamsburg, VA.
1988- 1992: Research Assistant Professor, Complex Systems Res. Cen., EOS, Univ. of New Hampshire, Durham, NH.
1992- 2001: Research Associate Professor, Complex Systems Res. Cen., EOS, Univ. of New Hampshire, Durham, NH.
1997- 2000: Associate Director of the Complex Systems Research Center
2000- 2003: Director of the Complex Systems Research Center
2001- 2003: Research Professor, University of New Hampshire, Durham, NH,
2003- 2005: Affiliate Professor of Earth Sciences, Univ of New Hampshire, Durham, NH
2007- present: Vice Prefect, Dept of Geology and Geochemistry, Stockholm University

Ten Publications:

Phillips, S.C., R.K. Varner, S. Frolking, J.W. Munger, J.L. Bubier, S.C. Wofsy and P.M. Crill (2009). Interannual, seasonal, and diel variation in soil respiration measured by autochambers along a wetland to upland slope at Harvard Forest. *J. Geophys. Res.,* in press.

Nisbet, R.E.R., R. Fisher, R.H. Nimmo, D.S. Bendall, P.M. Crill, A.V. Gallego-Sala, E.R. Hornibrook, E. Lopez-Juez, D. Lowry, P.B.R. Nisbet, E. F. Shuckburgh, S. Sriskantharajah, C.J. Howe, and E.G. Nisbet (2009). Emission of methane by plants. *Phil. Trans. Roy. Soc. B*, doi:10.1098/rspb.2008.1731.

Bäckstrand, K., P.M. Crill, M. Mastepanov, T.R. Christensen and D. Bastviken (2008). Nonmethane Volatile Organic Compound Flux from a Subarctic mire in Northern Sweden. *Tellus*, 60B, 226-237.

Bäckstrand, K., P.M. Crill, M. Mastepanov, T.R. Christensen and D. Bastviken (2008). Total hydrocarbon flux dynamics at a subarctic mire in northern Sweden. *J. Geophys. Res., 113:* G03026, doi:10.1029/2008JG000703.

Treat, C., J.L. Bubier, R.K. Varner and P.M. Crill (2007). Timescale dependence of environmental and plant-mediated controls on CH_4 flux in a temperate fen. J. Geophys. Res.-Biogeosci., 112: Art. No. G01014.

Johansson, T., N.Malmer, P.M. Crill, T. Friborg, J.A.Åkerman, M.Mastepanov and T.R.Christensen (2006). Decadal vegetation changes in a northern peatland, greenhouse gas fluxes and net radiative forcing, *Global Change Biology*, 12: 2352-2369.

Burrows, E.H., J.L. Bubier, A. Mosedale, G.W. Cobb, and P.M. Crill (2005). Net Ecosystem Exchange of Carbon Dioxide in a Temperate Poor Fen: A Comparison of Automated and Manual Chamber Techniques, *Biogeochemistry*, *76*: 21–45.

Christensen, T.R., T. Johansson, J. Åkerman, M. Masteponev, N. Malmer, T. Friborg, P. Crill and B. Svensson (2004). Thawing subarctic permafrost: Effects on vegetation and methane emission. *Geophys. Res. Lett.*, 31: L04501, doi:10.1029/2003GL018680.

Frolking, S. and P.M. Crill (1994). Climate controls on methane flux from a poor fen in southeastern, New Hampshire: Measurement and modelling. *Global Biogeochem. Cycles*, 8: 385-398.

Crill, P.M., K.B. Bartlett, D.I. Sebacher, R.C. Harriss, E.S. Verry, E. Gorham, L. Madzar and J. Sanner (1988). Methane flux from Minnesota wetlands. *Global Biogeochemical Cycles*, 2: 371-384.

Synergistic Activities:

ESF Workshop Belowground Carbon Pools in Permafrost Regions, Stockholm, 2005.
Tellus B advisory board 2006-present.
NCEAS workshop on Assessment of Global CH4 Sources, Santa Barbara, CA 2006-2009.
NordFlux, A research network supporting the study of greenhouse gas exchange from northern ecosystems sponsored by the Nordic Council of Ministers.
Management Council, EU COST action, ES0902: Permafrost and gas hydrate related methane release in the Arctic and impact on climate change, 2009-2016.

Collaborators and Close Colleagues:

J.A. Åkerman, Lund Univ.
F. Arghe, Stockholm Univ.
A. Arneth, Lund Univ.
G.P. Asner, Carnegie Inst, CA
K. Bäckstrand, Stockholm Univ.
D. Bastviken, Linköping Univ
D.S. Bendall, Cambridge Univ.
J.L. Bubier, Mt Holyoke College
E.H. Burrows, Oregon State Univ
T.R. Christensen, Lund Univ.
G.W. Cobb, Mt Holyoke College
R. Cosme de Oliveira Jr., EMBRAPA, Brasil
P.B. de Camargo, USP, Brasil
W.Z. de Mello, UF-Fluminense, Brasil
J.D. Dias, USP, Brasil
J.B. do Carmo, USP, Brasil

A. Ekberg, Lund Univ.
R. Fisher, Univ. London
T. Friborg, Univ. Copenhagen
S. Frolking, Univ New Hampshire
A.V. Gallego-Sala, Univ. Bristol
S. Hayward, Lund Univ.
T. Holst, Lund Univ.
M. Jackowicz-Korczynski, Lund Univ.
T. Johansson, Lund Univ.
M. Keller, NEON, Colorado
H. Koyi, Uppsala Univ.
A. Lindroth, Lund Univ.
N. Malmer, Lund Univ.
M.Mastepanov, Lund Univ.
M.E. McGroddy, UC-Berkeley
T. Moore, McGill Univ.
A. Mosedale, Univ New Hampshire
S.C. Mosedale, Univ New Hampshire
J.W. Munger, Harvard Univ.
R.H. Nimmo, Cambridge Univ.
E. Nisbet, Univ. London
R.E.R. Nisbet, Cambridge Univ.
M. Olsrud, Lund Univ.
A.M. Petrescu, Free Univ Amsterdam
S.C. Phillips, Univ New Hampshire
K. Savage, Woods Hole Res Cen
H. Silva, UF-Para, Brasil
W.L. Silver, UC-Berkeley
A. Skelton, Stockholm Univ.
L. Ström, Lund Univ.
A.W. Thompson, UC-Berkeley
C. Treat, Univ New Hampshire
J. van Huissteden, Free Univ Amsterdam
R.K. Varner Univ New Hampshire
E. Veldkamp, Univ Göttingen
M.L. White, Univ New Hampshire
R. Whitmarsh, Univ Southampton
B. Wick, Humboldt Univ, Germany
S.C. Wofsy, Harvard Univ.
A. Yurova, Lund Univ.

Graduate and Postdoctoral Advisees:

Graduate Students:
Evilene Lopes, Ph.D, UNH. 2005 now at U of Ill.
Hudson da Silva, M.S., UNH, 2005 now teaching at UF-Para, Brazil
Marco Ravenna, M.Sc.. 2007, External Examiner, KTH, Sweden.
Terhi Riutta, Ph.D. 2008, External Examiner, Helsinki Univ, Finland.
Kristina Bäckstrand, Ph.D., S.U., 2008
Marcin Jackowicz-Korczynski, Ph.D., Lund U., coadvisor, 2009, now in Poland
Maria Heinneman, Ph.D., S.U., current
Nguyen Duc, Ph.D., S.U., current
Gustaf Hugelius, Ph.D., S.U., co-advisor, current
Isabell Kiepe, Ph.D. Copenhagen U., co-advisor, current
Elin Sundqvist, Ph.D. Lund Univ, co-advisor, current

Post Doctoral Associates (continue to collaborate with all)
Steve Frolking (1994-5)
Jill Bubier (1997-8)
Ruth Varner (2000-2002)
David Basviken (2006-2009)

Education and Training

University of Wisconsin and Chinese Academy of Sciences, Ph.D., Biogeochemistry, 1988
Chinese Academy of Sciences, Beijing; Environmental Chemistry; M.S.; 1981
University of Science and Technology of China, Beijing; Geochemistry; B.S.; 1964

Research and Professional Experience

Research Professor, Complex Systems Research Center, Institute for the Study of Earth, Oceans, and Space, University of New Hampshire, September 1997 - Present,
Research Associate Professor, Complex Systems Research Center, Institute for the Study of Earth, Oceans, and Space, University of New Hampshire. November 1992 - September 1997.
Senior Scientist, The Bruce Company, Washington, D.C., consulting U.S. Environmental Protection Agency with Global Climate Change programs, August 1989 - October 1992.
Senior Administrator, National Environmental Protection Agency of China, Beijing. Managed scientific programs of environmental protection in China. April 1988 - August 1989.
Deputy Director, Chinese Academy of Sciences, Research Center for Eco-Environmental Sciences, Beijing. October 1985 - April 1988.

Ten Publications

1. Kurbatova, J., **Li, C.**, Varlagin, A., Xiao, X., and Vygodskaya, N. (2008) Modeling carbon dynamics in two adjacent spruce forests with different soil conditions in Russia. *Biogeosciences Discuss* 5: 969-980.
2. Li., C. 2007. Quantifying greenhouse gas emissions from soils: Scientific basis and modeling approach. Soil Science and Plant Nutrition, 53:344-352.
3. Sleutel S., De Neve S., Beheydt D., Li C. and Hofman G. 2007a. Regional simulation of organic carbon stock changes in cropland soils using the DNDC model: 1. Large scale model validation. Soil Use and Management, 22:342-351.
4. Miehle, P., S.J. Livesley, P.M. Feikema, C. Li, and S.K. Arndt. 2006. Assessing productivity and carbon sequestration capacity of Eucalyptus globulus plantations using the process model Forest-DNDC: Calibration and validation. Ecological Modelling 192:83-94.
5. Jagadeesh Babu, Y., C. Li, S. Frolking, D.R. Nayak, and T.K. Adhya. 2006. Field validation of DNDC model for methane and nitrous oxide emissions from rice-based production systems of India. Nutrien Cycling in Agroecosystems 74:157-174. doi:10.1007/s10705-005-6111-5.
6. Li, C., W. Salas, B. DeAngelo, and S. Rose, 2006. Assessing alternatives for mitigating net greenhouse gas emissions and increasing yields from rice production in China over the next 20 years. Journal of Environmental Quality 35:1554-1565, doi:10.2134/jeq2005.0208.
8. Cui, J., **Li, C.**, and Trettin, C. (2005) Analyzing the ecosystem carbon and hydrologic characteristics of forested wetland using a biogeochemical process model. *Global Change Biology* 11: 278-289.
9. **Frolking, S.**, **Li, C.**, Braswell, R., and Fuglestvedt, J. (2004) Short- and long-term greenhouse gas and radiative forcing impacts of changing water management in Asian rice paddies. *Global Change Biology* 10: 1180-1196.
10. Cai, Z., Sawamoto, T., **Li, C.**, Kang, G., Boonjawat, J., Mosier, A. et al. (2003) Field validation of the DNDC model for greenhouse gas emissions in East Asian cropping systems. *Global Biogeochemical Cycles* 17: 1107.

Synergistic Activities

Conducted research on biogeochemical theories and methodologies. Prof. Dr. Li has been engaged in biogeochemical studies of abundant and trace elements with a focus on modeling biogeochemical cycles of the elements and their impacts on human health and environmental safety. Since 1989, Li has been Principal Investigator (PI) coordinating the research projects as follows:

- "Developing Biogeochemical Model for Predicting N2O emissions from the US Agricultural Land" sponsored by EPA in 1989-1991;
- "Development of Biogeochemical Model of Carbon and Nitrogen Cycles in Agro-Ecosystems" sponsored by NSF in 1992-1994;

- "Quantifying Atmospheric Impacts of Rice Agriculture in China" sponsored by NASA in 1995-1997;
- "Assessing the Influence of Asian Rice Paddies on the Growth Rate of Atmospheric Methane 1980-2020" sponsored by NASA in 1998-2000;
- "Developing a Desktop DNDC Tool for Evaluating Best Management Practices for Reducing Nutrient Loading to Elkhorn Slough NERR" sponsored by NOAA in 2002-2004;
- "Disseminating a GIS Based Nutrient Management Training Tool for Coastal Managers" sponsored by NOAA in 2004-2006;
- "Quantifying CO2 Fluxes from Boreal Forests in Northern Eurasia (Russia): An Integrated Analysis of Flux Tower Data, Remote Sensing Data and Biogeochemical Modeling" sponsored by NASA in 2005-2007;
- "Predicting Impacts of Alternative Farming Management Practices on Crop Yield, Soil Carbon Sequestration and Trace Gas Emissions from Chinese Rice Agriculture" sponsored by EPA in 2005-2006;
- "Developing Manure-DNDC: Quantifying Ammonia and Methane Emissions from California Dairies" sponsored by USDA in 2006-2008;
- "Development of a Soil Carbon Model (Forest-DNDC) for Wetland and Upland Forests" sponsored by USDA Forest Service in 2000-2009.

Distributed basic knowledge of biogeochemistry to the public through training undergraduate and graduate students, writing textbooks, presenting lectures, and publishing papers.

Served government agencies - the National Environmental Protection Agency of China (1988-89) and the US EPA (1989-92), worked on scientific aspects of policies related to natural resource conservation, pollution control, and global climate change.

Established collaborative, academic ties between the biogeochemistry modeling research communities in North America, Asia, Europe and Oceania. In 2007-2008, mainly collaborated with international researchers in Canada, China, Japan, India, Germany, the U.K., Belgium, Finland, the Netherlands, Russia, Australia and New Zealand by hosting them in UNH or paying short-term visits to their countries through various US-based or international research projects. The major activities in the collaborations are (1) testing the DNDC model against crop yields, soil climate, soil C and N dynamics and greenhouse gas emissions observed in the countries, (2) modifying DNDC at science and code levels to make it applicable at global scale, and (3) applying DNDC for inventories and mitigations at regional, national or global scale.

Steve Frolking
Institute for the Study of Earth, Oceans, and Space
University of New Hampshire, Durham, NH 03824
Ph (fax): 603-862-0244 (0188); e-mail: steve.frolking@unh.edu

Experience
2006-2009: Director, Complex Systems Research Center, University of New Hampshire.
2003-2006: Associate Director, Complex Systems Research Center, UNH.
2002 - : Research Associate Professor, Inst. for the Study of Earth, Oceans, and Space, UNH.
1995-2002: Research Assistant Professor, Inst. for the Study of Earth, Oceans, and Space, UNH.
1993-1995: Post-Doctoral Fellow, NOAA Program in Climate and Global Change.
1989-1993: Graduate Fellow, Dept. of Earth Sciences, UNH.
1988-1989: Instructor in Physics, UNH.
1987-1988: Instructor in Physics, St. Anselm College, Manchester NH.
1986-1987: Instructor in Physics, UNH.
1984-1986: Research Scientist, Nuclear Physics Group, UNH.

Education
• Ph.D., Earth Sciences (Biogeochemistry), University of New Hampshire, 1989-1993.
• M.S., Physics, U. of New Hampshire, 1980-1983.
• B.S. (Summa Cum Laude), Physics, U. of New Hampshire, 1977-1980.

Some Relevant Publications
Frolking S. **2009-in press**. Permafrost, Ch. 5 in *Report on Methane and Nitrous Oxide Emissions from Natural Sources*, prepared for the Climate Change Division of the US EPA.

St-Hilaire F, JH Wu, NT Roulet, **S Frolking**, PM Lafleur, ER Humphreys, V Arora. **2009-in press**. McGill Wetland Model: Evaluation of a peatland carbon simulator developed for global assessments, *Biogeosciences*.

Brook E, Archer D, Dlugokencky E, **Frolking S**, Lawrence D. **2008**. Potential for Abrupt Changes in Atmospheric Methane, Ch. 5 in *U.S. Climate Change Science Program Synthesis and Assessment Product 3.4: Abrupt Climate Change*. [http://www.climatescience.gov/Library/sap/sap3-4/final-report/]

Frolking S, Roulet NT. **2007**. Holocene radiative forcing impact of northern peatland carbon accumulation and methane emissions, *Global Change Biology*, 13:1079–1088.

Frolking S, Roulet NT, Moore TR, Lafleur P, Bubier JL, Crill PM. 2002. Modeling the seasonal to annual carbon balance of Mer Bleue Bog, Ontario, Canada, *Global Biogeochem. Cycles*. 16(3): 10.1029/2001GB001457.

Frolking S, NT Roulet, TR Moore, PJH Richard, M Lavoie, & SD Muller (2001) Modeling northern peatland decomposition and peat accumulation, *Ecosystems,* 4:479-498.

Frolking S, McDonald K, Kimball J, Zimmermann R, Way JB, Running SW (1999) Using the space-borne NASA Scatterometer (NSCAT) to determine the frozen and thawed seasons of a boreal landscape, *J. Geophys. Res*. 104: 27,895-27,908.

Frolking, S, ML Goulden, SC Wofsy, S-M Fan, DJ Sutton, JW Munger, AM Bazzaz, BC Daube, PM Crill, JD Aber, LE Band, X Wang, K Savage, T Moore, and RC Harriss (1996) Modelling temporal variability in the carbon balance of a spruce/moss boreal forest, *Global Change Biol.*, 2:343-366.

Frolking, S and P Crill. **1994**. Climate controls on temporal variability of methane flux from a poor fen in southeastern New Hampshire: Measurement and modeling, *Global Biogeochem. Cycles*, 8:385-397.

Li, C, **S Frolking**, TA Frolking. **1992**. A model of nitrous oxide evolution from soil driven by rainfall events: I. model structure and sensitivity, *J. Geophys. Res.,* 97:9759-9776.

Synergistic Activities

- Associate Editor: *Journal of Geophysical Research – Biogeosciences,* 2005 – present.
- Lead author: report on mapping agricultural land use & management at sub-national scales, UN-FAO, 2006.
- Peer reviewer for ~40 journals, federal agencies, and international research organizations.

Confict of Interest List (non-UNH)

Brook E (Oregon State); Bubier JL (Mt. Holyoke College); Chambers JQ (Tulane); Clark DB (U Missouri); Crill PM (U Stockholm); Dlugokencky E (NOAA); Douglas E (UMass-Boston); Edmonds JA (PNNL); Fekete B (CCNY); Friedl M (Boston U); Fuglestvedt J (CICERO, Oslo); Harriss RC (HARC); Hollinger D (USFS); Houghton RA (WHRC); Keller M. (NEON Inc.); Kimball JS (U Montana); King AW (Oak Ridge); Lafleur PM (Trent U.); Lawrence D (NCAR); McDonald K (NASA JPL); McGuire AD (U Alaska); Melillo J (MBL); Moore B (Climate Central); Moore TR (McGill U); Munger JW (Harvard); Mysak LA (McGill); Niyogi D (Purdue); Pacala S (Princeton); Peterson B (MBL); Rawlins M (NASA-JPL) Richard PJH (U. Montreal); Richardson AD (Harvard); Roulet NT (McGill U); Salas W (Appl. Geosol'n.); Shevliakova E (Princeton); Shugart HH (U Virginia); Thomson AM (PNNL); Vörösmarty CJ (CCNY); Wofsy SC (Harvard); Xiao X (U Oklahoma).

Jeffrey Paul Chanton, Department of Oceanography, Florida State University, Tallahassee, Florida 32306.
850-644-7493, fax 850-644-2581, email jchanton@fsu.edu

A. Education

New College	Natural Science	A.B. 1975
UNC-Chapel Hill	Marine Sciences	M.S. 1979
UNC-Chapel Hill	Marine Science	PhD. 1985

B. Experience

2008. William H. Patrick Jr. Memorial Lecturer at the Soil Science Society of America Annual Meeting, Houston, TX

2006. Distinguished Research Professor Award, Florida St. Univ.

2005. Aldo Leopold Fellowship Award

2005. Florida Wildlife Federation Conservation Communicator of the Year

2003. Awarded named Professorship, John W. Winchester Professor of Oceanography.

1997. Professor, Dept. of Oceanography, Florida State University. Concurrent Doctoral Directive Status in the Department of Chemistry.

1993. Associate Professor, Dept. of Oceanography, Florida State University.

1988. Assistant Professor, Dept. of Oceanography, Florida State University.

1987. Research Assistant Professor, UNC Chapel Hill.

Jeff Chanton works on a variety of research problems that involve fluxes of greenhouse gases and isotopic chemistry. Current projects include the effect of permafrost decomposition on CH_4 release from boreal regions, the design of landfill covers to reduce CH_4 emissions and the study of ecosystem respiration in pine forests and peatlands. He's also involved in studies of gas hydrate stability and is a member of the Gulf of Mexico Gas Hydrate Research Consortium to establish a sea floor observatory.

C. Ten Related PUBLICATIONS. ***Italics = first author was a student when the work was done.***

1 Chanton, J. P. , D. K. Powelson, R. B. Green. 2009. Methane Oxidation in Landfill Cover Soils, is a 10% Default Value Reasonable? J. Environ. Qual. 38:654–663.

2 Walter, K. M., J. P. Chanton, E. A. G. Schuur, S. A. Zimov, & F. S. Chapin III, 2008. Methane production and bubble emissions from arctic lakes: Isotopic implications for source pathways and ages. J. Geophys. Res., 113, G00A08, doi:10.1029/2007JG000569.

3 Chanton, J. P., P. H. Glaser, L. S. Chasar, D. J. Burdige, M. E. Hines, D. I. Siegel, L. B. Tremblay, & W. T. Cooper (2008), Radiocarbon evidence for the importance of surface vegetation on fermentation and methanogenesis in

contrasting types of boreal peatlands, Global Biogeochem. Cycles, 22, GB4022, doi:10.1029/2008GB003274.

4 Hines, M.E., K N. Duddleston, J. Rooney-Varga, D. Fields and J. P. Chanton. 2008. Uncoupling of acetate degradation from methane formation in Alaskan wetlands: Connections to vegetation distribution. Global Biogeochemical Cycles, 22, GB2017, doi:10.1029/2006GB002903.

5 Prater, J. L., J. P. Chanton, and G. J. Whiting. 2007. Variation in methane production pathways associated with permafrost decomposition in collapse scar bogs of Alberta, Canada, Global Biogeochem. Cycles, 21, GB4004, doi:10.1029/2006GB002866.

6 Walter, K.M., S. A. Zimov, F. S. Chapin, III, J. P. Chanton, D. Verbyla. 2006. A positive feedback to climate warming through methane bubbling from Siberian thaw lakes. Nature 443, 71-75 doi:10.1038/nature050402005

7 Chanton, J.P., Fields, D. and Hines, M.E. 2006. Controls on the hydrogen isotopic composition of biogenic methane from high latitude terrestrial wetlands. J. Geophys. Res., 111, G04004, doi:10.1029/2005JG000134.

8 Chanton, J. P., D. K. Powelson, T. Abichou, D. Fields, & R. B. Green. 2008. Effect of Temperature and Oxidation Rate on Carbon-isotope Fractionation during Methane Oxidation by Landfill Cover Materials Environmental Science and Technology No 42, pp 7818-7823. DOI 10.1021/es80122y.

9 Chanton, J. P., D. K. Powelson, T. Abichou & G. Hater. 2008. Improved Field Methods to Quantify Methane Oxidation in Landfill Cover Materials Using Stable Carbon Isotopes Env. Sci and Tech. 42, 665-670.

10 *Lapham, L.L.***,** J .P. Chanton, C.S. Martens, P. D. Higley, H. W. Jannasch & J.R. Woolsey (2008). Measuring long term changes in dissolved ion and gas concentrations and stable isotopes at a hydrate site: Mississippi Canyon 118, Gulf of Mexico. Environmental Science and Technology, 42, 7367-7373.

D. Synergistic Activities

1. Director Aquatic Environmental Science Masters program, FSU
2. Associate editor, Journal of Geophysical Research
3. FSU Science Education Advisory Board
4. Panelist for President Bush's Ocean Commission in St. Petersburg, Florida.
5. Teacher of large undergraduate classes on Oceanography. Instructed 500 students in Fall, 01, 03, 05. Development of Course on Current Issues in Environmental Science. Offered to general public through the Florida State University Center for Professional Development.

E. Collaborators and Co-editors:

Tim Arkebauer, University of Nebraska, L. Boring, S. Opsahl Jones Research Center, Paul Glaser, University of Minnesota, Mark Hines, Univ. of Mass., G. Katul, Duke University, G. Lewis, NW Florida Management District, R. Oren, Duke University, Don Siegel, University of Syracuse, R. Streigel, USGS, S. Verma, University of Nebraska, D. Ward, University of Montana, , Gary Whiting, Christopher Newport University. Jean Bogner, Landfill Plus Inc. K. Walter, University of Alaska, Robert Woolsey, University of Mississippi.

F. Chanton's graduate and post graduate advisor, C.S. Martens, UNC-Chapel Hill.

G. Names and Affiliations of Recent Graduate Students and Post-docs.
Behzad Mortazavi, University of Alabama
David Powelson, Utah State University
Laura Lapham, Florida State University
Carl Childs, NOAA
James Prater, Office of Naval Research, Panama City Florida State University
Kelly Peeler, Northwestern University
Chad Hanson, Pew Trust
Dana Field, Rickards Public High School

Virginia Isabel Rich
Postdoctoral Researcher
Department of Ecology and Evolutionary Biology
BSW-224, University of Arizona, Tucson, AZ 85721
vrich@email.arizona.edu

Education and Training

University of California, Berkeley
(Integrative Biology and Molecular and Cell Biology) B.A. 1998

Massachusetts Institute of Technology/Woods Hole Oceanographic Institute
(Joint Program in Biological Oceanography: Microbial Oceanography) Ph.D. 9/2008

University of Arizona, Dept. of Ecology & Evolutionary Biology,
Post-Doctoral Researcher, 12/2008-present

Research and Professional Experience

Monterey Bay Aquarium Research Institute, DeLong Lab, *Intern* 2000

University of Washington, Departments of Biology and Biochemistry,
Lecturer and Teaching Associate 2000-2001

University of Washington, Friday Harbor Labs, Research Apprenticeship Program
Lecturer and Teaching Associate 2001-2002

University of Washington, Center for Cell Dynamics, *Laboratory Technician* 2001-2002

Publications

Rich, V, Pham V, Shi Y, Eppley J, DeLong EF. 2009. Time-series investigation of a coastal microbial community in Monterey Bay, CA, using the "genome proxy" microarray. *In preparation*.

Rich, V, Konstantinidis K, DeLong EF. 2008. Design and testing of "genome proxy" microarrays to profile marine microbial communities. *Environmental Microbiology*, 10: 506-521.

Preston, CM, Suzuki M, **Rich V**, Heidelberg J, Chavez F, DeLong EF. Detection and distribution of two novel form II RuBisCos in the Monterey Bay. *In preparation.*

DeLong EF, Preston CM, Mincer T, **Rich V**, Hallam SJ, Frigaard NU, Martinez A, Sullivan MB, Edwards R, Brito BR, Chisholm SW, Karl DM. 2006. Community genomics among stratified microbial assemblages in the ocean's interior. *Science*. 311:496-503.

Horz, H-P, **Rich V**, Avrahami S, and Bohannan BJ. 2005. Methane-oxidizing bacteria in a California upland grassland soil: diversity and response to simulated global change. *Applied and Environmental Microbiology*. 71(5): 2642-2652.

Synergistic Activities

- Presented posters and talks at domestic and international meetings including the 12th International Symposium on Microbial Ecology conference (Australia, 2008), the Gordon Conference for Marine Microbes (Italy, 2008), the11th International Symposium on Microbial Ecology (Austria, 2006), the 3rd Microbial Observatories and Microbial Interactions and Processes Program Principal Investigators' Workshop (Montana, 2004). *Received award for best graduate student poster at the Gordon Conference on Marine Microbes*, Il Ciocco, Italy, July 2008.
- *Microbial Ecology Journal Club* Co-Founder and -Organizer, MIT, 09/05 – 6/07.
- *Earth Systems Initiative's Microbial Systems Group Mini-symposium* Co-Organizer, MIT, 06/06: The first symposium of the nascent Microbial Systems Group at MIT, designed to foster communication among labs.
- *Path of Professorship* Workshop Organizer, MIT, 10/06, and consultant, 10/07: This two-day workshop for graduate and postdoctoral women scientists and engineers aimed to improve the retention of Science, Technology, Engineering, and Math (STEM) women in Academia, by providing information about this career path. It was sponsored by Dean Blanche Staton (bestaton@mit.edu) and I was the sole organizer, and was an organizational consultant in year 2. The workshop received very positive reviews, and has been repeated each year since, using the materials I developed.
- *Women-in-Science Seminar Series* Co-Organizer, MIT, 01/06 – 05/06.

Collaborators

David Bourne, Australian Institute for Marine Science; Jeff Chanton, Florida State University, Patrick Crill, University of New Hampshire/ Stockholm University; Ed DeLong, Massachusetts Institute for Technology; Katerina Dontsova, University of Arizona; John Eppley, Massachusetts Institute of Technology; Steve Frolking, University of New Hampshire; Changsheng Li, University of New Hampshire; Yanmei Shi, Massachusetts Institute of Technology; Matthew Sullivan, University of Arizona; Gene Tyson, Australian Water Quality Centre, University of Queensland; Vinh Pham, Massachusetts Institute of Technology; Christina Preston, Monterey Bay Aquarium Research Institute

Graduate and Post-Doctoral Advisers

Ed DeLong, U.C. Berkeley (PhD. advisor)

George Somero, Stanford University (PhD. co-advisor)

Brendan Bohannan, University of Oregon (PhD rotation advisor)

Scott Saleksa, University of Arizona (post-doctoral advisor)

Gene Tyson, University of Queensland (post-doctoral co-advisor)

Appendix 3: Bibliography & References Cited.

** names of proposal investigators have been highlighted.*

Åkerman, H.J., and Johansson, M. (2008) Thawing permafrost and thicker active layers in sub-arctic Sweden. *Permafrost and Periglacial Processes* 19: 279-292.

Bäckstrand, K., **Crill, P.M.**, Mastepanov, M., Christensen, T.R., and Bastviken, D. (2008a) Non-methane volatile organic compound flux from a subarctic mire in Northern Sweden. *Tellus B* 60: 226-237.

Bäckstrand, K., **Crill, P.M.**, Mastepanov, M., Christensen, T.R., and Bastviken, D. (2008b) Total hydrocarbon flux dynamics at a subarctic mire in northern Sweden. *J Geophys Res* 113.

Bäckstrand, K., **Crill, P.M.**, Jackowicz-Korczyñski, M., Mastepanov, M., Christensen, T.R., and Bastviken, D. (2009) Annual carbon gas budget for a subarctic peatland, northern Sweden. *Biogeosciences Discuss* 6: 5705-5740.

Beheydt, D., Boeckx, P., Sleutel, S., **Li, C**., and Van Cleemput, O. (2007) Validation of DNDC for 22 long-term N2O field emission measurements. *Atmospheric Environment* 41: 6196-6211.

Blodau, C., Bernhard, M., Peiffer, S., and Moore, T.R. (2007) Support for an anaerobic sulfur cycle in two Canadian peatland soils. *Journal of Geophysical Research* 112: G02004.

Bohannan, B.J.M., and Hughes, J. (2003) New approaches to analyzing microbial biodiversity data. *Current Opinion in Microbiology* 6: 282-287.

Bowling, D.B., Burns, S.P., Conway, T.J., Monson, R.K., and White, J.W.C. (2005) Extensive observations of CO2 carbon isotope content in and above a high-elevation subalpine forest. *Global Biogeochemical Cycles* 19: GB3023.

Bowling, D.R., Baldocci, D.D., and Monson, R.K. (1999) Dynamics of isotopic exchange of carbon dioxide in a Tennessee deciduous forest. *Global Biogeochemical Cycles* 13: 903-922.

Cai, Z., Sawamoto, T., **Li, C.**, Kang, G., Boonjawat, J., Mosier, A. et al. (2003) Field validation of the DNDC model for greenhouse gas emissions in East Asian cropping systems. *Global Biogeochemical Cycles* 17: 1107.

Chanton, J., Chaser, L., Glasser, P., and Siegel, D. (2005) Carbon and Hydrogen Isotopic Effects in Microbial, Methane from Terrestrial Environments. In *Stable Isotopes and Biosphere Atmosphere Interactions*. Flanagan, L.B., Ehleringer, J.R., and Pataki, D.E. (eds). Amsterdam: Elsevier-Academic, pp. 85-105.

Chanton, J.P. (2005) The effect of gas transport on the isotope signature of methane in wetlands. *Organic Geochemistry* 36: 753-768.

Chanton, J.P., Fields, D., and Hines, M.E. (2006) Controls on the hydrogen isotopic composition of biogenic methane from high latitude terrestrial wetlands. . *Journal of Geophysical Research—Biogeosciences* 111: G04004.

Chanton, J.P., Bauer, J., Glaser, P., Siegel, D., Ramonowitz, E., Tyler, S. et al. (1995) Radiocarbon evidence for the substrates supporting methane formation within northern Minnesota peatlands. *Geochim Cosmochim Acta* 59: 3663-3668.

Chanton, J.P., Glaser, P.H., Chasar, L.S., Burdige, D.J., Hines, M.E., Siegel, D.I. et al. (2008) Radiocarbon evidence for the importance of surface vegetation on fermentation and methanogenesis in contrasting types of boreal peatlands. *Global Biogeochemical Cycles* 22.

Chao, A. (1987) Estimating the population size for capture-recapture data with unequal catchability. *Biometrics* 43: 783-791.

Chao, A., and Lee, S. (1992) Estimating the Number of Classes Via Sample Coverage. *Journal of the American Statistical Association* 87: 210-217.

Chapelle, F.H., Vroblesky, D.A., Woodward, J.C., and Lovley, D.R. (1997) Practical considerations for measuring hydrogen concentrations in groundwater. *Environmental Science & Technology* 31: 2873-2877.

Chasar, L.S., **Chanton, J.P**., Glaser, P.H., and Siegel, D.I. (2000a) Methane concentration and stable isotope distribution as evidence of rhizospheric processes: comparison of a fen and bog in the Glacial Lake Agassiz peatland complex. . *Annals of Botany* 86.

Chasar, L.S., **Chanton, J.P**., Glaser, P.H., Siegel, D.I., and Rivers, J.S. (2000b) Radiocarbon and stable carbon isotopic evidence for transport and transformation of DOC, DIC and CH4 in a northern Minnesota peatland. *Global Biogeochemical Cycles* 14: 1095-1105.

Christensen, T.R., Ekberg, A., Ström, L., Mastepanov, M., Panikov, N., Öquist, M. et al. (2003) Factors controlling large scale variations in methane emissions from wetlands. *Geophys Res Lett* 30.

Conrad, R. (2002) Control of microbial methane production in wetland rice fields. *Nutrient Cycling in Agroecosystems* 64: 59-69.

Conrad, R. (2005) Quantification of methanogenic pathways using stable carbon isotopic signatures: a review and a proposal. *Organic Geochemistry* 36: 739-752.

Conrad, R., Claus, P., and Casper, P. (2009) Characterization of stable isotope fractionation during methane production in the sediment of a eutrophic lake, Lake Dagow, Germany. *Limnology and Oceanography* 54: 457-471.

Conrad, R., Chan, O.C., Claus, P., and Casper, P. (2007) Characterization of methanogenic Archaea and stable isotope fractionation during methane production in the profundal sediment of an oligotrophic lake (Lake Stechlin, Germany). *Limnology and Oceanography* 52: 1393-1406.

Cui, J., **Li, C.**, and Trettin, C. (2005) Analyzing the ecosystem carbon and hydrologic characteristics of forested wetland using a biogeochemical process model. *Global Change Biology* 11: 278-289.

Daniel, R. (2005) The metagenomics of soil. *Nature Reviews Microbiology* 3: 470-478.

Denman, K.L., Brasseur, G., Chidthaisong, A., Ciais, P., Cox, P.M., Dickinson, R.E. et al. (2007) Couplings Between Changes in the Climate System and Biogeochemistry. In *Climate Change 2007: The Physical Science Basis Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B. et al. (eds). Cambridge, United Kingdom and New York, NY, USA.: Cambridge University Press.

Dlugokencky, E.J., Houweling, S., Bruhwiler, L., Masarie, K.A., Lang, P.M., Miller, a.J.B., and Tans, P.P. (2003) Atmospheric methane levels off: Temporary pause or a new steadystate? *Geophysical Research Letters* 30: 1992.

Dlugokencky, E.J., Bruhwiler, L., White, J.W.C., Emmons, L.K., Novelli, P.C., Montzka, S.A. et al. (2009) Observational constraints on recent increases in the atmospheric CH4 burden. *Geophysical Research Letters* in press.

DOE (2008) Carbon Cycling and Biosequestration: Report from the March 2008 Workshop, DOE/SC-108. In. Science, U.S.D.o.E.O.o. (ed).

Ehhalt, D., Prather, M., and al., e. (2001) Atmospheric Chemistry and Greenhouse Gases In *IPCC Report: Climate Change 2001: The Scientific Basis*. Houghton, J.T., and *al., e.* (eds). Cambridge Cambridge University Press.

Eng, J.K., McCormack, A.L., and Yates III, J.R. (1994) An approach to correlate tandem mass spectral data of peptides with amino acid sequences in a protein database. *J Am Soc Mass Spectrom* 5: 976–989.

Finn, M. (1996) The mangrove mesocosm of Biosphere 2: Design, establishment and preliminary results. *Ecological Engineering* 6: 21-56.

Forster, P., Ramaswamy, V., Artaxo, P., Berntsen, T., Betts, R., Fahey, D.W. et al. (2007) Changes in Atmospheric Constituents and in Radiative Forcing. In *Climate Change 2007: The Physical Science Basis Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on*

Climate Change. Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B. et al. (eds). Cambridge, United Kingdom: Cambridge University Press.

Frias-Lopez, J., Shi, Y., **Tyson, G.W.**, Coleman, M.L., Schuster, S.C., Chisholm, S.W., and Delong, E.F. (2008) Microbial community gene expression in ocean surface waters. *Proceedings of the National Academy of Sciences U S A* 105: 3805-3810.

Frolking, S., **Li, C.**, Braswell, R., and Fuglestvedt, J. (2004) Short- and long-term greenhouse gas and radiative forcing impacts of changing water management in Asian rice paddies. *Global Change Biology* 10: 1180-1196.

Gabor, E.M., de Vries, E.J., and Janssen, D.B. (2003) Efficient recovery of environmental DNA for expression cloning by indirect extraction methods. *FEMS Microbiology Ecology* 44: 153-163.

Gauci, V., Dise, N.B., and Fowler, D. (2002) Controls on suppression of methane flux from a peat bog subjected to simulated acid rain sulfate deposition. *Global Biogeochemical Cycles* 16: 1004.

Gauci, V., Matthews, E., Dise, N., Walter, B., Koch, D., Granberg, G., and Vile, M. (2004) Sulfur pollution suppression of the wetland methane source in the 20th and 21st centuries. *Proceedings of the National Academy of Sciences U S A* 101: 12583-12587.

Gorham, E. (1991) Northern peatlands: role in the carbon cycle and probable responses to climatic warming. *Ecological Applications* 1: 182–195.

Griffis, T.J., Sargent, S.D., Baker, J.M., Lee, X., Tanner, B.D., Greene, J. et al. (2008) Direct measurement of biosphere-atmosphere isotopic CO2 exchange using the eddy covariance technique. *Journal of Geophysical Research, Biogeosciences* 113: D08304.

Happell, J.D., **Chanton, J.P**., Whiting, G.J., and Showers, W.J. (1993) Stable isotopes as tracers of methane dynamics in Everglades marshes with and without active populations of methane oxidizing bacteria. *Journal of Geophysical Research* 98: 14771-14782.

Hines, M.E., Duddleston, K.N., Rooney-Varga, J.N., Fields, D., and **Chanton, J.P.** (2009) Uncoupling of acetate degradation from methane formation in Alaskan wetlands: Connections to vegetation distribution. . *Global Biogeochemical Cycles* 22: GB2017.

Hirsch, A.I., Trumbore, S.E., and Goulden, M.L. (2002) Direct measurement of the deep soil respiration accompanying seasonal thawing of a boreal forest soil. *Journal of Geophysical Research* 107: 8221.

Holmgren, B., and Tjus, M. (1996) Summer air temperatures and tree line dynamics at Abisko. In *Plant Ecology in the Subarctic Swedish Lapland*. Copenhagen: Munksgaard International Booksellers and Publishers, pp. 159-169.

Hornibrook, E.R.C., Longstaffe, F.J., and Fyfe, W.S. (1997) Spatial distribution of microbial methane production pathways in temperate zone wetland soils: Stable carbon and hydrogen isotope evidence. *Geochim Cosmochim Acta* 61: 745-753.

Hornibrook, E.R.C., Longstaffe, F.J., and Fyfe, W.S. (2000a) Factors influencing stable isotope ratios in CH4 and CO2 within subenvironments of freshwater wetlands: Implications for delta-signatures of emissions. . *Isotopes Environ Health Stud* 36: 151-176.

Hornibrook, E.R.C., Longstaffe, F.J., and Fyfe, W.S. (2000b) Evolution of stable carbon isotope compositions for methane and carbon dioxide in freshwater wetlands and other anaerobic environments. . *Geochim Cosmochim Acta* 64: 1013-1027.

Horz, H.-P., **Rich, V.,** Avrahami, S., and Bohannan, B.J.M. (2005) Methane-Oxidizing Bacteria in a California Upland Grassland Soil: Diversity and Response to Simulated Global Change. *Applied and Environmental Microbiology* 71: 2642-2652.

Huson, D., Auch, A., Qi, J., and Schuster, S. (2007) MEGAN analysis of metagenomic data. *Genome Research* 17: 377–386.

Huxman, T., Troch, P., Chorover, J., Breshears, D.D., **Saleska, S.R.**, Pelletier, J. et al. (2009) The Hills are Alive: Earth Science in a controlled environment. *EOS* 90: 120.

IPCC (2007) *Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Geneva, Switzerland: IPCC.

Jackowicz-Korczynski, M., Christensen, T.R., Friborg, T., **Crill, P.M**., and Ström, L. (2009) CH4 exchange over Stordalen Mire by EC technique. In *Climate Change Impacts on Sub-arctic Palsa mires and Greenhouse Gas Feedbacks*. Fronzek, S. (ed): Finnish Environmental Institute, pp. 32-35.

Jagadeesh Babu, Y., **Li, C.**, **Frolking, S.**, Nayak, D.R., and Adhya, T.K. (2006) Field validation of DNDC model for methane and nitrous oxide emissions from rice-based production systems of India. *Nutrient Cycling in Agroecosystems* 74: 157-174.

Janssen, P.H., and Frenzel, P. (1997) Inhibition of methanogenesis by methyl fluoride -studies of pure and defined mixed cultures of anaerobic bacteria and archaea. . *Applied and Environmental Microbiology* 63: 4552-4557.

Johansson, M., Christensen, T.R., Åkerman, H.J., and Callaghan, T.V. (2006) What Determines the Current Presence or Absence of Permafrost in the Torneträsk Region, a Sub-arctic Landscape in Northern Sweden? *AMBIO: A Journal of the Human Environment* 35: 190-197.

Johansson, T. (2006) Temporal and spatial variability of carbon cycling in a subarctic landscape. In *Department of Physical Geography and Ecosystem Analysis*: Lund University.

Kohler, J., Brandt, O., Johansson, M., and Callaghan, T. (2006) A long-term Arctic snow depth record from Abisko, northern Sweden, 1913-2004. *Polar Research* 25: 91-113.

Kokfelt, U., Rosén, P., Schoning, N.K., Christensen, T.R., Förster, J., Karlsson, J. et al. (2009) Ecosystem responses to increased precipitation and permafrost decay in subarctic Sweden inferred from peat and lake sediments. *Global Change Biology* 15: 1652-1663.

Kurbatova, J., **Li, C.**, Varlagin, A., Xiao, X., and Vygodskaya, N. (2008) Modeling carbon dynamics in two adjacent spruce forests with different soil conditions in Russia. *Biogeosciences Discuss* 5: 969-980.

Lawrence, D.M., and Slater, A.G. (2005) A projection of severe near-surface permafrost degradation during the 21st century. *Geophysical Research Letters* 32.

Li, C. (2007) Quantifying greenhouse gas emissions from soils: Scientific basis and modeling approach. *Soil Science and Plant Nutrition* 53: 344-352.

Li, C., **Frolking, S.**, and Frolking, T.A. (1992) A model of nitrous oxide evolution from soil driven by rainfall events: 1. Model structure and sensitivity. *Journal of Geophysical Research* 97: 9759-9776.

Li, C., **Frolking, S.**, and Harriss, R.C. (1994) Modeling carbon biogeochemistry in agricultural soils. *Global Biogeochemical Cycles* 8: 237-254.

Li, C., Zhuang, Y., **Frolking, S.**, Galloway, J., Harriss, R., Moore III, B. et al. (2003) Modeling soil organic carbon change in croplands of China. *Ecological Applications* 13: 327-336.

Lozupone, C., Hamady, M., and Knight, R. (2006) UniFrac--an online tool for comparing microbial community diversity in a phylogenetic context. *BMC Bioinformatics* 7: 371.

Malmer, N., Johansson, T., Olsrud, M., and et al (2005) Vegetation, climatic changes and et carbon sequestration in a North-Scandinavian subarctic mire over 30 years. *Global Change Biology* 11: 1895-1909.

McManus, J.B., Nelson, D.D., Shorter, J.H., and Jiménez, R. (2005) A high precision pulsed QCL spectrometer dioxide. *Journal of Modern Optics* 52: 2309-2321.

Nelson, D.D., McManus, J.B., Herndon, S.C., Zahniser, M.S., Tuzson, B., and Emmenegger, L. (2008) New method for isotopic ratio measurements of atmospheric carbon dioxide using a 4.3 μm pulsed quantum cascade laser. *Appl Phys B* 90: 301-310.

Öquist, M.G., and Svensson, B.H. (2002) Vascular plants as regulators of methane emissions from a subarctic mire ecosystem. *Journal of Geophysical Research* 107: 4580.

Pataki, D. E., J. R. Ehleringer, L. B. Flanagan, D. Yakir, D. R. Bowling, C. J. Still, N. Buchmann, J. O. Kaplan, and J. A. Berry (2003). The application and interpretation of Keeling plots in terrestrial carbon cycle research, Global Biogeochem. Cycles, 17(1), 1022.

Popp, T.J., **Chanton, J.P.**, Whiting, G.J., and Grant, N. (1999) The methane stable isotope distribution at a *Carex* dominated fen In North Central Alberta. . *Global Biogeochemical Cycles* 13: 1063-1077.

Prater, J.L., **Chanton, J.P.**, and Whiting, G.J. (2007) Variation in methane production pathways associated with permafrost decomposition in collapse scar bogs of Alberta, Canada. *Global Biogeochemical Cycles* 21.

Rabenhorst, M. C., W. D. Hively, et al. (2009). Measurement of soil redox potential. *Soil Sci. Soc. Am. J.* 73: 668-674.

Reeburgh, W.S. (2005) Global Methane Biogeochemistry. In *Treatise on Geochemistry v 4: The Atmosphere*. Keeling, R. (ed), pp. 65-89.

Roesch, L.F.W., Fulthorpe, R.R., Riva, A., Casella, G., Hadwin, A.K.M., Kent, A.D. et al. (2007) Pyrosequencing enumerates and contrasts soil microbial diversity. *ISME J* 1: 283-290.

Saleska, S.R., Shorter, J.H., Herndon, S., Jimenez, R., McManus, J.B., Munger, J.W. et al. (2006) What are the instrumentation requirements for measuring the isotopic composition of net ecosystem exchange of CO_2 using eddy covariance methods? *Isotopes in Environmental and Health Studies* 42: 115-133.

Schloss, P., and Handelsman, J. (2005) Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. *Applied and Environmental Microbiology* 71: 1501-1506.

Schoell, M. (1980) The hydrogen and carbon isotopic composition of methane from natural gases of various origins. . *Geochim Cosmochim Acta* 44: 649-661.

Schoell, M. (1988) Multiple Origins of methane in the earth. *Chemical Geology* 71.

Schuur, E.A.G., and et al (2009) The effect of permafrost thaw on old carbon release and net carbon exchange from tundra. *Nature* 459: 556-559.

Shaw, A., Halpern, A., Beeson, K., Tran, B., and Venter, J. (2008) It's all relative: ranking the diversity of aquatic bacterial communities. *Environmental Microbiology* 10: 2200-2210.

Shi, Y., **Tyson, G.W.**, and DeLong, E.F. (2009) Metatranscriptomics reveals unique microbial small RNAs in the ocean's water column. *Nature* 459: 266-269.

Smith, W.N., Grant, B.B., Desjardins, R.L., Rochette, P., Drury, C.F., and **Li, C**. (2008) Evaluation of two process-based models to estimate N2O emissions in Eastern Canada. *Canadian Journal of Soil Scinces* 15: 31-51.

Sugimoto, A., and Wada, E. (1993) Carbon isotopic composition of bacterial methane in a soil incubation experiment: contributions of acetate and CO2/H2. *Geochim Cosmochim Acta* 57: 4015-4027.

Sun, G., **Li, C.**, Trettin, C., Lu, J., and McNulty, S.G. (2006) Simulating the Biogeochemical Cycles in Cypress Wetland-Pine Upland Ecosystems at a Landscape Scale with the Wetland-DNDC Model. In *Proceedings of the International Conference on Hydrology and Management of Forested Wetlands*. New Bern, NC, pp. 261-270.

Suyker, A., S. Verma, et al. (1997). Season-long measurement of carbon dioxide exchange in a boreal fen. *Journal of Geophysical Research* 102: 29021-29028.

Svensson, B.H. (1980) Carbon dioxide and methane fluxes from the ombrotrophic parts of a subarctic mire Ecology of a Subarctic Mire. *Ecological Bulletins* 30: 235-250.

Tabb, D.L., McDonald, W.H., and Yates, J.R., 3rd (2002) DTASelect and Contrast: tools for assembling and comparing protein identifications from shotgun proteomics. *J Proteome Res* 1: 21-26.

Treusch, A.H., Kletzin, A., Raddatz, G., Ochsenreiter, T., Quaiser, A., Meurer, G. et al. (2004) Characterization of large-insert DNA libraries from soil for environmental genomic studies of Archaea. *Environmental Microbiology* 6: 970-980.

Turetsky, M.R., Treat, C.C., Waldrop, M.P., Waddington, J.M., Harden, J.W., and McGuire, A.D. (2008) Short-term response of methane fluxes and methanogen activity to water table and soil warming manipulations in an Alaskan peatland. *Journal of Geophysical Research* 113: G00A10.

Tuzson, B., Mohn, J., Zeeman, M.J., Werner, R.A., Eugster, W., Zahniser, M.S. et al. (2008) High precision and continuous field measurements of $\delta^{13}C$ and $\delta^{18}O$ in carbon dioxide with a cryogen-free QCLAS. *Appl Phys B*.

UNEP (2003) Review of the interlinkages between biological diversity and climate change,and advice on the integration of biodiversity considerations into the implementation of the United Nations Framework Convention on Climate Change and its Kyoto Protocol. UNEP/CBD/SBSTTA/9/11. .

Updegraff, K., Pastor, J., Bridgham, S.D., and Johnston, C.A. (1995) Environmental and substrate controls over carbon and nitrogen mineralization in northern wetlands. *Ecological Applications* 5: 151-163.

VerBerkmoes, N.C., Denef, V.J., Hettich, R.L., and Banfield, J.F. (2009a) Systems biology: Functional analysis of natural microbial consortia using community proteomics. *Nat Rev Microbiol* 7: 196-205.

Verberkmoes, N.C., Russell, A.L., Shah, M., Godzik, A., Rosenquist, M., Halfvarson, J. et al. (2009b) Shotgun metaproteomics of the human distal gut microbiota. *ISME J* 3: 179-189.

Wagner, D. (2008) Microbial communities and processes in Arctic permafrost environments. In *Microbiology of Extreme Soils*. Dion, P., and Shekhar Nautiyal, C. (eds). Berlin: Springer.

Walter, K.M., Zimov, S.A., **Chanton, J.P**., Verbyla, D., and Chapin III, F.S. (2006) Methane bubbling from Siberian thaw lakes as a positive feedback to climate warming. *Nature* 443: 71-75.

Walter, K.M., **Chanton, J.P.,** Schuur, E.A.G., Zimov, S.A., and Chapin III, F.S. (2008) Methane production and bubble emissions from arctic lakes: Isotopic implications for source pathways and ages. *Journal of Geophysical Research* 113: G00A08.

Whiticar, M.J. (1999) Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane. *Chemical Geology* 161: 291-314.

Whiticar, M.J., Faber, E., and Schoell, M. (1986) Biogenic methane formation in marine and freshwater environments: CO_2 reduction vs. acetate fermentation - isotope evidence. *Geochim Cosmochim Acta* 50: 693-709.

Whiting, G.J., and **Chanton, J.P.** (2001) Greenhouse Carbon Balance of Wetlands: Methane Emission versus Carbon Sequestration. *Tellus B* 53: 521-528.

Yu, Z., Garcia-Gonzalez, R., Schanbacher, F.L., and Morrison, M. (2008) Evaluations of Different Hypervariable Regions of Archaeal 16S rRNA Genes in Profiling of Methanogens by Archaea-Specific PCR and Denaturing Gradient Gel Electrophoresis. *Applied and Environmental Microbiology* 74: 889-893.

Zahniser, M.S., Nelson, D.D., Barry, J., McManus, J.B., Kebabian, P.L., and Lloyd, D. (1995) Measurement of trace gas fluxes using tunable laser diode laser spectroscopy. . *Philosophical Transactions of the Royal Society of London, A* 351: 371-381.

Zahniser, M.S., Nelson, D.D., McManus, J.B., Herndon, S.C., Wood, E.C., Shorter, J.H. et al. (2009) Infrared QC laser applications to field measurements of atmospheric trace gas sources and sinks in environmental research: enhanced capabilities using continuous wave QCLs In *Proceedings of SPIE: Quantum Sensing and Nanophotonic Devices VI,*. Razeghi, M., Sudharsanan, R., and Brown, G.J. (eds).

Zeikus, J.G. (1977) The biology of methanogenic bacteria. *Bacteriological Rev* 41: 514-541.

Zhang, Y., **Li, C.**, Trettin, C.C., Li, H., and Sun, G. (2002) An integrated model of soil, hydrology and vegetation for carbon dynamics in wetland ecosystems. *Global Biogeochemical Cycles*

Zhuang, Q., Melillo, J.M., Sarofim, M.C., Kicklighter, D.W., McGuire, A.D., Felzer, B.S. et al. (2006) Co2 and CH4 exchanges between land ecosystems and the atmosphere in northern high latitudes over the 21st century. *Geophysical Research Letters* 33: L17403.

Zimov, S. A. et al. (1997) North Siberian lakes: A methane source fuelled byPleistocene carbon. *Science* 277, 800–802.

Appendices 4 and 5: Facilities, Equipment & Other Resources

At the University of Arizona

Facilities for Biogeochemistry

Saleska Laboratory: PI Saleska has ~1500 square feet of laboratory and office space for lab personnel. The laboratory is equipped with bench space for construction and testing of field equipment, a fume hood for chemical processing of samples, analytical balance, pH meters, and laptop/desktop computers for each member of the lab, including a dedicated 500 Gb RAID server for storage. All computers have extensive software installed for data processing and analysis.

Saleska lab equipment includes an arrary of micro-meteorological sensors, data-loggers, a gas exchange system (Li-Cor 6400); CSAT 3 sonic anemometers associated with the eddy-flux systems, Li-Cor 6262 closed path infrared gas analyzer, and a field portable gas chromatograph system with flame ionization and electron capture conductivity detectors for measurement of CH4, CO2, N2O, H2 trace gases in the field.

The University of Arizona's Biosphere 2 (B2): PI Saleska serves on the Biosphere 2 Science Steering committee of the UofAs $150 million Biosphere 2 facility, where he maintains a collaborative research program with full access to B2 facilities and resources for research complementary to this project. Relevant to this project, these resources includes a glass-enclosed model wetland ecosystem that will serve as a testbed for development and testing of sensors to be deployed to Stordalen Mire in Sweden, including soil profile gas sampling equilibrators. B2 maintains machine shop facilities that will also be available to this project. The facility also includes conferencing and lodging facilities which will be available for focused regular meetings of the research team.

The Biosphere 2 facility includes equipment that will be available to this project, including an extensive trace gas laboratory, equipped with: 1) A high sensitivity Proton Transfer Reaction Mass Spectrometer: an ultra-sensitive detector for volatile organic compounds (VOCs); (2) gas chromatagraph-ion trap mass spectrometer (Varian Saturn GC-ITMS) configurable for either liquid or gas samples, (3) Licor 840 and 7000, a high performance, dual cell, differential gas analyzer; and finally (most relevant for this project), (4) a real-time CO2 isotope QC laser spectrometer system built by Aerodyne, and identical to that proposed here for deployment to Stordalen Mire, for continuous, accurate, high-resolution measurement of CO2 isotopologues (acquiring 13C/12C and 18O/16O ratios of CO2 simultaneously). This can be plumbed to the B2 wetland for testing and development of the capacity of automated soil profile equilibrators to sample isotopes, before being deployed to the field.

Facilities for Molecular Microbial Ecology

Molecular Laboratory: Co-PI Rich will perform required molecular work as a guest in the Sullivan Lab (see Letter of Support from Matt Sullivan, Field 12). This lab is an environmental virology and microbiology lab that is fully equipped for molecular microbial research in ~1,500 square feet of space. Relevant research equipment includes 2 PCR gradient thermal cyclers, gel electrophoresis equipment and power supplies, digital gel documentation system (laser and UV-excitation), microcentrifuges, 96-well plate reader (fluorescence, absorbance, luminescence), analytical balances, pH meters, Q-water system, two -80'C freezers, 4'C refrigerator, -20'C freezer, laminar flow hood.

Computing: The University of Arizona Biotechnology Computing Facility provides community access to dedicated pipelines for high throughput data analyses; these pipelines include popular applications such as BLAST, FASTA, and CLUSTAL which are streamlined and optimized for the shared campus high

performance computing cluster. This setup includes pre- and post-processing of high-throughput data and associated data management tasks including coordination with public and private sequence repositories for acquiring and depositing generated data. The current hardware setup includes a cluster with 1392-core, 15.76 TFLOPS SGI Altix ICE and a shared memory SGI 568-core, 3.3 TFLOPS, Altix 4700 system with two SGI RC100 dual-core Xilinx FPGA blades. We are renting 1TB of data storage from the Facility, and will purchase an additional independent RAID data storage unit.

Core facilities: Building-specific shared facilities include nanodrop spectrophotometer, epifluorescence and conventional microscopy (with digital camera), pulse-field gel electrophoresis, high-speed ultracentrifugation, large-scale centrifugation, autoclaves, dishwashers, and media preparation.

DNA and cDNA sequencing will be performed at the on-campus Arizona Genomics Institute (see Letter of Support from Rod Wing, Field 12). AGI has myriad high-throughput sequencing machines of varied platforms (Sanger, pyrosequencing, etc), robotic liquid handlers, clone library hybridization screening equipment, imaging equipment for array work.

Subcontract Tyson, University of Queensland

Laboratory: PI Tyson has ~1400 square feet of laboratory space which is fully equipped for molecular microbiology. The laboratory can accommodate up to 14 people, and dedicated space is available for the research assistant supported by this grant. In addition, the lab is in the process of establishing a quarantine facility for importing and storing foreign soils.

Research equipment includes 2 PCR thermal cyclers, gel electrophoresis equipment (small, medium and large) and power supplies, digital gel documentation system (laser and UV-excitation), nanodrop spectrophotometer, centrifuges, analytical balances, pH meters, MilliQ water system, incubators, autoclave, two -80°C freezers, four -20°C freezers, 4°C refrigerator, cold room (4°C), fume and laminar flow hoods.

The laboratory is also equipped with a custom RNA extraction and processing hood designed to minimize RNA degradation and contamination.

Computing: Interpretation and analysis of the data generated in this study will require significant computational resources. Dr Tyson has access to high performance computing through Queensland Cyber Infrastructure (QCIF) at the University of Queensland. This includes access to two different computing clusters, “Gust” and **"Cyclone". “Cyclone”** is a SGI Altix Bx2 with 64 Itanium 2 CPUs and 121 gigabytes (Gb) of memory. System administrative support is available to help implement and optimize new software and pipelines.

Each member of the laboratory has a laptop/desktop computer (Mac or Dell) and in-house processing is done on a high-end Mac Pro server (8 processors, 32 Gb of memory and 4TB of disk storage). All computers have extensive software installed, including a suite of routine bioinformatics programs.

Core facilities: The University of Queensland houses a number of epifluorescence and confocal microscopes as well as state-of-the-art flow cytometry facilities. Since this project involves generating sequencing data from microbial communities, we will be benefited considerably by the proximity of one of Australia’s premier genome facilities, the Australian Genome Research Facility (AGRF), located at the University of Queensland. The AGRF has a number of different high-throughput sequencing machines of varied platforms (Sanger, Roche 454, Illumina), and robotic liquid handlers. Other facilities on campus provide access to pulse-field gel electrophoresis, high-speed ultracentrifugation, quantitative PCR machines (96- and 384-well blocks), electron microscopy, and high-throughput mass spectrometry for proteomics.

Subcontract Crill, Stockholm University

At present, on Stordalen Mire itself, there is a custom built (by the P.I.) IRGA based CO_2 and THC analysis system with 9 autochambers in three subhabitats. There are also three manually sampled diffusion gas arrays installed in the three habitats. We have 12 vdc Vaisala and LiCor IRGAs available for a portable manual flux system that will be built. We also have pumps, a compressor, thermocouples, a PAR sensor and a pressure sensor with computers and Campbell dataloggers at the heated instrument shack on the mire that houses the auto chamber instrumentation. We have 220 vac line power to the instrument shack and there is another house at the edge of the Mire to which the project will have access. Power is sufficient for the proposed instrumentation.

Stordalen Mire is also instrumented with an NEE and CH_4 eddy correlation tower and a full suite of meteorological measurements are made continuously over the fen portion of the mire (T. Friborg, Copenhagen Univ and T. Christensen, Lund Univ). This project will continue the measurements. There is additional meteorological and water level instrumentation on the mire operated by N. Roulet of McGill Univ as part of a Canadian funded hydrology study. All four groups have worked cooperatively in past years sharing ideas, expertise, data and even cosupervising graduate students.

We have laboratory space and a gas chromatograph with flame ionization and thermal conductivity detectors at the ANS (Abisko Scientific Research Station) 10 km to the west in Abisko. The research is a fully functional modern research station that offers accommodation. Details of the facilities can be found at http://www.linnea.com/~ans/ans.htm. Kiruna, a major mining town 110 km to the east, has commercial facilities for compressed and liquefied gases.

Manually sampled stable isotope measurements will be made at the Isotope Laboratory at the Department of Geology and Geochemistry of Stockholm University. The Department disposes over a variety of analytical equipment for research within geology, geochemistry and marine geoscience. Mass spectrometric capacity includes 4 analytical spectrometers for IRMS and GC-IRMS (2 Delta V, 2 Delta Plus and a Finegan MAT 252) with a variety of inlet and sample processing peripherals. There are also complete ion, liquid and gas chromatography facilities available including (most applicable to this project) flame ionization (CH_4 and CO2) detectors with methanizers and a HgO detector for H_2 analysis.

The soil gas sampling system is based roughly on that used by Hirsch et al. (2002) in which probes of microporous Teflon tube are buried at in the soils and automatically sampled with a pump through a manifold and solenoid system. Microporous Teflon was used because it is hydrophobic and because of rapid equilibration with dissolved gases. We propose to use closed loops of 3m of thin walled PTFE tubing that will be cut into four depths in each of the three habitat types at depths. Each tube at each depth will thus integrate the concentration of gas at that depth over the length of the equilibration tube. Wood or plastic depth guides spaced every 30 cm allow easy installation and depth maintenance until the cuts in the peat heal after about 1 month. The large surface to volume ratio will allow us to sample at least daily. Each depth will be switched in line in a closed loop and gas will flow through the analyzers at 1.5 slp.

There are three manual equilibration samplers of 2 m Teflon tubes length already installed in the mire buried at given depths that allow spatially integrated sampling of the soil gas. Low molecular weight gases, but not water, can diffuse through the Teflon and will equilibrate with the surrounding medium. High surface to volume ratios ensure rapid equilibration (less than one week at room temperature). Equilibration times for the particular tubing to be used will be tested before deployment.

Subcontract Chanton, Florida State University:

The FSU Oceanography Department occupies 5 floors of a modern laboratory building. Available for use on this project are a full array of radon and radium counting equipment, including scintillation and coincidence counters, alpha, gamma and beta counters, two Finnegan MAT Isotope Ratio Mass

Spectrometers, a Delta V IRMS and a new XP IRMS, recently purchased with an NSF equipment grant. These instrument measure $d^{13}C$, $d^{15}N$ and dD. We have gas chromatographs equipped with flame ionization, thermal conductivity and electron capture detectors, an Omni Star quadruple gas analyzer with a MEMs inlet system and other various analytical equipment including balances and other standard laboratory equipment. Vacuum lines for the preparation ^{14}C samples of CH_4, DIC, DOC and organic carbon are available. Field equipment includes a number of flux chambers and two LICOR IR gas (field CO2 analyzers) and a porometer. The NMR facility in the chemistry department at FSU (where Chanton has doctoral directive status) provides instrumentation facilities and expertise in NMR spectroscopy for the applications in chemistry, biology and physics of the FSU faculty. The FSU NMR Facility has six Fourier Transform NMR spectrometers. The facility is also equipped with considerable test equipment including an HP Vector Impedance meter, Tektronics 350 MHz and 400 MHz oscilloscopes, and two Wavetek Sweep Generators.

Subcontract Li, University of New Hampshire:

The *Complex Systems Research Center's (CSRC) Science Computing Facility (SCF)* has a wide range of computer servers, printers, plotters, archiving systems, software, data archives, and web based data distribution systems that are integrated using several internal networks and connected to the outside world through a high speed pipe. The overall *SCF* administration is provided by the *Research Computing Center* (*RCC*) located in the *Institute for the Study of Earth, Oceans and Space (EOS)*. Scientific data processing and analysis support is distributed throughout workgroups within the center with additional centralized expertise provided by *CSRC*'s *Laboratory for Remote Sensing and Spatial Analysis.* Within this proposal, we take advantage of this existing computer infrastructure, to meet our anticipated computational needs.

The main *CSRC* servers consist of high-end, multi-processor computing systems manufactured by Dell and SUN Microsystems. The Dell systems run Linux and are used for CPU intensive jobs, parallel modeling, and storage. They include several multi-node Beowulf clusters with over 25 Terabytes (TB) of RAID5 disk space, over twenty dual-CPU servers with a combined capacity in excess of 50 TB of RAID5 storage, and several other application and web servers. The Sun system is a Sunfire 280R that operates as both an application server and as the backup/archive server. Backups and archives are done using the Networker product from EMC. Most of the main servers share a gigabit (Gb) switch with the archive/backup system for high-speed communications. Nearline storage is done on a tape library unit.

The tape library is a 120 slot Qualstar 46120 unit with 4 AIT-3 drives capable of 12.0 TB of native storage. All of this equipment is kept within a physically secured, humidity and temperature controlled machine room with UPS power. Final data and image products are produced from several ink-jet plotters and laser printers within the department. Additionally, several CD/DVD writers are used for data distribution.

Our most recent additions to *EOS* include a 160 node cluster with 2 Quad Opteron head nodes and 2 Dual Opteron head nodes with over 4 TB of disk space and over 700 GB of memory, a 22 node 3 TB cluster, and a 32 node 2 TB cluster. In addition, our infrastructure has been strategically upgraded to provide gigabit networking to desktops.

Individual scientists and research groups have additional computing resources at their disposal. These include dedicated servers, individual workstations, and various peripheral devices. The group servers and individual workstations include: Linux and SUN workgroup servers and workstations, Windows workstations, Apple Macintoshes and laptop computers. All servers, user systems and networked peripheral devices are accessible within *EOS* through a 100/1000 Mb ethernet network. There are also wireless access points in many areas of the building. These systems also have access to both Internet 1 and Internet 2.

CSRC currently houses a 60TB+ geographically referenced data archive used for spatial data processing and analysis. This archive stored on RAID5 data disks served by a series of data servers, houses global, regional and local, Landsat, MODIS, IKONOS, Hyperion, ASTER, and SPOT satellite imagery, land cover classified products, vegetation and other indexes (EVI, LSWI, NDSI, NDVI, NDWI, LAI), aerial photography and GIS vector data layers for use by all projects within the department. Portions of this data, processed data products, and project results archive are disseminated and distributed through several dozen regularly updated and maintained *CSRC* operated websites. These websites are served through a variety of web servers running Apache web server software supported by other applications and libraries such as Tomcat, Web Mapping Server (WMS), OpenLayers and other geographically enhanced libraries such as GDAL, PROJ4, and GCTP.

CSRC also leverages the center's *Laboratory for Remote Sensing and Spatial Analysis*, a spatial information processing, analysis and distribution research laboratory. This laboratory provides geographic information system (GIS), Web Mapping, spatial data archiving, data distribution, remote sensing, image processing, cartography, large format printing and scanning support to several *CSRC* and *EOS* research projects. Staffed by professional geo-spatial information technicians, computer programmers, and graduate and undergraduate university students, the laboratory houses a multiple seat dual and quad core Linux, PC, and Mac OS computer cluster supplied with a variety of open source Remote Sensing, GIS, web mapping, image processing and cartography software and ESRI ArcGIS, Leica ERDAS Imagine, and IDL/ENVI, commercial site, block, and individually licensed GIS and Image processing software.

Plans for additional computing resources over the next two years include: new multi-CPU servers and clusters to be used for general computing needs and CPU intensive models, multiple TB of RAID storage, and infrastructure upgrades for faster networking.