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Biological Systems Research on the Role of Microbial Communities in Carbon Cycling

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Biological Systems Research on the Role of Microbial Communities in Carbon Cycling

163

With or Without You: Genome-Scale Analysis of Contrasting Strategies for Interspecies Electron Transfer

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http://www.geobacter.org

Project Goals: The long-term goal of our project, which is entitled "Systems Level Analysis of the Function and Adaptive Responses of Methanogenic Consortia", is to develop genome-scale metabolic models of microbial communities that play an important role in the global carbon cycle that can be coupled with the appropriate physical-chemical models to predict how the microbial communities will respond to environmental perturbations, such as climate change. The short-term objective in the current research is to determine if the syntrophic associations that are central to the functioning of methanogenic terrestrial wetlands can be predicatively modeled with genome-scale metabolic models.

Interspecies exchange of electrons is necessary for the proper functioning of the methanogenic ecosystems that are major contributors of atmospheric methane and for successful operation of methanogenic digesters, the most effective strategy yet devised for converting organic wastes to fuel. For over 40 years interspecies hydrogen transfer has served as the paradigm for anaerobic interspecies electron transfer.

In interspecies hydrogen transfer, hydrogen gas serves as the electron carrier, with electron-donating microorganisms reducing protons to hydrogen and hydrogen-oxidizing microorganisms extracting the electrons from hydrogen with the release of protons. Energetic inefficiencies inherent in the production and subsequent oxidation of hydrogen and limitations on the diffusion of hydrogen between partners may constrain the effectiveness of interspecies electron exchange via hydrogen and contribute to instabilities in methanogenic systems.

Recent studies with a model system of two *Geobacter* species revealed an alternative to interspecies hydrogen transfer for interspecies electron exchange (Z.M. Summers *et al.* 2010. Direct exchange of electrons within aggregates of an evolved syntrophic coculture of anaerobic bacteria. *Science* 330(6009): 1413-1415). *Geobacter metallireducens* and *Geobacter sulfurreducens* were cultured on ethanol under conditions in which *G. metallireducens* was required to provide electrons to *G. sulfurreducens* in order for ethanol metabolism to proceed. Strong selective pressure for rapid ethanol metabolism resulted in a co-culture that could metabolize ethanol much faster than the initial co-culture. Adaption for enhanced metabolism was associated with the formation of large (1-2 mm diameter) aggregates of the two species and selection for a mutation that enhanced the production of OmcS, a *c*-type cytochrome aligned along the electrically conductive pili of *G. sulfurreducens*. Introducing the mutation in *G. sulfurreducens* prior to initiating the co-culture accelerated aggregate formation and adaption for rapid ethanol metabolism. Additional genetic analysis and physiological studies ruled out the possibility of interspecies transfer of hydrogen or formate significantly contributing to the electron exchange and demonstrated that both pili and OmcS were essential for effective co-culture function. The aggregates were electrically conductive with sufficient conductance to account for the electron exchange within the aggregates. These studies suggest that the two *Geobacter* species directly exchanged electrons via long-range (μm) conduction through conductive pili with short-range (Å) electron exchange mediated by the OmcS of *G. sulfurreducens*, and potentially one or more cytochromes of *G. metallireducens*.

Aggregation of methanogens and the other organisms involved in the anaerobic conversion of wastes is essential for efficient conversion of wastes to methane in anaerobic digesters. It has previously been suggested that the close proximity of cells promotes interspecies hydrogen transfer. However, measurements of electron conduction through aggregates collected from commercial-scale methanogenic digesters yielded conductivities comparable to those of the *Geobacter* co-culture aggregates. Temperature studies indicated that conduction along organic filaments was the most likely mode of electron flux. Additional physiological studies ruled out hydrogen as a significant electron carrier. Studies on gene expression within the aggregates are underway to better understand the components responsible for the apparent direct electron transfer in these systems.

The direct electron transfer described above contrasts with the many instances of interspecies hydrogen transfer previously reported for a diversity of co-cultures in laboratory studies. It is clear that interspecies hydrogen transfer can take place under some conditions. For example, *Pelobacter carbinolicus,* a close relative of *Geobacter* species, grew syntrophically with the methanogen *Methanospirillum hungatei*, converting ethanol to methane. However, the two cell types did not aggregate as would be expected for direct cell-to-cell electron transfer. Furthermore, analysis of gene expression in both co-culture partners with whole-genome microarrays demonstrated that *P. carbinolicus* expressed genes for hydro-

gen production and that *M. hungatei* continued to express the genes for hydrogen uptake that are expressed during pure culture growth on hydrogen. *P. carbinolicus* did not increase expression of genes for components, such as pili and *c*-type cytochromes, that are hypothesized to be important in direct electron transfer. Co-cultures of *P. carbinolicus* with *G. sulfurreducens*, which is known to be capable of direct electron transfer under similar conditions with *G. metallireducens*, effectively metabolized ethanol, but did not form aggregates, further demonstrating that *P. carbinolicus* exchanges electrons via interspecies hydrogen transfer rather than direct cell-to-cell electron transfer.

It seems likely that the electron-donating microorganisms and methanogens that have routinely been used to study syntrophic metabolism in laboratory incubations were isolated with procedures that selected for organisms that exchange electrons via interspecies hydrogen transfer. It is expected that the study of pure cultures recovered with novel strategies that select for microorganisms that can directly exchange electrons, as well as metatranscriptomic and proteomic studies of natural methane-producing aggregates, will provide additional information on the mechanisms of direct electron exchange within methanogenic aggregates.

164

Systems Biology Approach to Elucidate Electron Transfer in Methanogenic Consortia

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http://systemsbiology.ucsd.edu
http://geobacter.org

Project Goals: The long-term objective of this research is to adopt systems biology approaches to predict the activity of microorganisms involved in important aspects of the global carbon cycle under multiple environmental conditions. The short-term goal of this project adresses the development of genome-scale metabolic models for methanogens of interest, couple them with the metabolic model of a syntrophic partner, and elucidate the electron transfer mechanisms of this syntrophy. These syntrophic associations are important in methanogenic terrestrial wetlands, one of the main sources of greenhouse gas.

Reconstruction of genome-scale metabolic networks has become a common denominator in systems biology. Following an established protocol, we have reconstructed the metabolic networks of two methanogens, *Methanococcus maripaludis* and *Methanospirillum hungatei,* representative species of Methanococcales and Methanomicrobiales, respectively. The reconstructions currently contain 441 and 420 reactions with 422 and 369 genes, respectively. The reconstructions capture all the major central metabolic, biosynthetic, methanogenesis pathways, as well as pathways for carbon dioxide fixation. These genome-scale models will be interrogated using constraint-based modeling approaches and validated based on physiological data. Taken together with the published *Methanosarcina barkeri* reconstruction, these models represent the three different classes of methanogens, thus paving the way for a systems level comparative analysis of methanogenesis.

In this project, we will also couple the generated methanogenic metabolic models with a syntrophic partner to interrogate mechanisms of direct electron transfer or hydrogen transfer in microbial communities. To establish the mathematical framework for modeling direct cell-to-cell electron transfer, we will first use the laboratory evolved syntrophic co-culture of *Geobacter metallireducens* and *Geobacter sulfurreducens* as a model system. For this purpose, we have expanded the existing models for the two *Geobacter* species to account for extracellular electron transfer pathways. We have accounted for all the possible routes of electron transfer in and out of the cell through the various electron carriers such as cytochromes, ferredoxin, quinones, NAD, and FAD. In addition to the stoichiometry associated with the respective redox reactions, these pathways also account for the appropriate gene association and cellular localization of the different electron carriers. This detailed representation of extracellular electron transfer pathways enables us to characterize the role of direct electron transfer in the functioning of the evolved syntrophic consortium. This mechanistic understanding is crucial for the functioning of the microbial community in carbon cycling.

165

Can Microbial Functional Traits Predict the Response and Resilience of Decomposition to Global Change?

Principal Investigators: Steven D. Allison[1,2]* (allisons@uci.edu), **Eoin L. Brodie**,[3] **Michael Goulden**,[2] **Adam Martiny**,[1,2] **Jennifer B.H. Martiny**,[1] and **Kathleen K. Treseder,**[1]
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http://allison.bio.uci.edu/research.html#traits

Project Goals: The main goal of this project is to connect diverse microbial groups with the extracellular enzyme systems that catalyze the decay of organic material. We will also determine whether different groups of microbes

and their enzymes respond to environmental changes, and whether they can recover from such changes. Finally, we will develop mathematical models to predict the responses of microbial communities and their associated functions under new environmental conditions.

Recent technological advances have revealed tremendous genetic and metabolic diversity in microbial communities of bacteria, fungi, and archaea. Microbes play fundamental roles in regulating carbon losses from terrestrial ecosystems by catalyzing the breakdown of dead plant and soil organic material. This process relies on the production of enzymes that act outside of microbial cells to convert complex molecules into available forms. Although these enzymes regulate carbon cycling and sequestration in nearly all terrestrial ecosystems, we do not currently know which microbes produce the diversity of enzymes observed in natural ecosystems. Without this information, we cannot accurately predict how much carbon will be lost from ecosystems under future environmental conditions that may occur with climate change or nutrient pollution.

Our research will take place in a grassland ecosystem in Southern California that hosts an ongoing environmental change experiment funded by DOE. We will assess microbial and enzyme responses to drought and nitrogen addition, two environmental changes likely to affect an increasing number of terrestrial ecosystems locally and globally (Figure 1). High-throughput DNA sequencing will reveal shifts in the composition of the microbial community, and novel gene cloning and expression techniques will link enzymes with specific groups of microbes. We will use this information to construct mathematical models of microbial and enzyme responses to environmental change. Our models will be tested by directly manipulating microbial communities and environmental conditions. The decay rates of specific chemical compounds will be assessed with a new nanotechnological tracer approach. Tracking microbial communities, enzymes, and rates of decay over time will verify if our models are correct and tell us how quickly microbes can recover from environmental perturbations.

The most important scientific impact of this work will be to establish a firm connection between the composition of microbial communities and the enzymatic functions that affect carbon cycling. In addition, our research will generate knowledge and models useful for predicting how ecosystems will store and release plant-derived carbon under future environmental conditions. The enzyme genes and microbes we identify may also have potential industrial applications, such as the processing and synthesis of biofuels.

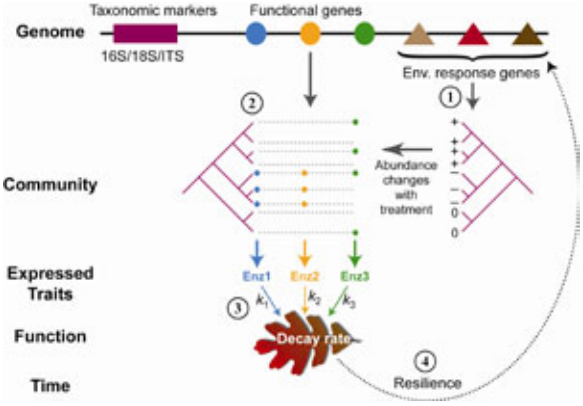

Figure 1. A trait-based framework for predicting the function of microbial communities under environmental change. Genes controlling enzymatic function and environmental responses are linked to taxonomic markers in the genomes of individual cells. In some cases, a particular trait is linked to a taxonomic group (Enz1 and Enz2), whereas others may be widely distributed among taxa (Enz3). Thus, changes in taxon abundance due to environmental drivers can affect the abundance distribution of functional genes in the community. Extracellular enzyme activities represent the expressed traits of the microbial community. Different enzymes control the decay rates (k1, k2, k3) of organic carbon compounds in litter. Circled numbers correspond to proposed research questions:

1. How do microbial taxa respond to environmental changes?
2. How are extracellular enzyme genes distributed among microbial taxa?
3. Can we predict enzyme function and litter decomposition rates by combining enzyme gene distributions with microbial taxa responses to environmental change?
4. Are microbial communities and their functions resilient to environmental change?

166

Phylogenetic Distribution of Functional Traits in Microbes

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Project Goals: The goal of this project is to determine how microbes respond to global environmental changes. As part of this project, we investigating the phylogenetic distribution of functional traits in microbes and whether this is related to the complexity of the trait

The goal of this project is to identify at what phylogenetic resolution do we find a specific functional trait. The hypothesis is that some traits are associated with large phylogenetic

clusters (e.g. oxygenic photosynthesis) whereas other traits are associated with small phylogenetic clusters (e.g. growth on small simple organic carbon molecules). To address this hypothesis, we mapped specific functional traits onto a 16S rRNA tree and determined the average phylogenetic cluster size where 90% or more of the organisms have the trait. The traits were defined either by genome annotation as subsystems for a function or the ability to grow on a particular carbon source. We detected only a few traits that are associated with clusters of 3% or more 16S rRNA sequence dissimilarity, whereas most traits are associated with small microdiverse clusters. However, most traits were non-randomly distributed. We next tested if the phylogenetic cluster size was related to the complexity of the trait. Complexity was defined either by the number of genes underlying the function or the structural complexity of a carbon substrate. We found that the average phylogenetic cluster size of a trait was significantly positively related to the complexity of the trait. This study demonstrates that microbial functional traits are related to phylogenetic groups of different sizes. Furthermore, the complexity of a trait influences the phylogenetic distribution of a trait, potentially as a result of different rates of gene gain and loss. This has general implications for our understanding of microbial evolution and biogeography.

167

Systems Level Insights Into Alternate Methane Cycling Modes in a Freshwater Lake Via Community Transcriptomics, Metabolomics and NanoSIMS Analysis

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Project Goals: The major goal of this project is to initiate a systems-level approach to studying natural communities involved in alternate modes of methane oxidation, such as aerobic versus anaerobic and nitrate (other factor)-dependent versus strictly oxygen dependent modes, in order to bridge gaps in understanding the specialized bacterial communities involved in these processes, including discovery of novel species and novel metabolic pathways involved in these processes. This project takes advantage of a large metagenomic dataset enriched in the DNA of the methanotrophic species employing different types of methane metabolism, generated in collaboration with the Joint Genome Institute. The specific objectives of this project are:

Objective 1. Identify actively transcribed pathways. Via next-generation sequencing-based transcriptomic profiling of four types of microcosm communities stimulated with methane (aerobic, anaerobic, aerobic with added nitrate and anaerobic with added nitrate), identify species actively involved in methane oxidation, based on expression patterns, and delineate specific pathways for methane oxidation and electron transfer.

Objective 2. Identify physiologically active pathways. Via methane and nitrogen flux analysis and via community metabolomics employing chromatography-tandem LC-MS/MS (quadrupole mass spectrometry), GC x GC-TOFMS (comprehensive two-dimensional Gas Chromatography-Time-Of-Flight Mass Spectrometry), follow dynamics of methane/ nitrate consumption and identify major metabolites indicative of specific metabolic pathways, and correlate gene expression profiles and predicted methane utilization pathways in the four simulated settings, as above.

Objective 3. Identify activity of individual cells. Via FISH (fluorescent *in situ* hybridization) or HISH (halogen *in situ* hybridization) -coupled nano-SIMS (Secondary Ion Mass Spectrometry) employing specific DNA probes and labeled methane and nitrate, couple a specific function in methane metabolism to a specific functional guild in the community, following the fates of methane and nitrate (and other characteristic ions) at the single cell resolution level.

In this work we integrate heterogeneous multi-scale genomic, transcriptomic, and metabolomic data to redefine the metabolic framework of CH_4 oxidation from single ecosystem-relevant microbial species (such as methanotrophic isolates from Lake Washington sediment) as well as a natural ecosystem (Lake Washington sediment). Draft genome sequences of model methanotrophic cultures were generated and manually annotated. Preliminary reconstruction of the C1-metabolic pathways was performed. In order to underline core methylotrophic functions a set of RNA-seq based transcriptomic experiments were carried out. In addition metabolomic experiments were performed to define C1-metabolic networks of type I and type II methanotrophic bacteria and to identify potential metabolic markers of methanotrophy. Around 60 metabolites including amino acids, carboxylic acids, sugar phosphates, nucleotides and CoA derivatives were quantified by LC-MS/MS and GC-MS. To elucidate the major metabolic flux for methane assimilation, ^{13}C –isotopic labeling experiments were performed.

New data reveal an unexpected carbon distribution in model proteobacterial methane oxidizers, providing a new version of the methanotrophy metabolic network. Similar system-level approaches were applied for characterization of the metabolic potential of yet uncultivable microbes from natural environments.

168

Genes, Isotopes, and Ecosystem Biogeochemistry: Dissecting Methane Flux at the Leading Edge of Global Change

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http://eebweb.arizona.edu/faculty/saleska/research.htm#isogenie
http://isogenie.pbworks.com/w/page/23915054/FrontPage

Project Goals: Our project focuses 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. To achieve these, we aim to: (A) Characterize microbial community composition and metabolic function associated with in situ methano -genic and -trophic pathways; (B) Characterize ecosystem biogeochemical cycling of CH_4 and CO_2, using isotopes to partition contributions from separate methano -genic and -trophic metabolisms.

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.

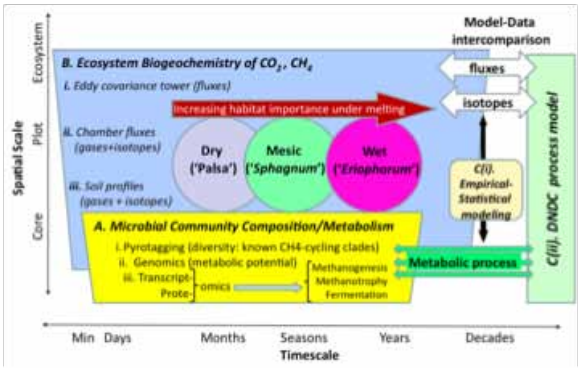
We are investigating how microbial community composition and function scale to ecosystem biogeochemistry of CH_4 and CO_2, and how such scaling is affected by climate change. To accomplish this, we employ a three-pronged interdisciplinary investigation of Sweden's Stordalen Mire, an established wetland field site at the thawing southern edge of the discontinuous permafrost zone:

(A) 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*)

(B) 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 laser 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*)

(C) 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 will 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*).

Overview of Technical Approach:



Project Grant DE-SC0004632

169

Plant Stimulation of Soil Microbial Community Succession: How Sequential Expression Mediates Soil Carbon Stabilization and Turnover

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Project Goals: The goal of our project is to understand the succession of soil microbial communities involved in degradation and transformation of C released from the roots of *Avena barbata*, a grass common to California. This project involves the identification of metabolic pathways of soil microorganisms responsible for C processing by labeling *A. barbata* with $^{13}CO_2$ and the stable isotope probing technique. Fluorescent in situ hybridization probing and NanoSIMS analysis will be used to identify active community members consuming ^{13}C derived from roots and visualize spatial associations between microbes and plant roots. The influence of elevated CO_2 on succession and sequential expression of microbial function will also be investigated using transcriptome-enhanced GeoChip, CAZy/FOLy functional microarrays, and second generation sequencing. Gene expression networks will then be identified in order to model the microbial community cascades which enable C stabilization and turnover.

Soil organic carbon (C) is the largest pool within the terrestrial C cycle and is derived from decomposed plant and soil microbial materials. The fluxes that control the size of this pool are critical to the global C cycle. It is known that plant roots and their exudates exert control over the microorganisms mediating decomposition of complex soil C compounds. Over time, living roots become root debris and undergo decomposition by soil microorganisms, ultimately entering stabilized pools. Therefore, the change over time of the composition and function of the C-degrading and transforming microbial communities associated with living and decomposing roots defines a central biological component of soil C stabilization. Our project focuses on the succession of soil microbial communities involved in degradation and transformation of C released from the roots of *Avena barbata*, a grass common to California. Metabolic pathways of soil microorganisms responsible for C processing will be identified by labeling *A. barbata* with $^{13}CO_2$ and distinguishing the functional C-cycling transcriptome by stable isotope probing of rhizosphere microbial communities. Fluorescent in situ hybridization probing and NanoSIMS analysis will be used to identify active community members consuming ^{13}C derived from roots and visualize spatial associations between microbes and plant roots. The influence of elevated CO_2 on succession and sequential expression of microbial function will also be investigated using transcriptome-enhanced GeoChip, CAZy/FOLy functional microarrays, and second generation sequencing. Gene expression networks will then be identified in order to model the microbial community cascades which enable C stabilization and turnover.

170

Microbial Food Web Mapping: Linking Carbon Cycling and Community Structure In Soils Through Pyrosequencing Enabled Stable Isotope Probing

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Project Goals: see below

The terrestrial biosphere contains a large fraction of global C and nearly 70% of the organic C in these systems is found in soils. The majority of C is respired and on an annual basis soil respiration produces 10 times more CO_2 than anthropogenic emissions, but it remains difficult to predict the response of soil processes to anthropogenic changes in the environment. Our difficulty in predicting how soil processes will respond to environmental change suggests a need for a greater understanding of the biotic mechanisms that govern the soil C-cycle.

In their metabolic diversity and their sheer numbers microbes dominate the soil processes that underlie ecosystem processes in terrestrial environments. Yet, the magnitude of their contribution lies in stark contrast to the little we

know about soil microorganisms and the principles that govern their function. Soil microorganisms do not behave as a homogeneous trophic level, and the composition of the soil microbial community can influence microbial processes in the soil both qualitatively and quantitatively. As a result, in order to understand changes in the terrestrial C-cycle in response to environmental change it is important to examine the internal dynamics of soil microbial communities and the manner in which they influence community function.

While we have made strides in understanding environmental controls on decomposition we still lack a coherent concept of the soil microbial food web. There is a general assumption of functional equivalence for different soil microbial communities with respect to the soil C cycle, but the validity of this hypothesis has been questioned. This deficiency in our knowledge results from the absence of in situ methods for identifying microorganisms involved in the soil C cycle and as a result we have a glaring lack of information about which organisms actually mediate critical soil processes. Our research program will approach this fundamental problem by developing a method for pyrosequencing enabled stable isotope probing (SIP) to dissect the microbial food web. Pyrosequencing enabled SIP offers a means to study the microorganisms that facilitate soil processes as they occur in soil, to characterize novel organisms that have escaped detection previously, and to make significant advances in our understanding of the biological principles that drive soil processes. We will use this groundbreaking approach to examine whether changes in soil community composition are ecologically significant with respect to their impact on the soil C-cycle. Project objectives include:

- Develop and validate protocols for pyrosequencing enabled ^{13}C-SIP of nucleic acids.
- Determine whether carbon input parameters (composition, quantity, timing) alter the route of C through the soil community and whether these shifts are driven by microbial community structure.
- Evaluate whether microbial community structure is functionally equivalent with respect to the C-cycle across edaphically related soils that differ in management history.

The method and the results generated by this project will improve our ability to examine the impacts of management decisions, soil history, and environmental change on the behavior of microbial communities in terrestrial ecosystems, revealing the ecological mechanisms by which microbes regulate both C mineralization and C retention in soils, and improving our ability to predict changes in terrestrial ecosystem processes in the face of accelerating global change.

171

Development of Novel Random Network Theory-Based Approaches to Identify Network Interactions in Soil Microbial Communities

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Project Goals: High-throughput metagenomics technologies can rapidly produce massive data sets, but one of the greatest challenges is how to extract, analyze, synthesize, and transform such vast amounts of information to biological knowledge. Thus, the goal of this study is to develop a novel conceptual framework and computational approaches based on a new mathematical approach, random matrix theory (RMT), using large scale, high throughput metagenomics sequencing and hybridization data.

The interactions among different microbial populations in a community play critical roles in determining ecosystem functioning but very little is known about the network interactions in a microbial community due to the lack of appropriate experimental data and computational analytic tools. High-throughput metagenomics technologies can rapidly produce massive data sets, but one of the greatest challenges is how to extract, analyze, synthesize, and transform such vast amounts of information to biological knowledge. Thus, the goal of this study is to develop a novel conceptual framework and computational approaches based on a new mathematical approach, random matrix theory (RMT), using large scale, high throughput metagenomics sequencing and hybridization data.

We have developed RMT-based conceptual framework for identifying functional molecular ecological networks (fMENs) with the high throughput functional gene array (GeoChip) hybridization data of soil microbial communities in a long-term grassland FACE (Free Air CO_2 Enrichment) experiment. Our results indicated that RMT is powerful in identifying fMENs in microbial communities. Both fMENs under elevated CO_2 (eCO_2) and ambient CO_2 (aCO_2) possessed the general characteristics of complex systems such as scale-free, small-world, modular and hierarchical. However, the topological structure of fMENs is distinctly different between eCO_2 and aCO_2, at the levels of the entire communities, individual functional gene categories/groups, and functional genes/sequences, suggesting that eCO_2 dramatically altered the network interactions among different microbial functional genes/populations. Such shifts in functional network structure are also significantly correlated with soil geochemical factors.

We have used this new approach to identify phylogenetic molecular ecological networks (pMENs) using metagenomics sequencing data of 16S ribosomal RNA (rRNA)

genes from soil microbial communities in the FACE site as well. Our results demonstrate that the RMT-based network approach is also very useful in delineating pMENs in microbial communities. The structure of the identified networks under aCO_2 and eCO_2 was substantially different in terms of overall network topology, network composition, node overlap, module preservation, module-based higher order organization (meta-modules), topological roles of individual nodes, and network hubs. These results suggested that eCO_2 dramatically altered the network interactions among different phylogenetic groups/populations. In addition, the changes in network structure were significantly correlated with soil carbon and nitrogen content, indicating the potential importance of network interactions in ecosystem functioning. To our knowledge, these are the first studies to demonstrate the changes in network interactions of microbial communities in response to eCO_2. In a word, elucidating network interactions in microbial communities and their responses to environmental changes is fundamentally important for research in microbial ecology, systems microbiology, and global change.

Student Oral Presentation–Tuesday

172

From Community Structure to Functions: Metagenomics-Enabled Predictive Understanding of Temperature Sensitivity of Soil Carbon Decomposition to Climate Warming

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Project Goals: see below

Understanding the responses, adaptations and feedback mechanisms of biological communities to climate change is critical to project future states of the earth and climate systems. Although a significant amount of knowledge is available on the feedback responses of aboveground communities to climate change, little is known about the response of belowground microbial communities due to the challenges in analyzing soil microbial community structure and functions. Thus, the overall goal of this study is to provide system-level, predictive mechanistic understanding of the temperature sensitivity of soil carbon decomposition to climate warming by using cutting-edge integrated metagenomic technologies. Towards this goal, the following four objectives will be pursued: **(i)** To determine phylogenetic composition and metabolic diversity of microbial communities in the temperate grassland and tundra ecosystems; **(ii)** To delineate the responses of microbial community structure, functions and activities to climate change in the temperate grassland and tundra ecosystems; **(iii)** To determine the temperature sensitivity of microbial respiration in soils with different mixtures of labile versus recalcitrant carbon, and the underlying microbiological basis for temperature sensitivity of these pools; and (**iv**) To synthesize all experimental data for revealing microbial control of ecosystem carbon processes in responses to climate change. We have carried out our studies at two contrasting long-term experimental facilities, the temperate grassland ecosystems in OK and tundra ecosystems in Alaska.

Feedback responses of microbial communities to warming. We have used integrated metagenomic technologies to analyze the responses of microbial communities in a long-term experimental warming grassland ecosystem in Oklahoma. Our results revealed at least three lines of evidence for microbial mediation of carbon cycle feedback to climate warming. First, long-term experimental warming induced a decline in temperature sensitivity of heterotrophic soil respiration due to soil microbial community adaptation. Second, warming significantly stimulated functional genes for labile but not recalcitrant carbon decomposition, which may promote long term stability of ecosystem carbon storage. Third, warming stimulated functional genes for nutrient cycling, favoring plant growth and vegetation carbon uptake. Our results indicate that microorganisms critically regulated ecosystem carbon cycle feedback to climate warming, with important implications for the carbon-climate modeling. Similar metagenomics analysis of soil microbial community in tundra ecosystems in Alaska are in progress.

Temperature sensitivity. We are conducting constant temperature (15°C) soil incubations from the two research sites. Temperature sensitivity is being measured by exposing soils to cycling temperatures at several time points during the 1-year incubation. In a single temperature cycle, soils are exposed to 6 temperature levels from 5 to 30°C over a 36 hour span; soil temperature is increased for 6 hours, with flux measurements made in the second half of that period after the temperature has equilibrated. In first 90-day period we are conducting this temperature cycle treatment 3 times. Then, we are performing this cycle 1 time in each 90-day period thereafter for a total of 6 temperature cycles over the period of 1-year. These temperature cycle experiments are a critical component of this study and are designed to disentangle the temperature sensitivity of the different microbial communities from differences in substrate availability that can confound comparisons between long-term incubations at different temperatures. From these experiments we can calculate Q_{10} relationships for different soils and different conceptual pools of soils within our modeling framework described later in this proposal. We expect that this experimental design will allow us to explore temperature sensitivity of different components of the organic matter

pool through time, and as it relates to the active microbial community as determined by the genomic analyses.

Development of amplicon-sequencing approaches for uncovering functional gene diversity. Functional genes involved in recalcitrant carbon degradation and N fixation were selected for pyrosequencing on the 454 titanium platform. The *ligDFEG* gene cluster is involved in the cleavage of the ß-aryl ether bond, the most abundant linkage in lignin (50-70% of total). Of these, *ligE*, a glutathione S-transferase domain protein, was targeted based on the sequence abundance in GenBank. Primer sequences were developed from a Hidden Markov Model derived compilation deposited into RDP's Fungene database. Second, the aromatic ring hydroxylating dihydrogenase (ARHD) gene, known for biphenyl degradation, was selected as this structure constitutes ~10% of lignin. The *nifH* gene was selected for its important contribution to soil fertility. Prior evidence indicates that *nifH* diversity responds to warming and is correlated with changes in soil carbon. Lastly, pyrosequencing will be performed on the *16S rRNA* gene to investigate changes in microbial community composition with treatment.

Evaluation of bioinformatic approaches for assembling short read sequences. We have also begun to explore the next-generation sequencing technologies (NGS) for characterizing our microbial communities. We have performed an initial assessment of the technical shortcomings of NGS such as what fraction of the total diversity in a sample can be recovered by NGS and what are the types and frequencies of errors in assembled genes from complex communities. To this end, we compared the two most frequently used sequencing platforms, the Roche 454 FLX Titanium and the Illumina Genome Analyzer (GA) II, on the same DNA sample obtained from a complex freshwater microbial community. We found that the two platforms provided a very comparable view of the community sampled, e.g., the derived assemblies overlapped in ~90% of their total sequences. Evaluation of base call error, frameshift rate, and contig length suggested that short reads (Illumina) offered equivalent, if not better, assemblies with longer reads (454). We are currently expanding our approaches to enable the use of higher volume data from more complex (soil) microbial communities. Such analyses will provide strategies for shotgun sequencing the entire microbial communities.

Modeling integration and development. We use data assimilation techniques to integrate soil incubation data into a model to optimize parameter estimation of different soil organic carbon (SOC) pools (labile to recalcitrant) with different turnover times. Soil carbon dynamics were described by a 1st-order differential equation. Model parameters were optimized using Bayesian probability inversion and a Markov chain Monte Carlo (MCMC) technique, which generates posterior probability density functions of model parameters. Preliminary results show that soil carbon efflux data of less than 200 days of incubation length only constrained parameters of a 1-pool model indicating that daily C decomposition by the end of the experimental period was still dominated by the CO_2 efflux of the labile SOC pool. Long-term datasets are required to constrain parameters of multiple carbon pool models in order to determine temperature sensitivities of carbon pools with slower turnover rates.

173

Meta-"omics" Analysis of Microbial Carbon Cycling Responses to Altered Rainfall Inputs in Native Prairie Soils

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Project Goals: see below

Soils process and store large amounts of C; however, there is considerable uncertainty about the details that influence microbial partitioning of C into soil C pools and what influential forces control the fraction of the C input that is stabilized. It is not clear how the microbial community will respond to climate-induced modification of precipitation and inter-precipitation intervals, and if this response will affect the fate of C deposited into soil by the local plant community. Part of this uncertainty lies with our ignorance of how the microbial community adapts physiologically or in composition to changes in soil moisture brought about by shifts in precipitation.

Our **goal** is to harness the power of multiple meta-omics tools to understand the functioning of whole-soil microbial communities and their role in C cycling. Towards this end, we will:

1. Further develop and optimize a combination of meta-omics approaches to study how environmental factors affect microbially-mediated C cycling processes.
2. Determine the impacts of long-term changes in precipitation timing on microbial C cycling using an existing long-term field manipulation of a tallgrass prairie soil.
3. Conduct laboratory experiments that vary moisture and C inputs to confirm field observations of the linkages between microbial communities and C cycling processes.

We will study the rainfall manipulation plots (RaMPs) established more than a decade ago at the Konza Prairie in Kansas. The RaMPs experiment consists of two treatments: (1) natural precipitation, and (2) extended precipitation interval. The extended precipitation interval consists of storing rainfall and reapplying it at an interval 50% longer than that between natural rainfall events.

We will employ a systems biology approach, considering the complex soil microbial community as a functioning system

and using state-of-the-art metatranscriptomic, metaproteomic, and metabolomic approaches. These omics tools will be refined, applied to field experiments, and confirmed with controlled laboratory studies. Our experiments are designed to specifically identify microbial community members and processes that are instrumental players in processing of C in the prairie soils and how these processes are impacted by wetting and drying events.

At this time, we have made progress towards the development and optimization of the methods we will use for metatranscriptomic and metaproteomic samples. We have set up a short-term laboratory experiment using soil collected from the Konza site. Soil received two treatments: (1) control or inoculated with an *Arthrobacter* strain whose genome has been sequenced, and (2) control or amended with acetate (a generic source of readily available C) or 4-chlorophenol (a xenobiotic C compound that is metabolized by the added *Arthrobacter* stain). After incubation, soils were extracted for either RNA or protein and gene expression and protein abundance have been assessed. See the David et al. poster for a summary of the initial results from this experiment.

Additional laboratory experiments have been planned, which will address the following questions: 1) Is there a temporal lag between peak transcriptional activity and peak biomass/protein production? 2) Do different soil microbial taxa display different strategies to cope with modified wet/dry cycles, both in terms of temporal patterns of response and physiological adaptations that control utilization of C and allocation of C within/without the cell? The designs of these experiments will be presented.

In early summer 2011 we will conduct the first of two samplings of the RaMPs experiment. The first sampling will occur when plant growth is optimal and will be timed to collect soils prior to a major rainfall event (when soils are relatively dry), shortly after wet-up, and at one more time as the soils begin to dry. At each of these times, we will measure soil community properties (pyrotagged analysis of rRNA DNA and rRNA, metatranscriptome, and metaproteome) and soil C cycling processes (soil respiration, extracellular enzyme activities, etc.).

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174

Assessing the Microbial Basis of Carbon Cycling in Prairie Soils with an Integrated Omics Approach

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Project Goals: This project aims to evaluate the carbon cycling potential of the soil microbiota in Kansas native prairie soils using different omics approaches.

Soils of the Great Prairie store more than 30% of the terrestrial organic carbon in the continental U.S. This area is expected to experience dramatic changes in precipitation patterns, with either longer drought periods or more rainfall, as a consequence of climate change. However, the impact of a changing climate on the stored carbon pools in these soils is currently not known. This project aims to evaluate the carbon cycling potential of the soil microbiota in Kansas native prairie soils using different omics approaches.

In order to generate a database for the "omics" analyses, 15GB of DNA sequence were obtained via Illumina sequencing from the Kansas prairie soil. A total of 102 million reads were assembled using a CLC Genomics workbench, and we obtained 1.4 million contigs of at least 300 bp in length. Those contigs were submitted to IMG for gene prediction and annotation.

To develop protocols for extraction of RNA and protein from soil, we used a model bacterium that has been genome sequenced, *Arthrobacter chlorophenolicus*, as an inoculum. The model strain was inoculated into liquid medium and non-sterile Kansas prairie soil, and acetate and 4-chlorophenol were added as general and specific carbon substrates, respectively. The same substrates were added to the soil without inoculum to assess the response of the indigenous microbes in the soil.

The RNA was extracted from soil using a MoBio kit and treated with DNase. Key target genes were quantified by quantitative PCR and RT-QPCR and the metatranscriptomes were obtained by Illumina sequencing. A first set of genes, 16SrRNA and gfp (encoding the green fluorescence protein that was stably inserted in the chromosome of the *A. chlorophenolicus* strain used) were chosen to estimate the *A. chlorophenolicus* cell number. Two other genes, *ICL* (isocitrate lyase, part of the 2-C bypass to the TCA cycle), and *suCAB* (succinyl CoA, part of the main TCA cycle) were used to track the pathways used by the microorganisms with different substrates.

To study the metaproteome, we first optimized a method for extraction of proteins from soil, based on a combination of lysis with SDS, boiling/sonication, followed by TCA precipitation, protein digestion and a size exclusion filtration (modified from Chourey et al, 2010). With this optimized protocol over 1000 proteins were identified via 2d-LC-MS/MS on an LTQVelos per soil microcosm. This protocol allowed us to directly characterize the metaproteome in soils with high levels of humic acids. The resulting peptides were searched against 4 databases: 1) the assembled reads of the metagenome from Kansas prairie soil, 2) this database complemented with the unmapped reads that didn't assemble into contigs, 3) 16S rRNA gene sequences data from the same soil was used to build a selected reference isolate genome database, and 4) the Arthrobacter isolate genome for those samples that had been inoculated with the model strain.

The first RNA results show that gfp transcript could be detected under most conditions and was thus a good estimator of the cell activity and abundance in the samples. Different metabolic pathways were more predominantly expressed depending on the substrates and incubation conditions used. In addition, the initial metaproteome data indicated that several of the enzymes involved in the degradation pathways of the particular organic substrate amended to the microcosms were expressed in soil and in liquid media. In general, several proteins involved in response to stress (thioredoxin, chaperonin, cold-shock proteins, etc.), were expressed in the prairie soil.

These initial studies will define the best techniques for omics applications in soil as well as baseline information about the carbon cycling potential in the Great Prairie of the U.S. The next step will be to determine the impact of altered precipitation on the soil microbial community and carbon cycling functions in these soils.

Reference

1. Chourey, K; Jansson, J; Verberkmoes, N; Shah, M; Chavarria, KL; Tom, LM; Brodie, EL; Hettich, RL. "Direct cellular lysis/protein extraction protocol for soil metaproteomics". *Journal of Proteome Research*. 2010 12:6615-22.

175

The Impact of Global Warming on the Carbon Cycle of Arctic Permafrost: An Experimental and Field Based Study

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http://www.princeton.edu/southafrica/DOEpermafrostproject

Project Goals:

1. **Perform ~2 years long, heating experiments on well-characterized, intact cores of Arctic active-layer and permafrost from a proposed reference ecosystem site where CO_2, N_2O and CH_4 fluxes, temperatures, humidity, soil moisture, nutrients, microbial diversity and activities and isotopic analyses are currently being measured in the field.**
2. **Perform phylogenetic, metagenomic, transcriptomics and proteomic analyses of the intact cores.**
3. **Characterize the abundance and composition of the solid and dissolved organic matter and the inorganic geochemistry in the active layer and permafrost.**
4. **Characterize changes in the organic matter composition, the vertical flux of volatile organic acids, O_2, H_2, CO_2 and CH_4 the isotopic systematics of CO_2 and CH_4 and changes in the transcriptomics, proteomics and C cycle networks in these cores during the long term heating experiments as the permafrost thaws under water saturated and water under saturated conditions.**
5. **Compare the results from intact cores and the heating experiments with field measurements.**
6. **Based upon these heating experiments and field measurements construct a 1D biogeochemical reaction/transport model that predicts the CO_2 and CH_4 release into the atmosphere as permafrost thaws and compare these predictions with observations at the reference ecosystem site.**

Permafrost, or perennially frozen ground, underlies ~24% of the Earth's surface and contains ~1/3 of the global soil organic C. It is, therefore, a possible source of extremely potent greenhouse gases, such as CH_4, N_2O and CO_2. Temperatures in the Arctic may increase 4-8°C over the next 100 years, thereby increasing the depth of the active-layer and thawing the underlying permafrost. Field observations and ice core records suggest that with thawing, the relatively undegraded permafrost organic C will be rapidly metabolized, creating a positive feedback to global warming through increased CH_4, N_2O and CO_2 emissions. Although many researchers have measured CO_2 and CH_4 fluxes and characterized the microbial diversity of the

Siberian and Canadian active-layer and permafrost, the relationship between methanogenic, methanotrophic and heterotrophic *in situ* activities within the active-layer and CO_2 and CH_4 fluxes as a function of temperature has not been delineated either in field or lab experiments. Defining these relationships is essential for determining the extent and rate of this positive feedback in order for these processes to be accurately reflected in global climate models.

To address this paucity of data we will collect 30 intact cores for long term heating experiments from the active layer and permafrost (0-1 meters below surface) at the McGill Arctic Research Station (MARS) on Axel Heiberg Island in the Canadian high Arctic. This site has been the location of climate investigations for the past 50 years. The extensive Arctic wetland area adjacent to the lake at this site will be the source of the intact cores (Fig. 1 top). CO_2 flux measurements indicate higher emissions during peak summer months compared to spring time when the ground is completely frozen. The microbial community of the active layer and associated permafrost varies as a function of depth with aerobic phyla dominant near the surface and anaerobic phyla dominant at greater depths. Archaeal phyla, including methanogens, comprise 0.1% of the microbial community. A low diversity fungal component is also present in the active layer and permafrost. Pore water geochemical results also indicate decreasing O_2 availability as a function of depth and proximity to the lake. The total organic carbon concentration is uniform with depth, but the dissolved organic carbon varies dramatically with depth with values as high as 100 mM. Aerobic viable cell counts from the active layer are also 100 times those of the permafrost. Anaerobic incubation experiments utilizing organic carbon amendments detect enhanced production of CO_2 and CH_4 with increasing temperature up to 15˚C, relative to undetectable CO_2 and CH_4 production at 0˚C.

Based upon last summer's coring campaign a new coring bit has been manufactured that will enable the collection of 1-meter long intact cores within polycarbonate tubes that can be sealed and frozen on site (Fig. 1 bottom). Heating experiments will be performed on these tubes in the lab where the temperature, precipitation and humidity of the headspace will be controlled and the gaseous, aqueous and solid phase constituents analyzed over time. Experiments will be performed under both water-saturated conditions and partially saturated conditions that reflect the observed variations in the water table depth of the site. The ongoing incubation experiments will be utilized to design the timing of sacrificial core analyses and the type of organic substrates that will be added in a subset of the cores. Organic and inorganic geochemical, metagenomic, metatranscriptomic and proteomic profiles will be performed on the core samples prior to and during prolonged heating to address questions of nutrient fluxes, diversity, abundance, activity and spatial relationships between microorganisms, respectively. A high sensitivity ^{14}C RNA isotope microarray will be developed based upon the observed 16S rRNA community structure that will map the carbon trophic cascade as permafrost with ^{14}C labeled compounds thaws. Cavity ring down spectrometers will be used to monitor C, O and H isotopic analyses of CH_4 and CO_2 from the heated cores, from cores amended with ^{13}C labeled compounds and from permafrost emissions at the MARS field site.

Figure 1. (top) Aerial view of the McGill Arctic Research Station showing polygonal terrain adjacent to Colour Lake. (bottom) Coring of wetland active layer and permafrost during July 2010.

176

Characterization of Active Layer and Permafrost from High Canadian Arctic Soil and Potential Effects of Climate Change on Greenhouse Gas Emissions

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http://www.princeton.edu/southafrica/DOEpermafrostproject

Project Goals: The goal of this project is to characterize changes in the geochemical profile and the microbial community of active layer and permafrost soils from a high Canadian Arctic environment based on long-term heating experiments.

Thawing of permafrost soils and the carbon stores that they contain has been of concern as a potential source of additional CO_2 and CH_4 to the atmosphere, providing a positive feedback on a warming climate. To assess the potential for increased release of greenhouse gases to the atmosphere, the active layer and permafrost from a moist, acidic soil in the high Canadian Arctic was characterized and used to perform microcosms with ^{13}C-labeled substrate additions under in situ and simulated warming conditions.

C/N ratios in this soil were between 15:1-18:1 and increased with depth, lower than the values observed in other Arctic soils (20:1-40:1) and exhibiting an inversion of typical soil C/N profiles. High levels of dissolved organic carbon (DOC; 100 mmol C/L) were observed in the subsurface at 30-35 cm depth and decreased into the permafrost while total dissolved nitrogen increased with depth. Porewater composition was dominated by SO_4^{2-} (~1 mM) and contained soluble Fe and Mn at depth, indicating a gradual transition to an anoxic region.

Microcosms from active layer (35 cm) and the permafrost (75 cm) samples were incubated anaerobically with acetate, CO_2/H_2, methanol, or unamended, under different temperature regimes, 4˚C and 20˚C. Production of CH_4 reached 2.8 nmol/g(FW)/day in CO_2/H_2 enriched samples, and CO_2 reached 3.5 nmol/g(FW)/day in acetate enriched samples. With warming from an in situ summer temperature of 4˚C to 20˚C, CO_2 production increased by an average of 36% across all samples, whereas CH_4 production did not increase on average.

This study suggests that upon warming these soils will likely be a net source of CO_2 to the atmosphere and the active layer deepens and additional soil carbon is available for degradation. Emissions of CH_4, however, do not seemed poised to substantially increase, likely due to the presence of more favorable electron acceptors that prevent the system from fully progressing to methanogenesis.

177

Multi-Scale Modeling of *Methanosarcina* Species

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Project Goals: The long-term goal of the proposed research will be the creation of integrated, multiscale models that accurately and quantitatively predict the role of methanogenic organisms in the global carbon cycle under dynamic environmental conditions. To achieve this goal the following specific aims will be addressed: (1) we will sequence the genomes of numerous members of the Order Methanosarcinales; (2) genomic sources of phenotypic variation will be identified through comparative genomics and tested experimentally using genetic approaches; (3) the transcriptional networks of two *Methanosarcina* species will be established using a combination of experimental and bioinformatic techniques; and (4) we will develop comprehensive metabolic network models across the order Methanosarcinales.

Methanogenesis is responsible for a significant fraction of the global carbon cycle and plays an essential role in the biosphere. In many anaerobic environments, turnover of organic matter is completely dependent on methanogenic archaea. Although a great deal is known about the physiology and metabolism of these organisms, our ability to incorporate methanogens into carbon cycle models remains in the "black box" stage. To address this issue we are developing systems level models that capture the metabolic and regulatory networks of *Methanosarcina* species, which are among the most experimentally tractable of the methane-producing archaea. Our initial goal is to sequence the genomes of approximately forty strains that span the taxonomic scale within the Order *Methanosarcinales*. These strains include isolates with thoroughly characterized physiology, biochemistry and genetics from both marine and freshwater environments. New strains from additional well-characterized ecosystems are being isolated and characterized for genome sequencing in later stages of the project. The genomic sources of phenotypic variation are being assessed by comparative genomic analysis and by development of comprehensive constraint-based metabolic network models for each strain. To understand the mechanism of adaptation of these organisms to changing environmental conditions, we are examining the evolutionary changes in global transcriptional responses of each organism to a range of growth conditions using the RNA-seq approach. Our ultimate goal is to incorporate the observed regulatory and metabolic networks into integrated, multi-scale models that accurately and quantitatively predict the role of methanogenic organisms in the global carbon cycle under dynamic environmental conditions. At present, we have completed draft sequences of four *Methanosarcina* genomes, with eight more in progress. Manually curated metabolic models have been created and tested for a model freshwater species (*Methanosarcina barkeri*) and a model saltwater species (*Methanosarcina acetivorans*), which will serve as starting points for modeling of our newly sequenced strains. Preliminary RNA-seq experiments have been conducted for *M. acetivorans*. To fully exploit these data, we have developed penalized linear regression for modeling the RNA-Seq read counts. These models explain more than 60% of the variations and can lead to improved estimates of gene expression.

178

Photoacclimation and Adaptation in the Green Lineage: A Case Study of the Picoeukaryote *Micromonas*

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Project Goals: To develop a systems biology approach to the study of the widespread marine alga *Micromonas* and investigate the impact of environmental perturbations on primary production.

Micromonas is a green alga that thrives from tropical to arctic marine systems and belongs to the picophytoplankton (unicellular algae ≤2 µm in diameter). Algae in this size class mediate a significant portion of CO_2 uptake in marine ecosystems. Green algae like *Micromonas* also provide simplified model systems for exploring the evolution of members of the 'green lineage', which includes all land plants. In 2009, we published the complete genome sequences of two isolates of *Micromonas*, namely RCC299 and CCMP1545, in collaboration with the JGI and community members. While the genomes provided a wealth of information, knowledge is lacking about the physiology of these algae and their ability to survive under changing conditions such as rising CO_2 levels or increased exposure to ultraviolet (UV) radiation resulting from shallowing mixed layers. Photosynthetic organisms have various acclimation mechanisms to compensate for changes in irradiance. Several gene suites have been identified that appear to be related to light-stress and photoprotection. For example, in higher plants such as *Arabidopsis*, ELIPs (early light induced proteins) accumulate transiently when exposed to stress, including high light (HL).

We tested the response of RCC299 to a rapid shift from the maximal-growth light level to HL or HL+UV. A combination of flow cytometry, quantitative PCR and short read transcriptome sequencing was used to explore cell physiology and putative photoprotective genes under experimental manipulations. Results showed that chlorophyll per cell decreased after short term (1 to 2.5 hours) exposure to HL+UV and was lower than in the control under both types of stress, as might be expected. Two general responses were observed for expression of chloroplast-encoded genes: i) those involved in transcription and translation were down-regulated in all conditions, however in HL and HL+UV this shift was visible earlier in the time course; ii) HL+UV treatment resulted in down-regulation of the photosystem II subunits. This effect was also seen, to a lesser extent, in HL conditions. Several nucleus-encoded genes were up-regulated under HL+UV, and to a lesser extent in HL, but not in controls—these genes are implicated in photoprotection. Moreover, ELIPs were more highly expressed in HL+UV but not HL or controls. Perhaps most exciting was expression of two divergent chloroplast-encoded genes—suggesting they represent novel functional aspects of the photosynthetic apparatus. Together, the results indicate that *Micromonas* is adapted for survival in dynamic light environments.

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179

Connecting Genomes to Physiology and Response: Systems Biology of the Green Alga *Micromonas*

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Project Goals: To develop a systems biology approach to the study of the widespread marine alga *Micromonas* and use it to investigate gene function, pathways and consequences of environmental perturbations on primary production.

Approximately half of global photosynthetic CO_2 uptake is performed by marine algae. Thus far, there are no model systems relevant to both marine carbon cycling and evolution of the eukaryotic supergroup Plantae. Development of such a system is important given the onset of climate change and limited understanding of how earth systems will move forward under current perturbations. Prasinophytes are a group of unicellular marine green algae that are evolutionarily distinct from the model green alga *Chlamydomonas*, but are related to land plants and thought to retain characteristics present in the Plantae ancestor. Here, we describe a prasinophyte model system that will be of broad ecological relevance to marine carbon cycling. Our system uses *Micromonas*, a widespread pico-prasinophyte that is exceptional in having a small genome (21 Mb), low gene redundancy and gene 'fusions', which join together domains typically encoded by separate genes. These features are valuable for investigating and assigning functions to genes and domains by their association with a known pathway or

physiological response. Moreover, given its relationship to plants the *Micromonas* system will enable modeling of more general primary producer responses across multiple ecological and evolutionary scales. The project builds on complete genome sequences from two *Micromonas* strains which share at most 90% of their protein encoding gene complements. Differences between these strains include elements of RNAi and vitamin biosynthesis pathways. We are using high-performance chemostats, transcriptomics and high-throughput proteomics to explore fundamental biological questions and their ecological implications. The data will address the known divide between transcriptional and translational responses. Comparative analyses will also allow investigation of pathways for which particular constituents appear to be derived from other lineages. Computational methods will be used to discover how gene activities are regulated and modulated in response to cellular events and processes. Our overall goal is for this system to provide insights on members of the green lineage—both marine algae and plants—including both novel and conserved genetic mechanisms.

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180

Soil Metagenomics and Carbon Cycling in Terrestrial Ecosystems

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Project Goals: The LANL Science Focus Area (SFA) in Soil Metagenomics and Carbon Cycling in Terrestrial Ecosystems combines field and model system studies to identify patterns of soil microbial community response to elevated CO_2 and other climate change factors (altered precipitation, soil warming), and to identify key microbial participants and processes in soil carbon partitioning across multiple terrestrial ecosystems.

Increased plant growth in response to elevated atmospheric CO_2 increases carbon inputs to the soil. The collective activities of complex soil microflora determine whether this additional carbon is sequestered in the soil or released back into the atmosphere and thus have local, regional and global impacts on terrestrial carbon storage and cycling. However, currently we only have a rudimentary understanding of the composition of microbial communities, their involvement in processes that represent control points in carbon flux, and the rates at which these processes occur in terrestrial ecosystems. Accurate climate modeling and carbon management scenarios require an understanding of these microbially-mediated processes.

The LANL Science Focus Area (SFA) in Soil Metagenomics and Carbon Cycling in Terrestrial Ecosystems combines field and model system studies to identify patterns of soil microbial community response to elevated CO_2 and other climate change factors (altered precipitation, soil warming), and to identify key microbial participants and processes in soil carbon partitioning across multiple terrestrial ecosystems. This poster highlights elements of the SFA directed toward (a) assessment of soil bacterial and fungal communities, and their responses to long-term elevated CO_2 across six of the DOE's FACE and OTC field research experiments (http://public.ornl.gov/face) using a variety of metagenomic approaches, (b) identification of active cellulolytic bacteria and fungi in different soils using stable isotope probing, and (c) development of a fungal large subunit rRNA gene databases and classification tool to enable interpretation of fungal metagenome data. Two associated posters provide more detailed information on metagenome projects within the SFA: (a) response of soil cellulolytic fungal communities, as measured by the cellobiohydrolase I gene (*cbhI*), to long-term elevated CO_2 in the DOE FACE and OTC sites, plus seasonal patterns and soil *cbhI* gene expression patterns at the NC pine FACE site, and (b) responses of soil bacterial communities comprising biological soil crusts and shrub root zones to long-term elevated CO_2 at the NV desert FACE site.

181

Impact of Ecosystem Type and Season on Responses of Fungal Communities and Their Cellulolytic Constituents to Elevated Atmospheric CO_2

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Project Goals: We investigated the impacts of ecosystem type and elevated atmospheric CO_2 on cellulolytic fungal communities by conducting a large-scale Sanger sequencing study of the fungal glycosyl hydrolase 7 family cellobiohydrolase I gene (cbhI) across five U.S. Department of Energy FACE sites. At the NC pine FACE sites, we determined seasonal patterns of diversity and composition for the total fungal community (LSU gene) and the cellulolytic fungal guild (cbhI gene), to determine how seasonal dynamics may affect community responses to elevated CO_2 and nitrogen (N)-fertilization at this site. The expression patterns of cbhI genes in soils from different depths and treatments are also being characterized.

Elevated atmospheric CO_2 generally increases plant productivity and subsequently increases the availability of cellulose in soil to microbial decomposers. As key cellulose degraders, soil fungi are likely to be one of the most impacted and responsive microbial groups to elevated atmospheric CO_2. However, we do not understand how fungal diversity is distributed *in situ,* which fungi contribute most to cellulose degradation, or how they respond to elevated CO_2. We investigated the impacts of ecosystem type and elevated atmospheric CO_2 on cellulolytic fungal communities by conducting a large-scale Sanger sequencing study of the fungal glycosyl hydrolase 7 family cellobiohydrolase I gene (*cbhI*) across five U.S. Department of Energy FACE sites. At the NC pine FACE sites, we determined seasonal patterns of diversity and composition for the total fungal community (LSU gene) and the cellulolytic fungal guild (*cbhI* gene), to determine how seasonal dynamics may affect community responses to elevated CO_2 and nitrogen (N)-fertilization at this site. The expression patterns of *cbhI* genes in soils from different depths and treatments are also being characterized.

To investigate the impacts of ecosystem type and a decade of elevated atmospheric CO_2 treatments on cellulolytic fungal communities, we sequenced 10,677 *cbhI* gene fragments across five distinct terrestrial ecosystem experiments. The composition of fungal *cbhI* genes was unique in each ecosystem. Using a 114-member *cbhI* sequence database compiled from known fungi, less than 1% of the environmental sequences could be classified at the family level indicating that cellulolytic fungi *in situ* are either dominated by novel fungi or by known fungi that are not yet recognized as cellulose degraders. Shifts in fungal *cbhI* composition and richness in response to elevated CO_2 exposure varied among the ecosystems. In aspen forest and desert creosote bush soils, *cbhI* gene richness was significantly higher after exposure to elevated CO_2 than under ambient CO_2. In contrast, richness was not altered in desert soil crusts, but the relative abundance of dominant operational taxonomic units (OTUs) was significantly shifted. Collectively, the data show that responses are complex.

Seasonal surveys of *cbhI* and the fungal LSU gene across treatment and control plots at the loblolly pine FACE site indicated that there is a trend of decreased fungal richness in elevated CO_2 plots compared to ambient plots across all seasons. Nitrogen fertilization in both ambient and elevated CO_2 plots increased richness in spring and summer sampling points indicating that some taxa are nitrogen limited during these seasons. In contrast, there did not appear to be any seasonal change in the cellulolytic fungal guild. These studies enable us to understand how much of a control seasonal fungal community dynamics exert on responses to climate change parameters.

Gene expression studies at the NC pine FACE site demonstrated that the expressed *cbhI* genes are dominated by Basidiomycota-like sequences that are not closely related to any of the available *cbhI* gene sequences from presently known fungi. In addition, the rank abundance of *cbhI* genes from DNA-based versus RNA-based clone libraries show that the most abundant *cbhI* genes are not always the most highly expressed. The DNA and RNA-based surveys provide complimentary information, revealing the community potentially able to carrying out a specific function and the populations that are most active under particular environmental conditions.

Collectively, these studies show that ecosystem type and seasonal dynamics play important roles in controlling responses to climate change parameters.

182

Response of Soil Microbial Communities in an Arid Ecosystem to Eight Years of Elevated CO_2

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Project Goals: The goal of this project is to describe the changes in community structure and ecosystem functioning expected to occur in response to elevated concentrations of atmospheric CO_2, in a manipulated and highly replicated field experiment. Our role in the larger context of the project is to characterize changes in the soil microbial communities.

Arid and semi-arid ecosystems, which cover ~40% of the terrestrial surface are predicted to be especially sensitive to climate change. We have built on previous studies conducted in the Nevada Desert FACE Facility (NDFF), a large scale, replicated Free Air CO_2 Enrichment (FACE) experiment to describe changes in an arid ecosystem associated with elevated atmospheric CO_2, a primary driver of climate change. The ecosystem is shrub-dominated with large surfaces lacking plant cover. Biological soil crusts exist in the inter-plant spaces and cover from 35 to 65% of the surface area. Soil crusts consist of cyanobacteria, algae, lichens and associated heterotrophic bacteria and fungi. Previous studies have identified several ecosystem responses to elevated CO_2. The plant populations at the NDFF responded to elevated CO_2 through increased biomass and changes in the commu-

nity structure, while the soil crusts responded to increased CO_2 through decreases in moss and lichen cover.

We have been employing a suite of molecular techniques, ranging from targeted gene sequencing to shotgun metagenomics, to characterize the soil microbial communities in the NTS-FACE site. Our preliminary studies have demonstrated that the bacterial populations are patchily distributed with only minimal species overlap between replicate samples. However, there was clear differentiation of the soil microbial populations by habitat. The bacterial populations associated with the creosote bush root zones were significantly different than those in the inter-space biologic soil crusts. Populations of Cyanobacteria, Actinobacteria and Proteobacteria dominated soil crusts whereas creosote root zone bacterial populations were dominated by Actinobacteria and to a lesser extent Proteobacteria. The differences in these communities also translated into differences in the response of these communities to elevated CO_2. However, the detected differences in response to elevated CO_2 were not consistent between the different metagenome approaches. The phyla found to respond to the CO_2 treatment were generally consistent between methods but the direction of their response differed. For example, 16S rRNA sequencing on Sanger or pyrotag platforms suggested a decline in the relative abundance of Cyanobacteria in response to elevated CO_2. In contrast, analysis of shotgun metagenomes using the SEED tool of MG-RAST suggested that Cyanobacteria were enriched in response to elevated CO_2. This disparity may be due to the presence of cyanobacteria in the soil crusts that are only distantly related to currently described species. The lack of reference genomes for these organisms renders them effectively invisible to metagenome taxonomy assignments and results in a reduction of Cyanobacteria calls compared to 16S rRNA sequencing. Nevertheless, these data suggest particular bacterial phyla were sensitive to elevated CO_2. We are now pursuing highly replicated field sampling coupled with pyrotag and shotgun metagenome sequencing to better define the fine scale changes in bacterial and fungal communities in response to elevated CO_2.

submitted post-press

182A‡

Bacterial Cheaters and Investors: Isolation of Non-Polymer Degraders and Extracellular Enzyme Producers from the Environment

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Project Goals: During the process of decomposition, extracellular enzymes catalyze breakdown of polymeric compounds into smaller molecules that can be taken up by cells. Little is known about the influence of "cheating" bacteria that do not secrete extracellular enzymes but use monomers resulting from the activity of extracellular enzymes secreted by other organisms ("investors"). The goal of this study was to isolate and identify cheating and investing bacteria from environmental samples.

We hypothesized that higher levels of fungal biomass would result in greater extracellular enzyme activity and consequently, a greater population of cheating bacteria. We also hypothesized that increased moisture content would increase diffusion of extracellular enzymes and monomers from investors to cheaters, and therefore would favor cheating bacteria. Samples were collected from Jennings Woods, an experimental forest in Northeast Ohio, USA. We sampled decayed leaves from three habitats in and around vernal pools comprising a complete factorial design of two treatments: high or low fungal biomass based on the observation of fungal hyphae, and high or low moisture. Samples were used to inoculate bi-layer gradient agar plates where the lower layer contained monomers and the upper layer contained polymers as the carbon source. After inoculation on the bi-layer gradient agar plates, the numbers of bacterial colonies growing on each plate were counted. Results showed that colony counts were higher on the side of the plate containing the higher concentration of monomers ($P<0.01$). However, differences among treatments (high or low fungal biomass and high or low moisture) were not statistically significant ($P>0.05$). Screening was carried out for the growth of the isolates on the polymer cellulose and its monomer cellobiose and results showed that 40 percent of the total isolates did not grow on cellulose while all of the isolates grew on cellobiose. Fungal biomass and moisture treatments did not differ significantly in the proportion of isolates that could grow on cellulose ($P>0.05$). Non-polymer degrading bacteria were isolated and appear to be cheaters, however, there was no detectable difference among treatments.