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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

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Approaches to Systematically Examine Carbon Flux in Microbial Communities Using 'Omics' and Stable Isotope Probing

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http://geomicrobiology.berkeley.edu/pages/amd.html

Project Goals: The primary goal is to develop integrated 'omics' methods, including stable isotope probing, for tracking carbon flows in microbial communities.

Earth's climate is regulated by biogeochemical carbon exchanges between the land, oceans and atmosphere that are chiefly driven by microorganisms, which produce and consume carbon dioxide (CO_2), methane, and organic matter and support the growth of higher organisms. Microbial communities are therefore indispensible to the study of carbon cycling and its impacts on the global climate system. However, approaches to examine carbon flux in communities systematically at the molecular level are inadequate. We are developing methods to track the flow of carbon into and through microbial communities using a well-characterized model system—acid mine drainage (AMD) biofilms.

We are using stable isotope probing (SIP)-proteomics and SIP-metabolomics to measure and characterize the incorporation of carbon compounds into proteins and metabolites from specific organisms. SIP-proteomics and SIP-metabolomics can determine ^{13}C or ^{15}N atom% of thousands of proteins and hundreds of metabolic features in the AMD community. Together with comprehensive community metagenomics, these novel approaches demonstrate which compounds are being consumed and which organisms are consuming them. Protein data from individual organisms, community metabolite profiles and measurements of carbon pools and fluxes will allow us to generate a model for carbon cycling in the community.

We have developed and validated a ^{15}N-based SIP-proteomic method using laboratory-grown AMD communities (Pan et al., 2011) and that has since been applied to further experiments (see below). We are now using ^{13}C-based SIP-proteomics to track the incorporation of $^{13}CO_2$ into microbial proteins. Newly developed methods for SIP-metabolomics with ^{13}C and ^{15}N constrain the biological origin and specific formulas of the metabolites generated by the AMD community, making metabolite identification more tractable. Together, these approaches will enable us to follow the movement of carbon through the community as it shifts from primary production in early growth stages to a mixture of heterotrophic and autotrophic metabolisms in the later stages of development.

The initial colonizer of the AMD biofilms, *Leptospirillum rubarum*, dominates early community development and carbon fixation in the system. *Leptospirillum ferrodiazotrophum* and other lower abundance community members appear later as biofilms develop and diversify. Using community genomic data collected over a period of 7 years, we assembled the partial genome of a new bacterial species that expands the *Leptospirillum* clade: *Leptospirillum* Group IV. The new species shares 97% 16S rRNA sequence identity and 70% identity between shared proteins with its closest relative, *Leptospirillum ferrodiazotrophum*. The presence of nitrogen fixation and reverse TCA cycle proteins suggest an autotrophic metabolism similar to that of *Leptospirillum ferrodiazotrophum*, while hydrogenase proteins unique to *Leptospirillum* Group IV suggest an active role in the anaerobic setting.

Fungi often colonize late-developmental-stage biofilms at low relative abundance and may play an important role in recycling carbon in the community. We are employing genomics, transcriptomics, and proteomics to link functional activities encoded and expressed by fungi with biogeochemical processes within the ecosystem (Miller et al., in prep). We reconstructed the near-complete composite genome (27 Mbp) of the dominant fungal AMD community member, *Acidomyces richmondensis*. Approximately 900 unique fungal proteins were detected by proteomics in field samples, covering many important metabolic pathways. Genes involved in heterotrophic carbon cycling, including several glycosyl hydrolases involved in polysaccharide hydrolysis, were expressed at varying levels based on transcript abundances inferred from ESTs recovered from field samples.

We have shown that Archaea from the Thermoplasmatales lineage dominate submerged anaerobic biofilms in the AMD system and play an important role in nutrient cycling in the anaerobic and degradative portion of the carbon cycle (Justice et al., in prep). Members of the novel ARMAN nanoarchaeal lineage are especially abundant in the sunken biofilms, comprising at least 10% of the community based on FISH and metagenomic data analysis. Comparative community proteomic analyses show a persistence of bacterial proteins in sunken biofilms, but there is clear evidence of deamidation caused by acid exposure. Given the low representation of bacterial cells in sunken biofilms, we infer that deamidated proteins are derived from populations of

lysed cells. Culture experiments demonstrated heterotrophic growth of A-plasma and *Ferroplasma* sp. on peptone, betaine, casein-derived peptides, and natural biofilm material, all coupled to iron reduction. The results demonstrate anaerobic archaeal growth via protein degradation, and possibly other organic carbon compounds. ^{15}N-based SIP-proteomics showed that archaea are the dominant species incorporating ^{15}N in sunken biofilms. The SIP-proteomic techniques also allowed us to profile the archaeal metabolic activities preferentially enriched for newly synthesized protein. These findings expand our understanding of the roles of Archaea in anaerobic nutrient cycling.

We are determining how a key parameter of global climate change, elevated temperature, regulates the flow of carbon through the microbial-based AMD ecosystem. Proteomic and metabolomic data highlight which community members and which modes of carbon cycling are affected by elevated temperature. This approach is establishing whether the carbon cycling pathways in the system are robust to the effects of climate change or, if not, where the cycling may break down.

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Estimation of Hydrogen Isotope Enrichment in Proteins and Lipids in a Microbial Community

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In the biosynthesis of lipids and amino acids, microorganisms may preferentially use one of the hydrogen stable isotopes (^2H and ^1H or D and H). This results in different fractions of hydrogen stable isotopes in synthesized biomass versus water in the growth medium. Isotope preference partially stems from the physiology of the microorganisms. Autotrophic microorganisms, for example, preferentially incorporate ^1H during lipid biosynthesis, possibly resulting from reactions involving NAD(P)H that forms isotopically light acetyl-CoA. Stable isotope fractionation is traditionally measured using isotope ratio mass spectrometry. However, it is challenging to measure and identify unknown molecules in a complex sample using this technique. Here we present a new approach for measuring hydrogen fractionation in complex samples of proteins and lipids from an acidophilic microbial community in acid mine drainage.

The analyses targeted coexisting bacteria and archaea grown in a 4 atom% ^2H-enriched medium. To measure average hydrogen fractionation in amino acids, the community proteome was digested using trypsin and measured using 2-dimension liquid-chromatography coupled with high-resolution tandem mass spectrometry analysis on an Orbitrap Velos. The Sipros algorithm (code.google.com/p/sipro) was used to search each MS^2 spectrum against all peptide sequences in the AMD protein database at every ^2H atom% level in 1% precision. The ^2H enrichment and sequence of 4,477 unique peptides from 36,090 spectra were identified. Further, to more precisely quantify isotopic enrichment, a MATLAB program was developed to find the optimum ^2H values which best fit the isotopic distributions of identified peptides in MS and MS^2 spectra. The results indicated that the microbial community has an average ^2H depletion of -360 ‰ relative to water in the growth medium for proteins. We applied a similar technique to the analysis of lipids identified using LCMS, and the lipids were found to be isotopically light by -260 ‰ relative to water in the growth medium.

This difference between -260 ‰ ^2H fractionation in lipids and -360 ‰ in proteins suggests that additional factors, that do not involve NAD(P)H, are also contributing to $^2H/^1H$ fractionation. Further, autotrophic bacteria sharing 94% 16S rRNA gene sequence identity displayed statistically significant differences in protein hydrogen isotope fractionation. This suggests these microbes have different metabolic traits determined by their distinct ecological niches. In addition, it was found that heterotrophic, archaeal members of the community had isotopically light protein and were significantly different from coexisting bacteria. Potentially, this could be attributed to metabolite transfer from autotrophs and unknown aspects of fractionation associated with iron reduction. Differential fractionation of hydrogen stable isotopes into metabolites and proteins may reveal trophic levels of members of microbial communities. The approach developed here provided insights into the metabolic characteristics of organisms in natural communities, and may be applied to analyze other systems.

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

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Project Goals: This research program explores fundamental aspects of carbon cycling in soil microbial communities. Major goals are to develop and validate methods for pyrosequencing-enabled stable isotope probing (SIP) and to use this approach to dissect the microbial food web in soil. 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. With this approach we will examine connections between microbial community composition and soil carbon cycle dynamics. Specific objectives include 1) determine whether carbon input parameters (composition, quantity, timing of carbon additions) alter the route of carbon through the soil community, 2) determine whether these shifts interact with respect to microbial community structure, and 3) evaluate whether microbial community structure is functionally equivalent across edaphically similar soils that differ in management history.

The terrestrial biosphere contains a large fraction of global C and nearly 70% of the organic C in these systems is found in soils. Much of the organic C in soils 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. It is important to examine the internal dynamics of soil microbial communities, and the manner in which they influence community function, in order to understand the how the terrestrial C-cycle responds to environmental change.

While strides have been made 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. The pyrosequencing enabled stable isotope probing approach that we are developing will allow for pulse chase style experiments that allow ^{13}C-isotopes to be tracked through the soil community over time. The approach will involve the application of synthetic biomass containing a mix of carbon sources designed to approximate the plant biomass. The use of synthetic biomass allows substitution of ^{13}C-labeled substrates into the mixture to track the manner in which different types of C (ie: polymers and sugars) are metabolized by different components of the community.

Initial experiments have explored 1) what are the dynamics of degradation for ^{13}C-cellulose plant simulant and how does the ^{13}C move through community nucleic acids over prolonged incubation, 2) how does the community respond to the addition of ^{13}C-cellulose plant simulant relative to the addition of only ^{13}C-cellulose, and 3) how does the ^{13}C assimilation into microbial nucleic acids from ^{13}C-xylose plant simulant vary from that of ^{13}C-cellulose plant simulant when all aspects of the experiment are identical except for the isotopic label. $^{12}CO_2$ and $^{13}CO_2$ generated from microbial respiration is determined over a month long incubation and soils are sampled destructively over time. Samples from different times are subject to DNA and RNA stable isotope probing and 454 pyrosequencing of gradient fractions. These data are used to determine the buoyant density profile for individual OTUs in ^{13}C treatments relative to unlabeled controls and to observe how the degree of ^{13}C-label incorporation by individual OTUs changes over time.

Future experiments will explore how food web dynamics differ between edaphically similar soils that differ in land management practice. To prepare for these experiments we have identified a series of suitable sites that represent a gradient of management impact resulting in accumulation of soil organic matter. The sites include an intensively managed corn field, fields that were removed from intensive management for 10 or 20 years and subsequently managed for organic grain crops, and long term pasture. Initial characterization of the soil bacterial and fungal communities by 454 pyrosequencing is currently underway in order to determine the community composition in these sites and develop protocols for pyrosequencing enabled ^{13}C-SIP. Using these data, OTUs identified by ^{13}C-SIP can then me mapped back to the landscape in space and time to evaluate their distribution and importance in native soil systems.

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.

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From Community Structure to Function: Metagenomics-Enabled Predictive Understanding of Microbial Communities to Climate Warming at the Temperate Grassland Ecosystems in Oklahoma

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Project Goals: Determining the response, adaptation and feedback mechanism of biological communities to climate change is critical to project future states of the earth and climate systems, but poorly understood in microbial com-

munities. 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 response of microbial community structure, function and activity 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 response to climate change. We have carried out our studies at two contrasting long-term experimental facilities, the temperate grassland ecosystems in OK (this poster) and tundra ecosystems in Alaska (Poster led by Schuur et al).

Feedback responses of microbial communities to climate warming. We have used integrated metagenomic technologies to analyze the responses of microbial communities in a long-term (10 years) experimental warming grassland ecosystem in Oklahoma. Our results showed that microorganisms play crucial roles in regulating soil carbon (C) dynamics through three primary feedback mechanisms: (i) shifting microbial community composition, which most likely led to the reduced temperature sensitivity of heterotrophic soil respiration, (ii) differentially stimulating genes for degrading labile but not recalcitrant C so as to maintain long term soil C stability and storage, and (iii) enhancing nutrient cycling processes to promote plant nutrient use efficiency and hence plant growth. Elucidating microbially mediated feedbacks is fundamental to understanding ecosystem responses to climate warming and provides a mechanistic basis for C-climate modeling.

Responses of microbial communities to clipping within the context of climate warming. We have also examined the responses of microbial communities to clipping within the context of climate warming using the GeoChip-based metagenomics technology. Various statistical analyses showed that clipping had significant impacts on microbial community structure, and altered the directions of the warming effects. The results clearly indicated that clipping substantially mediated warming effect on soil microbial community. The stimulated genes in relative more recalcitrant C decomposition under warming with clipping may have important implications for the stability of soil C storage. The diminished warming effect by clipping for N genes may affect plant production and further influence soil C cycling.

Metagenomic sequencing analysis of microbial communities under long-term warming. We employed the Illumina Hi-Seq2000 platform (2 X 100 paired end) to obtain shotgun metagenomes from 12 replicate samples, with 6 each for warming and control. Each sample yielded 10-15 Gb of sequence data with >60 Gb per community. Using an assembly-free bioinformatic pipeline, our results showed that more than 90% of the genes and organisms did not differ in abundance between the two communities while a higher abundance of genes related to sporulation was observed under warming. Significant differences were observed among the top four most abundant phyla (Proteobacteria, Acidobacteria, Planctomycetes, and Bacteriodetes) between warming and control. Interestingly, the microbial populations of these phyla from the warmed samples showed significant increase in G+C% content. Furthermore, several metabolic pathways were significantly increased under warming, including pathways directly related to the emission of greenhouse gases (e.g., CH_4, NO, and CO_2), nitrogen cycling (e.g., fixation, nitrification, nitrate/nitrite reduction), and organic carbon utilization (e.g., mixed acid fermentation, mannitol utilization).

Belowground net primary productivity under warming and clipping. The dynamics of belowground net primary productivity (BNPP) is of fundamental importance in understanding carbon (C) allocation and storage in grasslands. Thus we have examined the changes of BNPP under warming and clipping. Warming increased BNPP by 42-67% with a significant increase observed in wet years. Clipping also had significant positive impacts on BNPP. Overall, f_{BNPP}, the fraction of BNPP to NPP, increased under both warming and clipping treatments, more in dry years. Water availability (either precipitation or soil moisture) was the most limiting factor for both BNPP and f_{BNPP}. It strongly dominated the interannual variability of NPP and f_{BNPP} and their responses to warming and clipping. Our results suggested that water availability might regulate tallgrass prairie's responses to warming and land use change, which may eventually influence the global C cycling.

The stability of organic carbon in deep soils. The majority of C in deep soils is recalcitrant and old with turnover times of hundreds to thousands of years, whose fate is critical to project future climate warming. Therefore, we determined the warming effect on old, recalcitrant organic C decomposition by combining long-term (9 yr) in-situ field and short-term (9 wk) laboratory incubations. Our results showed that warming of approximately 2˚C significantly facilitated the loss of C with several thousand years old in the deep soil layer. Coupled stable isotope probing and meta-genomic analysis indicated that warming-induced old C decomposition was closely related to changes in the functional structure of microbial communities. Our findings suggest that warming may significantly reduce the size of the vast pool of old C in global soils and thus reinforce the positive feedback between the C cycle and climate.

Development of amplicon-sequencing approaches for uncovering functional gene diversity. Eco-functional genes involved in nitrogen and carbon cycling were targeted for pyrosequencing in order to resolve changes in microbial functional community structure underlying process changes. For nitrogen cycling, the functional genes nifH (N fixation), nirK (denitrification), amoA (nitrification), and Archaeal-amoA (nitrification) were initially targeted. Bacterial lac-

cases, which catalyze the oxidation of various substituted phenolic groups were also sequenced. The FunGene Pipeline and Repository has been enhanced to provide the full line of capability of processing/analyzing sequences of these eco-functional genes. In addition, primers are currently being developed for the nitrogen cycle (nosZ and nirS) and the carbon cycle (pmoA, ligE, and fungal peroxidase).

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From Community Structure to Functions: Metagenomics-Enabled Predictive Understanding of Microbial Communities to Climate Warming in Alaskan Tundra

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Determining the responses, adaptations and feedback mechanisms of biological communities to climate change is critical to project future states of the earth and climate systems, but poorly understood for microbial communities. We have carried out our studies at two contrasting long-term experimental facilities, a tundra ecosystem in Alaska (this poster) and a grassland ecosystem in Oklahoma (Poster led by Zhou et al).

Effects of experimental and natural warming of deep soil and permafrost on ecosystem carbon balance in Alaskan tundra. Approximately 1670 Pg (billion tons) of soil carbon are stored in the northern circumpolar permafrost zone, more than twice as much carbon than currently contained in the atmosphere. Permafrost thaw, and the microbial decomposition of previously frozen organic carbon, is considered one of the most likely positive feedbacks from terrestrial ecosystems to the atmosphere in a warmer world. Here we report results from two experimental systems that examine this question: **1)** a natural gradient of permafrost thaw where *minimal*, *moderate*, and *extensive* permafrost degradation has been observed over a decadal time scale, and **2)** a new ecosystem warming manipulation—the Carbon in Permafrost Experimental Heating Research (CiPEHR) project—where we increased air and soil temperature, and degraded the surface permafrost. Within these experiments, net ecosystem C exchange and the radiocarbon age of ecosystem respiration were measured to determine the influence of old C loss on ecosystem C balance. By partitioning respiration sources across the thaw gradient, we determined that areas that thawed over the past 15 years had 75% more annual losses of old C compared to minimally thawed areas, but had overall net ecosystem C uptake as increased plant growth offset these losses. In contrast, sites that thawed decades earlier lost an additional 25% more old C annually, which contributed to overall net ecosystem C release despite increased plant growth. These findings were mirrored by the warming experiment where increased plant uptake appears to compensate for microbial release of carbon, at least in the three years of warming that we have observed. Together, these data document significant losses of soil C with permafrost thaw that, over decadal time scales, overwhelms increased plant C uptake at rates that could make permafrost a large biospheric C source in a warmer world, similar in magnitude to current C fluxes from land use change.

Patterns of permafrost thaw influence on tundra microbial communities. Microbial communities from the permafrost thaw gradient were analyzed by Geochip 4.0. Six cores were taken from each of the minimal, moderate and extensive permafrost thaw sites; each core contained 6-7 sections by depth (2-3 organic and 3-4 mineral fractions). Various statistical analyses (detrended correspondence analysis, dissimilarity tests and multiple regression tree) all showed that the thaw gradient (sites) was more important than depth in influencing soil microbial community structure, though both had significant impacts. The Simpson diversity index was significantly different across the sites, with the highest value at minimal thawing site and lowest at moderate thawing site. For genes involved carbon degradation, there was no consistent trend across the thaw gradient, though significant differences were observed in some genes. Both methane production and oxidation genes were significantly affected by permafrost thaw, with highest abundances at minimal site and lowest at moderate site. For nitrogen cycling, the abundance of denitrification genes was generally higher at minimal site with only one exception of *narG*, likely due to a more anaerobic condition at minimal site. The genes in assimilatory nitrogen reduction were generally higher at the moderate thaw site, though *nasA* did not differ among three sites, indicating high plant and microbes activity at the moderate site.

Laboratory determination of microbial temperature sensitivity. To determine the temperature sensitivity of microbial respiration (Q_{10}) in soils with different mixtures of labile versus recalcitrant carbon, we are conducting soil incubations of soils from different depths at two constant temperatures (15°C and 25°C). These soils came from a warming experiment in a tundra ecosystem in Alaska and a warming experiment in a grassland in Oklahoma. Three different layers were incubated from Alaska: two surface soils (0 – 15 cm and 15 – 25 cm with high carbon content) and a horizon deeper (>50 cm) within the surface permafrost. From the Oklahoma site, we used soils from control and warmed plots combined with a root exclusion treatment that had kept new root inputs out over the 8 years of the experiment. Instantaneous Q_{10} was measured by exposing soils to 6 different temperatures ranging from 5 to 30°C while measuring CO_2 flux over this range. For Alaskan soils,

warmed soils from 0 – 15 cm layer had a slightly higher, but not significant, average Q_{10} than the control plots. No other differences in average Q_{10} were detected for different soil layers or treatments. However, carbon fluxes in the first 15 cm were 10 and 35 times higher than fluxes at 15 – 25cm or from the permafrost soil, respectively.

Modeling integration and development. A new model was developed based on the soil C dynamics model developed at the early stage of the project. The main objectives of the model are to calculate Q_{10} using a data-model fusion technique, and to evaluate dynamics of Q_{10} with recalcitrance of soil organic C (SOC). Q_{10} and turnover rates for different C pools (labile, slow and passive pools) and fraction of each C pool can be optimized using Bayesian probability inversion and a Markov chain Monte Carlo (MCMC) technique. This approach generates posterior probability density functions of model parameters. A previously published dataset using three incubation temperatures (15, 25 and 35 ˚C) and an incubation period of 588 days was used to test the model. Preliminary results show that Q_{10} values for labile and slow C pools can be constrained very well at temperature regimes of both 15-25 ˚C and 25-35 ˚C, but only the Q_{10} value at 25-35 ˚C can be well constrained for the passive pool. Based on the dataset, Q_{10} values increase with recalcitrance of SOC. This preliminary result also indicates that longer incubation studies are needed in order to assess the temperature sensitivity of slower turnover pools, especially at low temperature regimes. Further work will push towards integrating microbial community into an ecosystem modeling framework.

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Microbial Communities Generating Greenhouse Gases in Thawing Permafrost

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http://www.ecogenomic.org/melting-permafrost

Project Goals: The IsoGenie project ("Genes, isotopes, and ecosystem biogeochemistry: dissecting methane flux at the leading edge of global climate change") focuses on integrating microbial and geochemical studies to improve our understanding of carbon cycling in a subarctic wetland where climate change induced permafrost thawing is transforming carbon sinks into sources. Our research goals are: (1) to discover relationships between microbial community composition and metabolism to ecosystem carbon cycling; (2) to learn how these relationships are affected by shifting environmental variables, and (3) apply this knowledge to better understand and predict changing carbon budgets in subarctic ecosystems experiencing substantial climate change. One of the aims of this research was to characterize microbial communities along a degradation gradient using 16S rRNA gene amplicons, meta-genomics, -transcriptomics and -proteomics.

High northern latitudes are at the leading edge of global climate change with the effects of warming already evident in degrading permafrost. Increased thawing of permafrost, a significant global carbon sink, releases previously sequestered labile carbon. Thawing initiates a transition from intact permafrost (Palsa hummock) through an intermediate thaw state (collapsed Palsa; hollow) to fully degraded inundated end state (fen). The transition to a fen state has been associated with dramatic increases in biogenic methane production and other greenhouse gases (GHGs). GHGs produced by peat microorganisms are 10-fold and 100-fold higher in the hollow and fen samples respectively, as compared to hummock samples (100 year CO_2 equivalents). The exact combination of *in situ* ecological conditions triggering GHG efflux is unknown, however the rising water table associated with thawing permafrost is believed to lead to anoxic conditions favourable to microbial methane production.

Here, microbial communities along a degradation gradient in Stordalen Mire (Abisko National Park, Sweden) were characterized using 16S rRNA gene amplicon pyrosequencing. Sampling sites and depths were selected based on geochemical data, including GHG flux. Changes in microbial community structure along the degradation gradient were substantial and occurred at the high water mark (middle sampling depth) in the hollow sites (Figure 1). Microbial communities below the waterline in hollow peats (positive methane flux) were relatively low complexity and were dominated by a single archaeal species within the order *Methanomicrobiales* and two bacterial species of the order *Acidobacteriales*. Microbial community richness and composition in the hummock samples (low to negative GHG flux) approached that of non-permafrost soils. *Acidobacteria* were dominant across all sample sites and depths, comprising between 20% and 42% of the OTUs observed. The ubiquity of *Acidobacteria* across all samples is consistent with members of this phylum being adapted to low energy, low nutrient, highly acidic and water stressed environments.

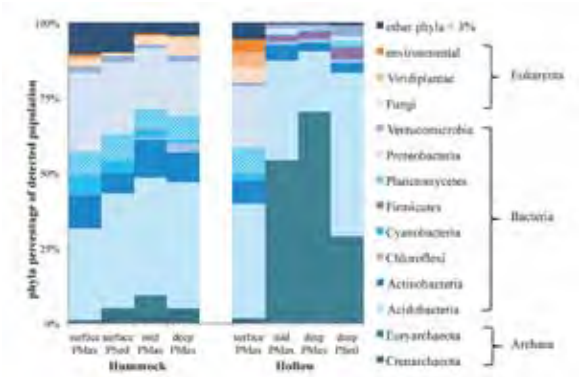

Figure 1. Microbial community composition of active layer peat in (low GHG) Hummock and (mid GHG) Hollow. Two extraction methods, PMax (PowerMax kit, MoBio) and PSoil (PowerSoil Total RNA/DNA co-elution kit, MoBio), were tested in this study.

To further elucidate these emerging patterns in microbial community structure and diversity, samples collected during 2010 and 2011 (June, July, August, and October) are currently being sequenced. The third year of sample collection is scheduled for the 2012. Metagenomic sequencing of select samples are being undertaken to facilitate the recovery of the dominant *Methanomicrobiales* and *Acidobacteriales* genomes. In addition, microbial community profiles generated from all samples, together with GHG data will be used to select samples for combined metagenome, metatransciptome and metaproteome analysis. Integration of meta'omic data with the isotopic geochemistry will clarify ecosystem variables related to GHG production. Insights gained regarding the ecological triggers of GHG emission over the degradation gradient will inform emission projections, filling a gap in current climate modelling scenarios.

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

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https://www.princeton.edu/southafrica/permafrost-project/

Project Goals:

1. **Perform ~2 year long, thawing experiments under water saturated and water under saturated conditions on well-characterized, intact cores of Arctic active-layer and permafrost from a proposed reference ecosystem site where CO_2 and CH_4 fluxes, temperatures, humidity, soil moisture, nutrients, microbial diversity and activities, as well as C isotopic analyses, are currently being measured in the field.**
2. **Perform phylogenetic, metagenomic, transcriptomic and proteomic analyses of these cores prior to and during the experiments.**
3. **Characterize the composition of the solid and dissolved organic matter, the inorganic geochemistry, the vertical flux of volatile organic acids, O_2, H_2, CO_2 and CH_4 and the isotopic systematics of CO_2 and CH_4 during the experiments.**
4. **Compare the results of these experiments with field measurements, and based upon both data sets construct a 1D biogeochemical reaction/transport model that predicts the CO_2 and CH_4 release into the atmosphere as permafrost thaws.**
5. **Development of a high sensitivity ^{14}C RNA isotope microarray and a CRDS for C and H isotopic analyses of atmospheric CH_4.**

Forty 1 meter long cores were collected from a 7 meter diameter polygon located near the McGill Arctic Research Station during April 2011 where the depth to permafrost is 70 cm. Total community genomic DNA (cgDNA) was isolated from the 2011 samples using four different commercially available kits to determine the best DNA extraction protocol for metagenomics. The v1 region of the 16S rRNA gene was amplified directly from cgDNA or re-amplified from 16S rRNA amplicons. Taxonomic analysis revealed similarity in bacterial communities derived from different extraction kits. The significant variation in the bacterial community structure as a function of depth in the active layer previously reported in the 2010 core samples does not appear to be an artifact of extraction protocols. Gemmatimonadetes is the dominant bacterial phylum in the uppermost active layer, whereas Firmicutes and Actinobacteria are the dominant phyla near the permafrost table and within the permafrost consistent with geochemical evidence of increasing anaerobicity with depth. The organic matter from these cores ranged from 6% total organic C at the surface to 1% at 10 cm depth with C/N = 13-16. NMR and FT-ICR-MS reveal the composition of this organic matter is significantly different from that reported in boreal soils and peat deposits (1). ^{14}C analyses are underway.

Microcosms of subsamples from the cores amended with select nutritional amendments revealed significant CO_2 production and CH_4 consumption and the presence of stress responsive proteins (e.g. DnaK, GroEL) and proteins essen-

tial for energy production and survival under carbon starvation (e.g. F0F1 ATP synthase, acyl-CoA dehydrogenase). Proteins from the genera *Bradyrhizobium, Sphingomonas, Lysinibacillus* and *Methylophilaceae* were detected, and these bacteria were also identified by 454 pyrosequencing on the same samples. Metaproteomics of the pristine core samples, however, yielded relatively few protein identifications suggesting a relative lack of microbial activity and limited microbial biomass. Metaproteome efforts have been focused on evaluation and optimization of experimental protocols to efficiently extract proteins from these low biomass cores. Lipidomic analyses of the core samples are underway.

Continuous *in situ* gas flux analyses of CO_2 and CH_4 fluxes from permafrost and ice-wedge active layer (AL) soils were conducted using CRDS's during July 2011. Polygon AL soil flux showed a net outward CO_2 flux (175 to 3,155 mg/m^2-day) and consumption of atmospheric CH_4 (-1.2 mg/m^2/day). Gas flux from the ice-wedge AL surface was in a similar range as the polygon, having slightly higher maximal CO_2 flux (3670 mg/m^2/day) and net CH_4 consumption (-2.0 mg/m^2/day). The $\delta^{13}C$ of the CO_2 efflux from the surface were consistent with microbial activity, ranging from -10.6 ‰ to -15.5 ‰ for the polygon and ice-wedge soils, respectively. In both AL soils, gas flux fluctuates diurnally. The CO_2 out flux was found to be anti-correlated to surface temperature (R = -0.67), whereas the CH_4 in flux was found to be correlated to surface temperature (R = 0.76). Using a vertical gas probe, the CO_2 concentrations increased with depth and corresponded to a CO_2 flux 19 to 41 mg/h in the polygon AL soils *vs*. 18 to 54 mg/h in the ice-wedge soils. Through the same profile, the CH_4 concentration decreased from 0.59 ppmv to <0.1 ppmv within 30 cm of the surface in the ice wedge and from 0.62 to 0.18 ppmv at the base of the polygon AL. Below the surface, the $\delta^{13}C$ of CO_2 was more ^{13}C depleted than at the AL surface, reaching -18.9 ‰ and -21.3‰ at the base of the AL in the polygon and ice wedge soils, respectively. These data suggest that both polygonal and ice-wedge AL soils contribute net efflux of CO_2 and consumption of CH_4 during the summer season and that the more saturated ice-wedge soils may have a more favorable environment for methanogenic bacterial activity with depth with greater stratification in fluxes of gases through the profile. Defining these relationships is critical for accurately modeling the extent and rate of + or - feedback in global climate models.

Thawing experiments on the 2011 cores have begun with an initial active layer thaw over a one-month period to simulate Arctic spring thaw. Previous reports of dramatic shifts in the phylogenetic and functional gene structure of active layer and permafrost after only 2 weeks of warming (2, 3) motivated us to relate this sudden change to initial C and N fluxes in our thawing experiments. Once the community and fluxes have stabilized we will initiate permafrost thawing.

All components for the Scintillator Layered Imaging Microscope for Ecological Research (SLIMER) have been assembled and the spatial accuracy and resolution validated with an α source. The next step will be to increase S/N for low energy β sources. The sensitivity and reproducibility of the PU-CRDS has been significantly increased to that it is now capable of measuring the $\delta^{13}C$ of 2 ppmv CH_4 with a precision of ±2‰. Modifications are underway to make the PU-CRDS robust under field conditions for transport to the Arctic.

Two posters given at the Fall Meeting of AGU and data shared with the RCN on Permafrost Carbon stimulated much discussion and new collaborations.

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IsoGenie: Microbes, Ecosystem Biogeochemistry, and Climate Change: Dissecting Methane Flux in a Thawing Permafrost Peatland in Northern Sweden

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Project Goals: As permafrost thaws, increasing CH_4 emissions from northern wetlands are likely to cause positive feedback to atmospheric warming. The IsoGenie project seeks to discover how functional relations between biogeochemical processes, particularly methane production, and the underlying microbial community dynamics are affected by climate change-induced permafrost thaw. This goal is enabled by recent transformative methodological advances in both ecosystem science and molecular biology that allow high-volume tracking of isotopic composition

of carbon gases, as well as the genetic potential and gene expression in the microbial communities which produce those gases.

To achieve this goal, we measured concentration profiles and surface fluxes of carbon gases (CH_4 and CO_2), along with their isotopic compositions, across a gradient in permafrost thaw from palsa (with underlying permanently frozen peat), to recently collapsed and flooded palsa dominated by *Sphagnum spp.* (intermediate thaw), to fully inundated sites dominated by *Eriophorum angustifolium* (fully thawed). At the same time, we sampled soil microbial communities in these sites to characterize composition, metabolic potential and gene expression. Both isotopic composition of carbon gases, and meta-genome/-transcriptome/-proteome data can be used to identify active metabolic pathways of methane production, and we hypothesized that the two methods would give consistent results. This poster focuses on biogeochemical and isotopic results, and a companion poster focuses on microbial community results (Mondav et al, "Microbial Communities Generating Greenhouse Gases in Thawing Permafrost").

Along this environmental gradient, from permafrost to fully thawed sites, the lability of the peat increases significantly as determined in incubations of peat material and monitoring of methane and carbon dioxide production rates. Coincident with this trend is an increase in methane surface fluxes and an increase in $_{13}C$ isotopic composition of methane (from ~ -80‰ in intermediate thaw sites to ~-65‰ in fully thawed sites) suggesting a shift in methane metabolism towards acetate fermentation and away from CO_2 reduction. We also observed significant temporal variation during the 2011 growing season in the carbon isotopic composition of methane fluxes, indicative of shifts in methanogenic and methanotrophic activity. These observations will be used to test a biogeochemical model (the DNDC model) of methane production that includes alternative production pathways under different redox conditions.

Together, these initial results suggest that thaw-induced changes in hydrology and plant community composition increase peat lability, stimulating acetate fermentation and yielding increased methane emissions. We conclude that the biological controls on metabolic pathways of methanogenesis, though poorly represented in most ecosystem models, may nonetheless be important, in interaction with permafrost thaw dynamics, in determining future CH_4 emissions under changing climate.

The IsoGenie Project is supported by the Office of Biological and Environmental Research in the U.S. DOE Office of Science, Project Grant DE-SC0004632.

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The Impact of Precipitation and Nitrogen Deposition Changes on the Microbial Community and Greenhouse Gas Cycles of a Southern California Grassland

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Project Goals: The overall project goal is to describe the impact of changes in precipitation regimes and nitrogen deposition levels, predicted by regional and global models for Southern California, on grassland leaf litter decomposition and associated microbial community. In this presentation we describe a piece of the project, the goals of which are to investigate the impact of these treatments on the structure and function surface soil microbial composition, with specific focus on greenhouse gas fluxes. Further, this project seeks to strengthen the connection between microbial community structure and function, and potentially enhance our understanding of the biospheric role in greenhouse gas cycles.

Fluctuations in greenhouse gas (GHG) concentrations in the atmosphere can lead to profound climatic and environmental changes. Likewise, environmental changes can influence variation in soil consumption and release of key greenhouse gases. Carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) are currently increasing in the atmosphere at high rates due to human activities. Accurately understanding natural and anthropogenic roles in ever-changing greenhouse gas cycles is necessary for a more complete understanding of biosphere-atmosphere interactions. It is only with this understanding that decisions can be made at local, national and international levels to mitigate potentially harmful climatic changes. This project examines the biological mechanisms behind the response and resilience of soil microbial communities to change. This is examined in both community composition as well as function, in terms of production and consumption of CH_4, CO_2 and N_2O, thereby enhancing our understanding of the biospheric role in greenhouse gas cycles.

Southern California provides an excellent natural laboratory for investigating the relationships between climate, plant composition, net primary production, soil microbial ecology, and ecosystem function. California's coastal climate is Mediterranean, with a mild wet winter, a warm or hot dry summer, and extreme inter-annual precipitation variation associated with ocean currents. Soil trace gas fluxes may be variable in this ecosystem due to this high climatic variability on seasonal and inter-annual scales, with intense pulses of precipitation and extensive drought. This region is likely to experience profound environmental change in the next 50 years, due to high densities of fossil fuel combustion, NOx emission, and nitrogen deposition (Fenn et al. 2003). Total nitrogen deposition is 2.5 to 4.0 gN $m^{-2}yr^{-1}$ in many regions of the San Gabriel and San Bernardino Mountains,

and increasing (Fenn et al. 2003). Global Circulation Model (GCM) and Regional Climate Model (RCM) runs almost uniformly project significant warming in California (IPCC 2007), which can lead to increased drought conditions. Projections of future precipitation are less certain, with many GCMs projecting increased winter precipitation, and some models predicting a dramatic change (IPCC 2007). Hence the study of the impacts of increased N deposition within the context of both increased and decreased precipitation is relevant to understanding the response of this system to projected changes in this environment.

This experiment, performed at Loma Ridge in the Irvine Ranch Conservancy, manipulates N and rainfall in a grassland community in a factorial combination of replicated plots. Nitrogen is added in fall and mid-spring as slow-release granular ammonium nitrate (osmocote) pellets at 6 $gN\ m^{-2}\ yr^{-1}$. This level of N addition in treatment plots is comparable to the higher levels of nitrogen deposition in the region (Fenn et al. 2003) and is similar to that used in other studies. The 50% precipitation increase and decrease treatments, which have been in place since 2007, are achieved by manipulating both the average size and number of storms (Hanson 2000), to simulate anticipated precipitation shifts. The experiment uses rainout shelters with clear polyethylene retractable roofs to remove ~50% of the annual precipitation from the low water plots by selective closure during a subset of the winter storms. The water draining off the shelters is collected with metal gutters and PVC pipe, stored and applied to increased precipitation plots.

Gas flux rates of N_2O, CH_4 and CO_2 have been measured across the start of the rainy season of 2011-2012. All gases are measured monthly using static vented chambers and gas chromatography. In addition, CO_2 is measured constantly using automated flow-through chambers. The initial data show some striking trends. As predicted, decreased rainfall is associated with lower ecosystem respiration across plots, but increased rainfall was also shown to coincide with lower respiration rates than controls. Increased N deposition was found to correlate with increase N_2O release, as expected. No trends have yet been observed in CH_4 flux, but both consumption and release have been observed in the site.

Concurrent with gas flux measurements, *in situ* soil moisture and temperature data are being collected. Soil cores are removed from each plot when gas flux measurements are performed. Cores are frozen immediately and returned to the lab where DNA and/or RNA are extracted. Pyrosequencing (454) analysis of the microbial community has been performed on a subset of samples using the same 16S primers and bioinformatics pipeline currently employed to examine litter microbial communities. The initial data from this subset of samples will be presented.

This research has only just started, with several molecular techniques to be employed later on on samples across time and treatments. Changes in the relative abundances the functional genes *pmoA* (for methanotrophy), *nirS*, *nirK* and *nosZ* (for denitrifiction), and *amoA* (for nitrifiction), along with associated mRNA activity, will be measured by qPCR and rt-qPCR, respectively. Also, samples of pooled replicate plots are being analyzed by a full meta-genomic Illumina sequencing protocol, with data forthcoming.

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Reconciling Phylogeny and Function During Plant Litter Decomposition by High-Throughput Functional Metagenomics

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Project Goals: The overall aim of this project is to link functional traits that influence carbon cycling with individual microbial taxa in order to build predictive trait-based models of ecosystem responses to global change. The functional traits are connected to microbial taxa with DNA sequencing, novel functional assays, experimental manipulations and mathematical models that are used to investigate the distribution of extra-cellular enzyme genes and functions involved in plant litter decomposition across taxonomic groups of microbes. The resulting trait-based frameworks are then used to model how altered water and nitrogen availability as well as plant species composition impact plant litter decomposition rates in annual grassland.

This presentation focuses on one aspect of the discovery and identification of functional traits involved in plant litter decomposition, the functional screening of metagenomic libraries. A total of twenty-four metagenomic fosmid libraries have been constructed from high molecular weight DNA isolated from decomposing plant litter collected over one year period from a global change experiment undergoing

rainfall and nitrogen manipulations. The libraries encompass over 20 Gb of metagenomic DNA and cover a range of microbial taxonomic groups including Actinobacteria, Bacteroidetes and different classes of Proteobacteria as well as Fungi.

The 24 libraries are currently being screened for activities involved in cellulose, hemicellulose, chitin, lignin and starch degradation as well as peptide breakdown and mineralization of organic phosphate using novel functional assays. These automated high throughput assays are based on colorimetric and fluorescent substrates and have been optimized to allow multiplexed screening of both endolytic and exolytic hydrolysis activities required during different stages of decomposition. Several cellulose, hemicellulose, chitin and starch degrading as well as phosphatase and proteinase producing clones have been identified.

Clones expressing the targeted activities are sequenced with the Illumina sequencing platform and the identified hydrolytic genes are characterized for enzyme kinetics and given phylogenetic context for incorporation into trait-based microbial decomposition models.

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Atmospheric Nitrogen Deposition and Microbial Mechanisms Enhancing Soil Carbon Storage

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http://www.webpages.uidaho.edu/nitrogen-gradient/Default.htm

Project Goals: Since the industrial revolution, the amount of reactive nitrogen (N) deposited from the atmosphere has increased across large areas of the Northern Hemisphere (e.g., from 50-100 to 1500-2000 mg N m^{-2} y^{-1}). Agricultural and industrial activities are largely responsible for this increase, which has the potential to disrupt many of the fundamental ways in which ecosystems function—ranging from the growth and vigor of trees to the microbial mechanisms mediating the cycling and storage of carbon (C) in soil. Our long-term experiment was established to understand the mechanisms by which atmospheric N deposition could alter the ecosystem processes in sugar maple dominated forests of the Great Lakes region. Exposure to 18 years of experimental N deposition, at a rate expected mid-century, has slowed the decay of plant litter, which has increased soil C storage as well as export of phenolic DOC; this biogeochemical response has occurred in the absence of a change in litter biochemistry or production. Presently, we are testing the hypothesis that the slowing of litter decay and the accumulation of soil organic matter in response to anthropogenic N deposition is governed by the environmental regulation of microbial gene expression. We predict that chronic experimental N deposition has down regulated the transcription of fungal genes encoding lignocellulolytic enzymes, thereby slowing litter decay and opening a niche for other, less effective lignocellulolytic soil microorganisms to occupy. To understand whether these hypothesized mechanisms are at work in our experiment, we have employed molecular genetic approaches to examine the community and physiological responses of saprotrophic fungi and *Actinobacteria* residing in forest floor and surface soil.

Figure 1. Distribution of replicate sites spanning the north-south range of northern hardwood forests in the Upper Great Lakes region.

Experimental Design: Since 1994, we have experimentally simulated increased atmospheric N deposition in replicate stands of a northern hardwood forest ecosystem stretching across a 500-km climatic gradient (Fig. 1). Our study sites deliberately encompass the north-south geographic range of the of the sugar maple-dominated (*Acer saccharum* Marsh.) northern hardwood forest in the Great Lakes region of North America, enabling us to generalize our experimental results across this geographic region. These sites are floristically and edaphically matched (>80% sugar maple on Typic Haplorthods), but they differ in climate along the north-south latitudinal gradient (Fig. 1). The study sites also span an atmospheric N deposition gradient, over which NO_3^--N composes ~60% of wet-plus-dry deposition. There are six 30-m x 30-m plots at each study site; every plot is surrounded on all sides by a 10-m wide treated buffer. Three plots at each site receive ambient atmospheric N deposition. The other three plots at each site receive ambient N deposition plus 3 g NO_3^--N m^{-2} y^{-1}, a rate approaching that expected by 2050 across large portions of North America and other regions of the Earth. The additional N is delivered over the growing season in six equal applications (0.5 g N m^{-2} $month^{-1}$) of solid $NaNO_3$ pellets, which are broadcast over the forest floor.

Hypothesis Testing: Basidiomycete and ascomycete fungi are the primary agents of plant cell wall decay, and a change in their composition or decline in activity could slow litter decay under experimental N deposition. To characterize the actively metabolizing community of these organisms, we used cDNA clone libraries constructed from 28S fungal rRNA. The active basidiomycete communities under ambient and experimental atmospheric N deposition differed significantly in terms of membership as well as their dispersion across a phylogenetic tree. Furthermore, suggestive, albeit nonsignificant, differences in the fraction of unique phylogenetic branch length (i.e., the UniFrac metric) between ambient and experimental atmospheric N deposition were observed for forest floor basidiomycetes. In contrast, the active ascomycete communities under ambient and experimental atmospheric N deposition did not exhibit significant differences in these same metrics. Collectively, our observations indicate that experimental N deposition has altered the composition of litter decaying fungi and that these changes have ecosystem-level implications for the cycling and storage of C in forest ecosystems.

Actinobacteria also are one of the few groups of saprotrophic microorganisms which oxidatively depolymerize lignin, producing substantial soluble polyphenolics during the process. These organisms could plausibly become more important agents of lignin decay, if atmospheric N deposition suppresses the activity or alters the composition of lignolytic basidiomycete and ascomycete fungi. To test this idea, we quantified actinobacterial abundance and community composition under ambient and experimental N deposition. Actinobacterial abundance was assessed using quantitative PCR of 16S rRNA and community composition was evaluated using clone libraries and phylogenetic community analyses (i.e., Libshuff and Unifrac). Contrary to our expectation, experimental atmospheric N deposition had no effect on actinobacterial abundance in the forest floor (~10^{10} gene copies/g); however, it significantly decreased actinobacterial abundance by 47% in surface mineral soil. Our analyses revealed experimental N deposition further elicited a significant membership change in forest floor and surface soil communities, as well as significant differences in the phylogenetic diversity of forest floor *Actinobacteria*. This shift in community composition occurred in concert with the slowing of plant litter decay, the accumulation of soil organic matter, and the greater production of phenolic DOC.

To determine whether slower decomposition rates resulted from down-regulation of the transcription of key lignocellulolytic genes, we quantified the expression of fungal genes encoding key cellulolytic (cellobiohydrolase, *cbhI*) and lignolytic (laccase, *lcc*) enymes. Our results indicate that the community-scale expression of *cbhI* under experimental N deposition did not differ significantly from that under ambient N deposition. In contrast, expression of *lcc* was significantly down-regulated by a factor of 2-4 fold relative to its expression under ambient N deposition. Our results suggest that chronic atmospheric N deposition may lower decomposition rates through a combination of reduced expression of ligninolytic genes such as *lcc*, as well as compositional changes in the fungal community. More importantly they indicate that ecosystem response to atmospheric N deposition, a wide-spread agent of global change, is controlled by the environmental regulation of fungal gene expression.

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Microbial Response to Modified Precipitation Patterns in Tallgrass Prairie Soil: Molecular Mechanisms, Activity Rates, and Organic Matter Dynamics

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http://cropandsoil.oregonstate.edu/soils/research/myrold/konza

Project Goals: Identify microbial physiological responses to modified precipitation in Great Plains prairie soils, and assess the implications for carbon cycling dynamics.

A significant amount of carbon (C) is processed and stored in prairie soils: grasslands cover 6.1-7.4% of the earth's land surface and hold 7.3-11.4% of global soil C. Global change models predict that the future precipitation regime across the North American Great Plains will entail less frequent but larger rainfall events. The response of prairie soil microbial C processing and allocation to this scenario of higher hydrologic variability is not known, but will be a key determiner of the future capacity for prairie soil C sequestration. We are approaching this problem by assessing soil microbial function (respiration, C utilization efficiency, extracellular enzyme activity) and molecular indicators of dominant C allocation pathways (soil transcriptome, proteome and metabolome) under ambient and experimentally modified precipitation regimes.

The Rainfall Manipulation Plots (RaMPs) at the Konza Prairie Long-Term Ecological Research (LTER) site in north-eastern Kansas, USA is a replicated field manipulation of the timing and magnitude of natural precipitation that was established in 1998. This experiment does not modify the total amount of growing season rainfall, it imposes extended dry periods and larger, less frequent rainfall events. We collected soil before, during and after rainfall events in both ambient and extended precipitation interval (more "droughty") treatments and measured microbial growth, respiration and potential organic matter degradation responses. Notable results include (1) Equivalent rainfall

events caused equivalent microbial respiration responses in ambient and interval manipulation plot soils, but biomass increased after the rainfall in the extended precipitation interval plots only. This implies a greater C use efficiency, or greater potential for belowground C retention, in "droughty" soils. (2) C:N ratio of biomass was increasingly high as soil water content decreased. This implies a physiological and/or population-level shift in the microbiota at low soil water content. (3) Extracellular enzyme activity responses were mixed across the suite of functional groups measured, with one consistent response: cellulose hydrolysis potential was always lower 5 days after rainfall. This implies a decreased dependency on soil organic matter degradation, with a lag period, after rainfall events; perhaps related to plant activity and root exudate deposition belowground.

These results lead to hypotheses regarding microbial physiological adaptation to drought stress in prairie soils. We are collecting molecular data (454 sequencing and QPCR of bacterial 16S rRNA and fungal ribosomal genes and transcripts, full transcriptomes and proteomes) to test these hypotheses. (H1a) Microbial taxa that respond quickly to increased water availability after drought are more active in soil with an altered precipitation regime history. (H1b) Transcripts and proteins from COGs indicative of growth, not maintenance, will be more abundant after rainfall in the "droughty" plots. (H2a) In soils with low water contents, transcripts and proteins driving trehalose (or other compatible solute) production will be more abundant. (H2b) In soils with low water contents, fungal cells will be more abundant. (H3) Higher root exudate uptake and metabolism 5 days after rainfall events will be evidenced by higher abundance of sugar and amino acid transporters. Directly addressing these mechanistic hypotheses would not be possible without "Omics" approaches.

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Early Career Award

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Microbial Communities in Restored Wetland Sediments

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http://www.jgi.doe.gov/sequencing/why/CSP2011-tringe.html

Project Goals: In this project, we use high-throughput sequencing tools to characterize microbial communities in restored wetland sediments, aiming to understand how the biotic and abiotic environmental factors govern microbial community structure and how microbial communities influence carbon flux, and thus impact long-term biological carbon sequestration.

On peat islands previously drained for agriculture, aerobic decomposition of peat has led to substantial land surface subsidence, thereby increasing risk of levee failure. Restoration of wetlands has a great potential to reverse land subsidence by slowing down decomposition and favoring new peat accumulation. In addition, the high primary production and slow decomposition rates found in restored wetlands may result in a net atmospheric CO_2 sequestration. However, one major concern is the emission of CH_4 that could potentially offset the carbon captured due to primary production. In wetland ecosystems, microbial communities play key roles in governing greenhouse gas flux, yet they are poorly characterized due to their high complexity. By using powerful high-throughput sequencing tools, we aim to identify community patterns, indicator species, genes or pathways that are associated with peat accretion rates and CH_4 flux, and these will providing vital information for better modeling of wetland carbon flux and management.

In this preliminary study, we collaborated with scientists at the U.S. Geological Survey (USGS), and collected belowground samples from a restored wetland from a USGS pilot-scale restoration project on Twitchell Island in the Sacramento/San Joaquin Delta, CA. The wetland is continuously fed by water from San Joaquin River, and is primarily vegetated with cattails (*Typha* spp.) and tules (*Schoenoplectus acutus*). We selected three sites that have varied proximity to the inflow, thus exhibiting gradients in physicochemical conditions and peat accretion rates. From each site, we collected three sample types, including the bulk decomposed material, cattail rhizomes and tule rhizomes. Pyrosequencing of amplified V8 regions of 16S rRNA genes was used to generate microbial community profiles. In parallel, mesocosm anaerobic incubation was conducted to evaluate CO_2 and CH_4 flux.

Our sequencing data indicate that wetland community composition is primarily governed by sampling site, and secondarily by sample type. Particularly, wetland communities from these three sites transited in a direction largely consistent with the physicochemical gradients along these sites. The mesocosm incubation experiment showed that CO_2 flux was significantly higher in the rhizome samples than in the bulk samples. By contrast, difference in CH_4 flux was more related to sample sites. Low CH_4 flux communities were associated with the site closest to the inflow, correlated to higher availabilities of electron acceptors, particularly sulfate and nitrate. Some of their more abundant microbial populations, as compared to high CH_4 flux communities, are likely reducers of these electron acceptors, as suggested by their closest microbial isolates. High CH_4 flux communities were associated with sites further from the wetland inflow, which have shown higher peat accretion rates. These sites harbored more abundant methanogenic archaeal populations, which likely contributed to the higher methane flux observed.

Currently, additional samples collected in a different season are being analyzed to identify seasonal effects, and comparative metagenomic analyses are being conducted to reveal differences in community functional profiles.

This project was funded by DOE Early Career Research Program, and was also supported by DOE JGI Community Sequencing Program. The work conducted by the U.S. Department of Energy Joint Genome Institute is supported by the Office of Science of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.

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Great Prairie Grand Challenge Soil Metagenome Sequencing Project

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Project Goals: Our overarching goal is to use deep metagenome sequencing to gain an understanding of the soil microbial community phylogenetic and functional gene repertoire in the U.S. great prairie and adjacent cultivated soils. This information will be used to determine the impact of land management (i.e. cultivation) on microbial community structure and function. In addition, this information will provide a database of microbial genes in native prairie soils that can serve as a baseline for understanding the impacts of perturbation and climate change on processes including soil carbon cycling. In order to achieve these goals the consortium is developing novel tools for metagenome sequencing, assembly, annotation and gene mining.

The United States Great Prairie contains approximately 35% of the total soil organic carbon stocks in the continental U.S. These carbon pools may be impacted in unknown ways by altered climate conditions and by land management. At the Joint Genome Institute, the U.S. Great Prairie was chosen as a "grand challenge" project for deep metagenome sequencing of a complex microbial community. This project is particularly challenging because of the high microbial diversity in soil that complicates metagenome assembly.

To date we have sequenced nearly 2 terabases of DNA from eight locations: 1) Wisconsin native prairie, 2) Wisconsin cultivated corn, 3) Wisconsin cultivated switchgrass, 4) Wisconsin restored prairie, 5) Iowa native prairie, 6) Iowa cultivated corn, 7) Kansas native prairie and 8) Kansas cultivated corn. Each site was sampled along a quadrant for a total of 8 samples per site. All of the samples were first profiled for their microbial community compositions by 454 pyrotag sequencing. In addition, the DNA from a central core from each location was extensively shotgun sequenced using the Illumina platform. A new algorithm was developed for assembly of the metagenome data. Different screening tools were developed to screen the assembled data and raw reads for key genes of interest in the carbon and nitrogen cycle.

454 pyrotag sequence data. We found that cultivation has a major impact on the composition of the soil microbial communities at all locations. There was similarity in the microbial community structures from the prairie soils in the three different locations. Some specific microbial species were more or less abundant in prairie soils, compared to cultivated soils, thus potential indicators of land use history. For example some Bradyrhizobia were more abundant in all of the native prairie soils. The restored prairie site had a microbial community composition that was intermediate between the cultivated corn and the native prairie soils, suggesting that this prairie community was progressing towards a restored (native) state.

Metagenome assembly. A novel approach was developed for metagenome assembly based on data reduction by filtering and normalization, followed by dividing the data into smaller disconnected sets to aid in assembly. This approach was tested on the Iowa corn and Iowa prairie metagenomes and is currently being applied to the remaining metagenomes. For more details about this approach, please see the poster presented by Adina Howe.

Metagenome mining. We first used a Hidden Markov Model (HMM) approach to screen for *nif*H genes in the Iowa corn and prairie assemblies. In addition, we developed a set of validated genes for different key pathways, including cycling of carbon and nitrogen. This gene database consists of "maudules" that represent individual pathways of interest. Currently we are using the maudules to mine the Iowa corn and prairie and the Kansas prairie metagenomes.

The work conducted by the U.S. Department of Energy Joint Genome Institute is supported by the Office of Science of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.

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Development of Integrated "Omics" Approach for Assessing Microbial Cycling of Carbon in Prairie Soil Using a Model Soil Bacterium: *Arthrobacter chlorophenolicus*

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http://www.lbl.gov/

Project Goals: Soils of the U.S. 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 and more extreme precipitation events,

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 impact of altered rainfall patterns on the carbon cycling dynamics of the soil microbiota in Kansas native prairie soil using a combination of omics approaches.

To develop protocols for RNA and protein extraction from soil, we spiked the soil with a soil bacterium that has been genome sequenced, *gfp* (green fluorescent protein)-tagged *Arthrobacter chlorophenolicus*. The *gfp* gene was used as an internal standard for accurate quantification. The model strain was inoculated into sterile and non-sterile Kansas prairie soil, amended or not with general (acetate) and specific (chlorophenol) carbon substrates.

Total RNA was extracted from the samples and is currently being sequenced using the Illumina platform to obtain metatranscriptomes. Target genes of interest were quantified by quantitative PCR and RT-QPCR. Total proteins were also extracted from the same samples to obtain metaproteomes. Metaproteomics proved particularly challenging due to the high humic acid content in the Kansas prairie soil. Therefore, we tested and optimized methods for extraction of proteins from the soil for subsequent measurement by shotgun metaproteomics via 2d-LC-MS/MS on an LTQ Velos mass spectrometer.

The first RNA-based results confirmed that the *gfp* transcript could be detected under most conditions and was thus a good estimator of *A. chlorophenolicus* abundance and activity. The initial metaproteome data indicated that several of the enzymes involved in acetate and chlorophenol degradation pathways were expressed in soil. In addition, several proteins involved in response to stress (thioredoxin, chaperonin, cold-shock proteins, etc.) were expressed. We also detected high levels of a flagellin protein in the soil amended with chlorophenol. Comparison of *A. chlorophenolicus* protein yields from sterile and non-sterile soil showed the impact of high background soil diversity on complicating the proteomic results.

These methods are now being applied to the samples acquired from the rainfall manipulation plots at the Konza Prairie Long-Term Ecological Research station. At JGI we have sequenced over 150 Gb of DNA from Kansas soil. To analyze the data using omics we have developed a new comprehensive functional database. We also focused on improving the sequence annotation by screening sequences belonging to particular KO (KEGG Orthology) families. We used this information to build a HMM (Hidden Markov Model). The database was structured into "maudules", which were chosen to represent specific functions with ecological context (such as denitrification, methanogenesis, etc.). This validation approach will enable us to directly explore omics data for key functions of interest, and to integrate the different omics analyses to improve our understanding of carbon cycling processes in the prairie.

This research is sponsored by the DOE-BER, Biological Systems Research on the Role of Microbial Communities in Carbon Cycling Program, under Contract No. DE-SC0004953.

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Microbial Mediation of Litter Decomposition in Soil: The Role of Plant Roots

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Project Goals: Our project (*Plant Stimulation of Soil Microbial Community Succession: How Sequential Expression Mediates Soil Carbon Stabilization and Turnover*) focuses on a fundamental understanding of C cycling in soil as mediated by soil microorganisms and their interactions with plants. Our work investigates how the interactions between roots and soil microorganisms affect transformations of root derived C, decomposition and loss as CO_2, as well as C sorption and stabilization in soil at ambient and elevated levels of atmospheric CO_2. Through our research we seek to provide a mechanistic understanding of the conversion of root C to stabilized soil C, clarify the impacts of increased concentration of CO_2 on soil C sequestration, and substantially expand our understanding of molecular regulation of terrestrial C cycling.

Soil organic C is the largest component of the terrestrial C cycle, with fluxes through the C pool mediated by soil microorganisms and modulated by their interactions with plant roots and root exudates. We characterized the metabolic profiles of *Avena barbata* root exudates collected in sterile hydroponic systems using gas chromatography-mass spectrometry (GC-MS). A variety of compounds were detected in exudate samples, including carbohydrates (e.g. glucose, fructose, galactose), low molecular weight organic acids (e.g. oxalic, malic, maleic acids), amino acids and amides (e.g. lysine, serine, glycine), fatty acids (e.g. arachidic, lauric, oleic acids), sterols (e.g. cholesterol) and others (e.g. hydroxylamine, glycerol). We are using this library of identified exudate compounds to inform spatially explicit analysis of *A. barbata* root exudates patterns in soil. To do so, *A. barbata* seedlings grown in microcosms were pulse labeled with $^{13}CO_2$ and the exudates were sorbed to an initiator-treated silicon wafer. Organic compounds on the wafer were then analyzed by nanostructure-initiator mass spectrometry (NIMS). Using the hydroponically-generated library, we are currently identifying compounds detected using the NIMS approach.

A concurrent effort examines the effect of live *A. barbata* on the mineralization of ^{13}C-labeled root litter in soil over two growing seasons. The mineralization rates of labeled

root litter in the presence of *A. barbata* were determined and compared with those in a no-plant treatment by measuring total CO_2 and $^{13}CO_2$ fluxes. $^{13}CO_2$ flux in the no-plant treatment was significantly higher than in the presence of live *A. barbata* in the early litter decomposition stage, suggesting an initial negative priming effect of live roots on litter mineralization. However, the trend changed after 40 days; after 70 days, the $^{13}CO_2$ flux rates from decomposing root litter became higher in the presence of live *A. barbata* plants, suggesting a positive priming effect.

The metabolic data and mineralization rates measured in the presence and absence of live plant roots will guide future investigations of the microbial metabolic pathways responsible for rhizosphere C processing. This work will be extended to distinguish the C-cycling transcriptome of rhizosphere microbial communities utilizing ^{13}C-labeled exudates to provide a mechanistic basis for understanding organic matter priming in the rhizosphere.

Funding for this research was provided under contract FOA DE-PS02-09ER09-25 through Genomic Sciences program of the Office of Biological and Environmental Research, U.S. Department of Energy to the University of California, Berkeley. Part of this work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under contract DE-AC52-07NA27344 and at Lawrence Berkeley National Laboratory under the auspices of the University of California—contract DE-AC02-05CH11231.

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Linking Microbial Identity and Succession Patterns to Uptake of Plant-Derived Carbon in the Rhizosphere

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Project Goals: Our project (*Plant Stimulation of Soil Microbial Community Succession: How Sequential Expression Mediates Soil Carbon Stabilization and Turnover*) focuses on a fundamental understanding of C cycling in soil as mediated by soil microorganisms and their interactions with plants. Our work investigates how the interactions between roots and soil microorganisms affect transformations of root derived C, decomposition and loss as CO_2, as well as C sorption and stabilization in soil at ambient and elevated levels of atmospheric CO_2. Through our research we seek to provide a mechanistic understanding of the conversion of root C to stabilized soil C, clarify the impacts of increased concentration of CO_2 on soil C sequestration, and substantially expand our understanding of molecular regulation of terrestrial C cycling.

Soil organic C is the largest pool within the terrestrial C cycle and fluxes within this pool are regulated by complex interactions between edaphic factors, plants, soil microorganisms and minerals. Plant roots and their exudates exert control over the microorganisms mediating decomposition of complex 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.

In this research we are assessing structure and successional patterns within the microbial community mediating carbon transformations in the rhizosphere of *Avena barbata* (slender wild oat), by assessing community transcription and substrate (i.e., root exudates or root litter) use profiles along actively-growing roots (i.e., root tip to fully mature root) and through time as roots grow and eventually decompose. Initial results suggest that the rhizosphere microbial community differs along a root age gradient and we hypothesize that these differences will be reflected in resource use measured by stable isotope probing (SIP). We have constructed a suite of stable isotope labeling growth chambers and microcosms, which we are using to pulse label *A. barbata* plants and monitor the fate of added $^{13}CO_2$ as it moves into the soil C pool both as root exudates and decomposing root litter. We are specifically interested in the differential response of rhizosphere communities exposed to either independent or simultaneous additions of ^{13}C-live roots and ^{13}C root litter.

High throughput next generation sequencing is being used to measure rhizosphere microbial community diversity (16S rRNA) as well as being combined with community separation based upon uptake of added ^{13}C labels (SIP). 16S rRNA survey also forms the basis for development of probe sets for phylogentic microarray chips to be used for stable isotope probing via Chip-SIP, a new method that combines community identification with high density RNA microarrays and substrate use profiling by NanoSIMS isotopic analysis of the array spots. We are also trialing existing probes and using the acquired 16S rRNA data to develop probes for fluorescent in-situ hybridization (FISH) to spatially examine the rhizosphere microbial community. The FISH method will be combined with NanoSIMS isotopic analysis to simultaneously observe the fate of added ^{13}C label in intact soil/root samples.

Funding for this research was provided under contract FOA DE-PS02-09ER09-25 through Genomic Sciences program of the Office of Biological and Environmental Research, U.S. Department of Energy to the University of California, Berkeley. Part of this work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under contract DE-AC52-07NA27344 and at Lawrence Berkeley National Laboratory under the auspices of the University of California—contract DE-AC02-05CH11231.

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Soil Fungal Community Responses to Long-Term Elevated CO_2 and N Deposition Conditions in a Temperate Pine Forest (DOE Duke Forest FACE site)

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Project Goals: The simultaneous increase of atmospheric CO_2 and nitrogen (N) deposition to forest ecosystems is predicted to alter plant productivity and, consequently, to change the amount and quality of above and belowground carbon entering forest soils. It is not known how such changes will impact the composition and function of soil microbial communities, particularly fungal communities, that play a key role in degrading complex carbon. Our recent studies explored the composition of soil fungal biomass after ten years of elevated CO_2 conditions, and under five years of combined elevated CO_2 and increased soil N conditions, in a temperate pine forest (DOE Duke Forest FACE site). We used a combination of DNA- and RNA-based surveys and sequencing of target genes to compared features of the resident and active soil fungal community.

Ecological studies: First, we conducted a comparative study of fungal cellobiohydrolase I genes (*cbh*I), representing the resident (DNA-based) and expressed (cDNA-based) communities in surface soil (0-10 cm depth) across the elevated CO_2 and N-fertilization treatments at the Duke Forest FACE site. Our study demonstrated that the richness and composition of the soil cellulolytic fungal community was distinct between the DNA- and cDNA-based gene surveys. Richness or composition of the cellobiohydrolase-containing fungal community was not altered by elevated CO_2 and/or N-fertilization conditions relative to the ambient controls. The soil fungal community was dominated by members of the Basidiomycota that have minimal or no representation in current sequence databases.

Second, we conducted seasonal surveys of the fungal *cbhI* gene and the fungal LSU gene (phylogenetic marker) across the treatments at the Duke Forest FACE site, over two years and five seasonal time points to determine how fungal community responses to climate change parameters may vary with season. In all seasons, soil fungal community richness (LSU gene) was decreased in elevated CO_2 plots compared to ambient plots. In both ambient and elevated CO_2 plots, N fertilization increased richness in spring and summer sampling points indicating that some taxa are nitrogen limited at this site during these seasons. In contrast, we did not detect a change in *cbhI* richness that correlated with season.

Third, we conducted an intensive LSU sequencing survey of soil fungal communities present in forest floor and in underlying soil to determine the response to elevated CO_2 and N deposition treatments manifest across soil depths of a few cm (forest floor, 0-2 cm, 2-5 cm, 5-10 cm). Soil chemistry and fungal community (LSU gene) richness and composition differed significantly across shallow changes in soil depth. Fungal community response to elevated CO_2, N deposition, and the combined treatment was also highly stratified by depth, illustrating that fungal community roles in this forest ecosystem cannot be accurately predicted using bulk, homogenized samples typically employed in large field studies.

Method development and database resources: Defining the factors underpinning methodological biases is required to optimize the design of gene expression studies in soils. As a prerequisite to the ecological studies described above, we compared the richness and composition of the fungal *cbhI* gene in forest floor and underlying soil, that could be captured using two cDNA preparation methods and two different cDNA priming methods. Richness, composition and reproducibility of gene expression profiles of the fungal *cbhI* gene were examined when amplified from sscDNA, or from dscDNA synthesized using SMART PCR. In the dscDNA libraries from soil or litter samples, richness was significantly reduced and the composition was altered relative to sscDNA libraries. Library composition was significantly more reproducible among replicate sscDNA libraries than among parallel dscDNA libraries from litter. We also performed comparative richness and compositional analyses of the fungal *cbhI* gene amplified from soil cDNAs that had been generated using either oligo(dT) primers or random hexamers. Our results demonstrated that similar *cbh*I richness and composition were recovered using either priming method.

To support taxonomic interpretation of the soil fungal community sequences obtained in the ecological studies, we established a taxonomic database for the *cbhI* gene, and for the fungal LSU gene. The accuracy of the LSU database using multiple sequence lengths was determined using naïve Bayesian classifier and BLAST approaches. These classification resources are publicly available to the scientific community through the Ribosomal Database Project.

This project was supported by the U.S. Department of Energy Biological System Science Division, through a Science Focus Area Grant (2009LANLF260) to CRK and a Los Alamos National Laboratory Director's Postdoctoral Fellowship to CFW. Sequencing was made possible through the DOE Joint Genome Institute and the Los Alamos National Laboratory LDRD program.

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Arid Land Ecosystem Responses to Long-Term Elevated Atmospheric CO_2 in a Large Manipulated Field Experiment

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Project Goals: Atmospheric CO_2 levels are expected to double within the next century. Arid land ecosystems, which comprise over 40% of the earth terrestrial surface, are predicted to be particularly vulnerable to changes in climate. Processes mediated by soil microorganisms responsible for carbon and nitrogen turnover are critical in arid land ecosystems where plants are sparse. We conducted highly replicated surveys of soil microbial communities associated with the dominant shrub species (creosote bush) and in interspace biological soil crusts (biocrusts) at the DOE Nevada Test Site Free Air CO_2 Enrichment (FACE) site to determine the effects of over ten years of elevated CO_2 conditions on the soil biota.

First, the ability of a targeted metagenomic approach (small subunit (SSU) rRNA pyrosequencing) and shotgun metagenome approaches were compared to identify known distinguishing features between the creosote root zone communities and biocrusts, and differences due to the more subtle elevated CO_2 treatment. The biocrust datasets were clearly differentiated from root zone datasets using either of the sequencing approaches. However, different compositional features were identified using the different approaches. The ability to detect possible treatment effects was largely approach-dependent, as the magnitude of resolved differences due to elevated CO_2 was smaller when shotgun metagenome reads were used, compared to pyrosequenced SSU datasets or even SSU reads recruited from the shotgun metagenomes. Based on prior knowledge of the biocrust communities, the SSU-based datasets more accurately identified the dominant biocrust cyanobacteria populations compared to the shotgun metagenome datasets.

Second, using quantitative PCR (qPCR) of cyanobacteria 16S rRNA genes, 16S rRNA pyrotag sequencing, and shotgun metagenome sequencing, we explored the response of biocrust cyanobacteria to elevated CO_2 in more detail. In years with sufficient moisture for growth, higher plants in this ecosystem have responded to elevated CO_2 with increased biomass. We hypothesized that the photosynthetic cyanobacteria that are dominant in the biocrusts would respond similarly. In contrast to our prediction, the relative abundance of cyanobacteria biomass (qPCR) was not significantly different between biocrusts under ambient or elevated CO_2 conditions, and trended toward a decrease in cyanobacteria biomass under elevated CO_2 conditions. Similarly, the proportion of cyanobacteria in 16S rRNA gene libraries or in shotgun metagenomes was either not significantly different or was reduced in biocrusts under elevated CO_2 conditions relative to the ambient controls. Comparison of the shotgun metagenomes provided information to suggest physiological and functional differences in the biocrust cyanobacteria under the elevated and ambient CO_2 conditions. Taken together these results indicate long-term elevated CO_2 produced shifts in both the structure and function of the biocrust cyanobacteria.

Employing highly replicated field sampling and DNA sequencing, we identified that a major effect of elevated atmospheric CO_2 on the biocrusts was a divergence in microbial community composition, resulting in increased spatial heterogeneity. The increased variability of the microbial communities in response to elevated CO_2 was robust using multiple sequencing approaches and was observed in both taxonomic and functional profiles. These results will inform future studies to quantify natural variability of soil microbial communities and for the design of future climate change experiments having predictive potential.

This project was supported by the U.S. Department of Energy Biological System Science Division, through a Science Focus Area Grant (2009LANLF260) to CRK. Sequencing was made possible through the DOE Joint Genome Institute and the Los Alamos National Laboratory LDRD program.

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Direct Interspecies Electron Transfer in Methanogenic Environments: Genome-Scale Analysis of Mechanisms in Defined Co-Cultures and Natural Aggregates

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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 predictively 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 digestors, the most effective strategy yet devised for large-scale conversion of organic wastes to fuel. For over 40 years interspecies hydrogen transfer has served as the paradigm for anaerobic interspecies electron transfer. However, our recent studies demonstrated that direct interspecies electron transfer is possible[1] and may be the predominant mechanism for electron exchange in some methanogenic environments[2].

In order to elucidate the mechanisms of direct electron exchange under methanogenic conditions, and the factors controlling the rate of this process, studies were conducted with natural methanogenic aggregates as well as defined co-cultures. Co-cultures were established with *Geobacter metallireducens* as the electron-donating partner and either *Methanosarcina bakeria* or *Methanosaeta harudinacea* as the methanogenic partner. A *Geobacter* species was chosen because *Geobacter* species are often abundant constituents of methanogenic aggregates in anaerobic wastewater digestors as well as in methanogenic soils, such as rice paddies. *Methanosaeta* are the most abundant methanogens in similar methanogenic environments, except when they are replaced by *Methanosarcina* species.

Co-cultures of *G. metallireducens* and *Msr. barkeri* formed aggregates that effectively converted ethanol to methane. Formate could be ruled out as a potential electron shuttle between the two organisms because *Msr. barkeri* is unable to use formate as an electron donor. Although *Msr. barkeri* has the potential to use hydrogen, hydrogen did not appear to be an important intermediate for electron exchange because the cells within the aggregates were not adapted for hydrogen utilization. These results are consistent with direct interspecies electron transfer between *G. metallireducens* and *Msr. barkeri*.

The possibility of direct interspecies electron transfer is being further evaluated with studies in which on or more of the co-culture partners are strains that are deficient in components that are considered to be important for this process. For example, the PilA-pili of *Geobacter sulfurreducens* were recently shown to possess metallic-like conductivity that permits long-range electron transfer along their length and play a role in interspecies electron transfer[3]. Therefore, a strain of *G. metallireducens* that cannot produce PilA-pili was constructed and it is being determined whether this mutant can form syntrophic associations with *Msr. barkeri*. Furthermore, gene expression patterns in the two microorganisms are being compared with gene expression patterns determined in studies with previously described[1] co-cultures of *Geobacter* species conducting interspecies electron transfer.

Co-cultures of *G. metallireducens* and *Mst. harudinacea* that effectively converted ethanol to methane were also established. The ability to these two organisms to form syntrophic aggregates is significant because *Mst. harudinacea* is unable to use either hydrogen or formate as electron donors. Therefore, direct interspecies electron transfer is likely in this co-culture system as well. This is consistent with our previous finding that *Geobacter* and *Methanosaeta* species were the predominant microorganisms in natural methanogenic aggregates that were exchanging electrons via direct interspecies electron transfer[2]. Gene expression analysis of these co-cultures is being initiated and expected to be complete by the time of the meeting.

Analysis of gene expression of natural methanogenic aggregates provided additional evidence for direct interspecies electron transfer to the *Methanosaeta* species, which accounted for over 90% of the methanogens in aggregates[2]. Although *Methanosaeta* are unable to use hydrogen or formate as electron donors, a full pathway for carbon dioxide reduction could be detected in *Methanosaeta* genomes. Genes in the carbon dioxide pathway of the *Methanosaeta* in the natural aggregates were expressed at high levels. These results indicate that *Methanosaeta* receive low-potential electrons that can drive carbon dioxide reduction, consistent with the concept that *Methanosaeta* species can directly accept electrons from other members of the consortia.

As summarized in a companion poster, a genome-scale metabolic model that can describe the growth of a *G. metallireducens*/*G. sulfurreducens* co-culture that functions via direct interspecies electron transfer has been constructed. The gene expression and other physiological data needed for similar genome-scale metabolic models of methanogenic communities functioning via direct interspecies electron transfer is now being collected from the defined co-cultures and natural aggregates.

The discovery of direct interspecies electron transfer is a paradigm shift in anaerobic microbial ecology. The studies summarized here are expected to provide models that will be able to predict rates of electron exchange in methanogenic communities under a diversity of environmental conditions and the impact of environmental perturbations, such a climate change, on the rates of methanogenesis. Furthermore, an understanding of the mechanisms for direct interspecies electron transfer for methane production is leading to new concepts to either promote this process for bioenergy applications or to inhibit undesirable release of methane from terrestrial environments.

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Integrated Genome-Scale Modeling of Syntrophic Consortia Reveals Microbial Community Dynamics and Mechanisms of Electron Transfer

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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 of the current research is to determine if the syntrophic associations that are central to the functioning of methanogenic terrestrial wetlands can be predictively modeled with genome-scale metabolic models.

The previously described[1] syntrophic co-culture of *Geobacter metallireducens* and *Geobacter sulfurreducens* serves as a genetically tractable model for the process of direct interspecies electron transfer hypothesized to be important in terrestrial methanogenic environments. Therefore, this co-culture is serving as an initial test case to determine if it is possible to use genome-scale metabolic models to predict the physiological responses of microorganisms engaging in direct interspecies electron transfer in complex communities and to characterize the relative efficiencies of direct electron exchange versus other alternatives, such as interspecies hydrogen or formate transfer.

In order to model different modes of electron transfer, it is important to accurately account for the energetics involved in extracellular electron transport. Hence, the existing genome-scale reconstructions of *G. sulfurreducens* and *G. metallireducens* were expanded to reflect the most updated annotations, a distinct periplasm compartment, and detailed biosynthetic pathways. Importantly, both these models now include detailed representation of the energy metabolism involved in extracellular electron transfer. The models now account 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 thermodynamic consistency, appropriate gene association and cellular localization of the different electron carriers. The *G. sulfurreducens* model now consists of 829 genes and 1079 reactions, while the *G. metallireducens* model consists of 974 genes and 1173 reactions.

We further developed a modeling framework to integrate these two genome-scale models into a combined model to study the metabolic interactions. This framework includes a shared metabolite pool to account for the metabolic exchanges between the constituents of the consortia. Computational simulations revealed that the optimal ratio of the constituents contained 21% *G. metallireducens.* This prediction is in accordance with experimental observations of a composition of 15% *G. metallireducens*.

Flux balance analysis simulations indicated that direct interspecies electron transfer is more efficient than interspecies hydrogen transfer for the growth of the consortia. This prediction is consistent with the previous observation[1] that selective pressure for rapid syntrophic growth selected for a mutation that promoted direct electron transfer. Acetate secreted by *G. metallireducens* was used as a carbon source as well as an additional source of electrons for *G. sulfurreducens*.

We also modeled a newly developed co-culture in which the *G. sulfurreducens* strain was incapable of acetate oxidation because the citrate synthase gene was deleted. This eliminated additional grow of *G. sulfurreducens* with acetate as the electron donor. The optimal ratio of the constituents in this co-culture was predicted to be 45% *G. metallireducens*, which compared well with the experimental observation of 50%. Again, direct electron transfer was predicted to be preferred over interspecies hydrogen transfer.

We further performed high-throughput transcriptomic profiling (RNA-seq) and physiological screens of both the wild type and mutant aggregates and analyzed them in the context of the metabolic model to gain insights into the mechanisms of electron transfer. Differential expression analysis revealed that 481 genes had significant changes in expression levels between wild-type and citrate synthase mutants. The global effects of these gene expression changes on the dynamics of the microbial community are being investigated with the aid of the integrated community model. We have also performed genome re-sequencing of the aggregates and identified SNPs that have accumulated as a result of the selective pressure of syntrophy.

In summary, this study represents an integrated multi-omic approach to elucidate the electron transfer mechanism and to characterize the metabolic phenotype of a laboratory evolved syntrophic consortium. The models being developed in these studies will be important for understanding the functioning of anaerobic terrestrial microbial communities and predicting the influence of environmental changes on methane emissions and other aspects of carbon cycling.

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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 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-dependent versus oxygen-dependent modes, in order to bridge gaps in understanding the specialized bacterial communities involved in these processes.

The 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.

Our research was focused on the following three objectives:

Objective 1: Identify actively transcribed pathways. Next generation sequencing-based transcriptomic profiling (RNA-seq platform) was used to perform global characterization of C_1-metabolism in lake sediment, as well as community responses to stimulated environmental perturbation, such as low oxygen and additional nitrate**.** Reconstruction of the related C_1-metabolic function was performed. The results demonstrated that the sediment C_1- community is represented by microbial species adapted to low oxygen and low methane flux.

Objective 2: Identify physiologically active pathways. A highly comprehensive ion exchange solid phase extraction (SPE) liked with hydrophilic interaction liquid chromatography (HILIC-MS/MS) and liquid chromatography with a pentafluorophenylpropyl column (LCPFPP-MS/MS) was developed to understandcentral carbon metabolites involved in methane assimilation and adjacent pathways in complex natural mixtures. The recovery of 51 targeted metabolites from five compound classes (amino acids, carboxylic acids, sugar phosphates, nucleotides, and acyl-CoAs) was investigated. The following SPE procedures were employed: (a) mixed mode strong cation exchange, (b) mixed mode strong anion exchange, and (c) mixed mode weak anion exchange. We analyzed 32 of the targeted 51 metabolites using either HILIC-MS/MS or LCPFPP-MS/MS after SPE sediment samples cleanup and pre-concentration. The remaining 19 targeted metabolites were either at, or below, the detection limit. The current approach provides a good workflow for absolute quantification of intermediates in C_1-carbon metabolismin naturalmicrobial communities.

Objective 3: Identify activity of individual cells. As part of Objective 3, 10 individual active cells with unusual phylogeny were selected for whole genome amplification, sequencing, assembly, and functional characterization as a result of metabolic reconstruction. The result couples genomics to function, and therefore, has the potential to reveal newinsights into the physiological capabilities of yet uncultured members of natural microbial communities.

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Vitamin Biosynthesis and Regulation in Marine Algae

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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.

Phytoplankton are important primary producers in marine environments. While the regulatory role of macronutrients on algal growth has been clearly demonstrated, the potential influence of vitamins and small metabolites remains largely unknown. Several groups of organisms have developed independence from vitamin B12, however vitamin B1 (thiamine) is required by all known members of the three domains of life. Still, it is unclear to what extent ecologically relevant marine algae require exogenous sources of B1 and which taxa can synthesize this vitamin. Furthermore, there is little information about the algal genes involved in thiamine biosynthesis, which limits hypothesis development and experimentation.

Here, we present an analysis of the thiamine biosynthetic pathway in eukaryotic algae, with targeted experimental work in the marine green alga *Micromonas*. To explore the evolutionary history of thiamine biosynthesis genes and elucidate which marine algae may have the genetic potential to synthesize B1 we performed a comparative genomics analysis of thiamine biosynthetic genes encoded by algae from different eukaryotic supergroups and by cyanobacteria. These genes were compared to those in bacteria, yeast and

land plants. The analysis revealed that green algae are quite different from other eukaryotic algae (e.g. diatoms and pelagophytes), and that even within the green algae there is significant variation. For example, within the *Micromonas* genus, one strain (RCC299) appears to lack key thiamine biosynthetic enzymes, and therefore presumably requires an exogenous source, while another strain (CCMP1545) appears to encode a more complete pathway. In addition, at least one thiamine biosynthetic gene, found in *Micromonas*, and other green algae, is more akin to homologs in fungi than to the gene that performs the same biosynthetic step in land plants.

Because *Micromonas* has a widespread distribution in the ocean, we examined several of the identified putative thiamine biosynthesis genes experimentally. In addition to sampling for complete transcriptome sequencing, qPCR primers were designed to a suite of genes in each strain. Experiments were conducted to explore growth responses to thiamine deprivation in the two *Micromonas* strains. When cultured without thiamine, *Micromonas* CCMP1545 up-regulated key thiamine biosynthetic genes however neither strain seemed able to grow successfully in the absence of thiamine. qPCR primers were also designed to genes that have no known role in thiamine biosynthesis, but, based on genomic analysis, appear to be under the control of a thiamine sensing molecular switch. *Micromonas* RCC299 up-regulated these genes in response to thiamine deprivation and we hypothesize they represent previously unrecognized thiamine transporters. The disparity in expression responses between the two *Micromonas* as well as the unusual pattern of gene homology indicate that thiamine plays a role in algal physiology and ecology. However, experimental validation is critical for developing true understanding of ecological controls and niche differentiation.

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Computational and Experimental Approaches to Systems Biology of Marine Green Algae

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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.

We are developing a model system for eukaryotic marine algae. Thus far there are no model systems relevant to both marine carbon cycling and evolution of the Viridiplantae—the eukaryotic lineage containing all land plants and green algae. The primary drivers for developing the dual-*Micromonas* system are: *i*) approximately half of global photosynthetic CO_2 uptake is performed by marine algae yet there is little understanding of the physiological consequences of current global change scenarios and *ii*) green algae provide insights to eukaryotic cellular processes and the ancestor of land plants.

Prasinophytes are a group of unicellular marine green algae that are evolutionarily distinct from the model green alga *Chlamydomonas*, but are related to both the latter and land plants. *Micromonas* is a widespread prasinophyte that is exceptional in its size (<2 micrometer diameter) and having a small genome (21 Mb). The genomes of two *Micromonas* strains share 90% or less (depending on criteria used) of their protein encoding genes, have low gene redundancy and contain 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. Our strategy is to subject the two different strains to ecologically relevant perturbations and use whole transcriptome and proteome profiling, as well as traditional cellular measures, to understand their responses to perturbation and develop knowledge of cellular pathways. The genomes of *Arabidopsis thaliana*, *Physcomitrella patens*, *Chlamydomonas reinhardtii*, *Ostreococcus* RCC809, *Saccharomyces cerevisiae* and *Escherichia coli* are being used for comparative purposes.

To investigate genetic pathways in *Micromonas*, a mixed computational inference strategy is being utilized that integrates data from diverse sources, including the transcriptome and proteome expression. As a first step, we have implemented a scoring system to track supporting evidence for all predicted genes in the two strains. Every possible annotation is scored, in a database that holds all annotations and their evidence, and the highest scoring gene at a locus then selected automatically. We are currently expanding the database to hold all biologically relevant information for different experimental conditions. This database provides a foundation for pathway and perturbation analyses. Interaction orthologs (interologs) are included to help identify putative interaction pathways. An integrative multi-species biclustering algorithm is also being used to identify putatively conserved modules that are shared by the two *Micromonas* species, as well as species-specific differences in these modules and modules that are unique to each organ-

ism. In turn, these modules were used in conjunction with several network-inference methods to identify additional putative pathways whose activity levels were identified using PARADIGM, a state-of-the-art pathway prediction algorithm. Given its relationship to plants the dual-*Micromonas* system will enable modeling of more general primary producer responses across ecological and evolutionary scales. Our overall goal is to develop an efficient system for gaining insights to the green lineage, including novel and conserved genetic mechanisms, with high relevance to marine carbon cycling. 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.

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Early Career Award

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Linking Phylogeny and Function of Methanotrophic Archaeal-Bacterial Consortia in Deep-Sea Methane Seeps Using *in situ* Targeted Metagenomics, Molecular Ecology, and Stable Isotope Tracer Experiments

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Project Goals: We are exploring the biogeochemical functions and ecological relationships between ANaerobic MEthane oxidizing Archaea (ANME) and Sulfate Reducing Bacteria (SRB) and associated prokaryotes. We have pursued the following approaches: 1) Magneto-FISH to isolate the ANME-SRB consortia, 2) molecular investigation of phylogeny and function of the ANME-SRB metagenomes and the *in situ* community, 3) stable isotope tracer experiments plus single-cell FISH-nanoSIMS analysis to link organisms with function, and 4) bulk geochemical measurements to observe biogeochemical significance. Targeting ANME-SRB metagenomes has allowed comparison with other metagenomes and supported the reverse-methanogenesis hypothesis for methane oxidation. *In situ* measurements and stable isotope tracer experiments enabled testing the functionality of genes identified by metagenomic analysis within the ANME-SRB consortia, and revealed an unexpected role for these organisms in the nitrogen cycle. Although valuable in isolation, combining these techniques yields new insights into these uncultured methane-based syntrophic partnerships.

Targeting the uncultivable ANME-2c and their bacterial consortia in methane seep sediment from Eel River Basin, we have built on the Magneto-FISH technique pioneered by our lab group (Pernthaler et al 2008). We have generated and analyzed more than 10 times the sequence from our initial effort and, using the Community cyberinfrastructure for Advanced Microbial Ecology Research and Analysis (CAMERA) pipeline, identified a greater number of ORFs and gene/protein families. Due to the unique environment of the methane seep, the proportion of sequence assigned to COG or pfam database sequences has remained low (e.g. ~8% currently compared to 4% previously); also, when compared to the NCBI RefSeq database only 50-70% of ORFs in assembled contigs were similar to any protein sequences of characterized organisms. Similar to what we found in earlier targeted metagenomes, there was a wide variety of ANME-2c-associated bacteria, dominated by δ- and γ-*Proteobacteria*. δ-*Proteobacteria*-derived sequences were primarily affiliated with *Desulfobacterales*, *Desulfuro-monadales*, and *Syntrophobacterales*. Many different γ-*Proteobacteria* were present with no single group predominating. Archaeal sequences made up a smaller percentage of the metagenome and were mostly from the *Methanosarcinales, Methanomicrobiales*, and *Methanocellales* and other methanogenic groups. Using 16S rRNA gene quantitative PCR, we were able to confirm our ability to enrich for specific syntrophic partners with Magneto-FISH, with genomic DNA extracts from the *Desulfobulbus*-targeted magneto-FISH revealing a greater proportion of *Desulfobulbus* 16S rRNA genes compared with the original sediment.

To generate better contig assembly and to overcome limitations in annotation we have combined our individual ANME-2c targeted metagenome libraries from a single sample in the Eel River Basin into a single contig assembly. This combined metagenome was compared to publicly available metagenomes from methane seep sediments within the Integrated Microbial Genome (IMG) database and to recently published genomes of ANME-1, *Desulfosarcina*, and *Desulfobulbus*. Recruiting our contigs to previously published methane seep fosmid libraries, revealed up to 30% coverage of ANME-2 and SRB fosmids and little to no recruitment to taxonomically identified ANME-1 fosmids. All of the genes necessary for the reverse methanogenesis pathway are present in the ANME-2c enriched metagenome, confirming earlier findings. Genes for carbon fixation, sulfate reduction, nitrate reduction, and nitrogen fixation are also present. Grouping the reads and contigs using sequence-based categorization (e.g. tetranucleotide correlation and self organizing map algorithms) will increase confidence in these characterizations. Further association of identity and function using 16S rRNA genes and other housekeeping genes (e.g. RecA/RadA, RecG, leuS etc.) correlated to functional genes involved in sulfate reduction (aprBA, dsrAB), nitrogen metabolism (nifHDK, nirK, napG, etc.) and methanotrophy (e.g. mcrA) in the ANME-

2c enriched metagenome and in other methane seep habitats. Combining these techniques should provide phylogenetic characterization that is less dependent on databases from cultivated organisms.

We leveraged information from the ANME-2c metagenome to show ANME archaea are involved in N_2 fixation at methane seeps. We initially identified the potential for ANME related *nif* genes in the metagenome (Pernthaler et al. 2008) and bolstered this with a survey of the *nifH* genes in the ANME-2c enriched sample and the microbial community at the Eel River Basin (ERB) methane seep. We showed direct incorporation of $^{15}N_2$ into ANME-2 archaea collected at the ERB using FISH-nanoSIMS (Dekas et al. 2009). We have expanded our study to other methane seeps (Costa Rica; Hydrate Ridge, USA; Monterey Canyon, USA) and to potential bacterial diazotrophs. Nitrogen fixation rates are highly spatially variable at methane seeps, both laterally and with sediment depth, with the highest rates observed within the methane-sulfate transition zone. Quantitative comparison between the EA-irMS bulk rates and the NanoSIMS single-cell rates of nitrogen fixation suggest that the ANME are responsible for the majority (~80%) of seep nitrogen fixation, but that the remainder is fixed by other diazotrophic organisms, likely including free-living sulfate reducing bacteria.

Publications

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Correlative Compositional Imaging and Protein Profiling in Marine Anaerobic Methane Oxidizing Microbial Communities

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Project Goals: Multiple experimental lines of inquiry are being applied in an effort to shed light on the metabolic activity and genomic capacity of organisms responsible for the anaerobic oxidation of methane. Characterization by secondary ion mass spectrometry, transmission electron microscopy, and fluorescence *in situ* hybridization reveals organism specific composition and structure, while metaproteomics and histological approaches reveal biochemical functionality and distribution within these uncultured multicellular consortia.

Large amounts of methane are oxidized in anoxic marine sediments by symbiotic microorganisms whose physiology is largely unknown. These uncultured organisms, comprised of consortia of methane-oxidizing archaea (ANME) and sulfate reducing bacteria (SRB), form highly structured physical associations and participate in a syntrophic relationship. To characterize these metabolic partnerships, complimentary microscopic and spectrometric techniques are being developed, providing high resolution compositional and phylogenetic information. These analyses indicate significant differences between cell types and create the opportunity for conducting comparative and quantitative image analysis between samples experiencing shifts in environmental conditions. Integration of these approaches with metagenomics and metaproteomics has the potential to result in direct correlation of form and function in these organisms.

Methodologies appropriate for isolating high concentrations of biological material from environmental samples have been recently developed by our team, making possible the application of high resolution imaging techniques including electron microscopy and spatial mass spectrometry. These techniques have proven suitable for the analysis of microbial consortia with a diversity of imaging methodologies. Characterization of ANME/SRB consortia by transmission electron microscopy (TEM) reveals a diversity of cellular morphologies and intercellular components. Many of these intercellular features can be understood partially from comparison with those present in organisms in pure culture, however other features appear to be novel and undescribed. In addition, TEM analysis reveals the presence of an inorganic iron-bearing phylosilicate crust which surrounds some, but not all of the aggregate types. Material composition of these features, as well as compositional differences between organism types are being probed by secondary ion mass spectrometry (nanoSIMS) at spatial resolutions previously not possible. These investigations indicate the presence of internal polyphosphate storage granules in ANME cells, and numerous storage granules with unknown composition in the associated SRB partner. In addition, high resolution imaging reveals close physical contact between cells of different types, underscoring the possibility of metabolic coupling between them. Concomitant to these imaging techniques, taxonomic identification of individual microorganisms in prepared thin sections of the consortia is made possible by employing florescence *in situ* hybridization (FISH). The pairing of these techniques gives the ability to study these highly structured consortia at unprecedented spatial resolution and deliver information as to individual organism roles within this complex ecosystem.

As an extension to these studies, histological approaches are being developed to query the presence of functional gene products. The enzymes methyl-coenzyme M reductase

(Mcr) as well as nitrogenase (Nif) are being targeted to probe enzyme distribution within microbial consortia. These enzymes represent carbon and nitrogen entry points into the metabolism of ANME/SRB consortia and their identification holds the potential to directly reveal specialization and resource partitioning at the cellular level.

In tandem to conducting cell specific image based analyses, environmental metaproteomic data from methane-seep sediments and microcosm experiments containing ANME/SRB consortia are being used to survey the distribution of expressed proteins. Preliminary proteomic profiling of methane seep sediments reveal many enzymes known to be involved in methane oxidation and sulfur reduction pathways. These analyses indicate that it is possible to extract a significant number of proteins from environmental samples, and furthermore that these proteins can be identified using a locally created metagenomic database. We have found that the efficiency of protein extraction from soil microbes is influenced strongly by the type of soil matrix in which they reside and early results show that protein extraction is inhibited by the presence of clays. These findings will help tailor microcosm setups for in-depth exploration and validation of partnership between ANME and SRB consortia in deep sea methane seep sediments. The ability to profile proteomes from samples of variable environments allows for the identification of gene products of known and unknown function, and will thus be of value in identifying the metabolic modes utilized in the anaerobic oxidation of methane.

Collectively, these investigations indicate that many of the processes occurring in anaerobic methane oxidizing microbial communities are discernable utilizing culture independent techniques and aid in understanding the role of these organisms in global elemental cycling.

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How Monomer Availability Impacts the Scramble for Carbon During Plant Polymer Decomposition: Using Pure Cultures as a Predictive Model System

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Project Goals: Microbes are responsible for decomposing plant materials in terrestrial systems, which has an important impact on carbon sequestration. During the decomposition of plant materials, microorganisms take up monomers for use in respiration and biosynthesis. Plant cell wall polymers, such as cellulose, pectin and lignin, are degraded into monomers by microorganisms capable of producing extracellular enzymes. However, production of extracellular enzymes is an energy-intensive process. Therefore, our project goal is to describe in detail how monomer availability affects microbial physiology and gene transcription. Furthermore, from this information we will predict the identity and importance of "cheating" organisms during decomposition. In this project, we categorize microorganisms involved in decomposition as 1) Investors, the microorganisms that produce extracellular enzymes to break down complex plant material into simple molecules; 2) Obligate cheaters, who do not carry genes of extracellular enzymes but who take up monomers released from plant polymers; 3) Opportunistic cheaters, who do have genes of extracellular enzymes, but suppress expression of these enzymes and still take up degradation products.

In order to understand the interaction between decomposer activity and carbon supply during the decomposition process, we grew *Talaromyces stipitatus* NRRL 1006, a potential investor/opportunistic cheater, in sand microcosms with cellulose as the sole carbon source. We amended microcosms with monomers in two experiments to monitor shifts in gene expression and microbial physiology in response to different amounts and types of labile carbon monomers. Here we describe patterns in respiration; responses in extracellular enzyme activity, biomass, and gene expression will also be presented. In the first experiment, xylose was amended at four concentrations ranging from 10 mM to 120 mM in order to examine concentration thresholds resulting in a physiological and transcriptomic response to monomers. Respiration responded to all xylose amendments, but was highest for the 60 and 120 mM amendments. In the second experiment, we amended microcosms with 60 mM solutions of glucose, xylose, galacturonic acid, and vanillin to determine the effect of switching from growth on cellulose and corresponding glucose monomers, to other monomer types. Surprisingly, respiration increased the most due to xylose amendment, followed by glucose and galacturonic acid. Respiration was suppressed by vanillin.

These experiments will be repeated with 13 additional model fungal and bacterial species with completed genomic sequences. These organisms include potential investors and cheaters. In initial experiments, the respiration rate of investors growing on cellulose in sand microcosms showed periodic increases and decreases during 20 days incubation, whereas the respiration rate of cheaters peaked quickly and then went flat. Small subunit RNA copy numbers of cheaters and potential investors continued to shift following leveling off of respiration rate.

Combining data from these experiments, it is hypothesized that "cheaters" will be identifiable as the organisms with the highest monomer concentration thresholds resulting in suppression of extracellular enzyme genes.

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Methanogenic Archaea and the Global Carbon Cycle: A Systems Biology Approach to the Study of *Methanosarcina* Species

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Project Goals: 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. Strains from additional well-studied and important ecosystems will be isolated and examined at later stages of the project. 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.

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. One of the initial successes of the first year of funding was to develop a genome-scale model of *Methanosarcina acetivorans* metabolism.

Methanosarcina acetivorans strain C2A is a marine methanogenic archaeon notable for its substrate utilization, genetic tractability, and novel energy conservation mechanisms. To help probe the phenotypic implications of this organism's unique metabolism, we have constructed and manually curated a genome-scale metabolic model of *M. acetivorans*, iMB745, which accounts for 745 of the 4540 predicted protein coding genes (16%) in the *M. acetivorans* genome. The reconstruction effort has identified key knowledge gaps and differences in peripheral and central metabolism between methanogenic species. Using flux balance analysis, the model quantitatively predicts wild type phenotypes and is 96% accurate in knockout lethality predictions compared to currently available experimental data. The model was used to probe the mechanisms and energetics of byproduct formation and growth on carbon monoxide, and the nature of the reaction catalyzed by the soluble heterodisulfide reductase HdrABC in *M. acetivorans*. The genome-scale model provides quantitative and qualitative hypotheses that can be used to help iteratively guide additional experiments to further the state of knowledge about methanogenesis.

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Identification of S-layer Proteins in the Methanosarcinaceae

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Project Goals: One major goal of this collaborative project addresses the identity, structure and function of archaea envelopes that provide cell protection from environmental challenges. Our model organisms include key methanogenic species involved in anaerobic carbon cycling and methane production. An understanding of their ability to adapt and thrive in changing habitats impacts fundamental processes of microbial biomass transformations, CO_2 sequestration, and energy generation by anaerobic microorganisms.

The cell envelopes of many archaeal species[1] have a proteinaceous surface or lattice termed the surface-layer (S-layer). It is typically composed of only one or two abundant, often post-translationally modified proteins that self-assemble to form a highly organized surface-exposed array. Currently, very little is known about the properties of such surface arrays in any archaean. Surprisingly, over a hundred proteins were annotated to be S-layer or surface associated components in the *Methanosarcina mazei, Methanosarncina acetivorans,* and *Methanosarcina barkeri* genomes, reflecting limitations of current bioinformatics predictions[2,3]. To experimentally address what proteins are present, we devised an *in vivo* biotinylation technique to affinity tag all surface-exposed proteins that overcame challenges in working with these fragile microorganisms. The *Methanosarcina* species were adapted to growth under N_2 fixing conditions to minimize the level of free amines that would interfere with the NHS-label acylation chemistry used[4]. A

3-phase separation procedure was then employed to isolate the intact labeled cells from any lysed-cell derived proteins. The Streptavidin affinity enrichment was followed by stringent wash to remove non-specifically bound proteins, and LC-MS-MS methods were employed to identify the labeled surface proteins. The major surface layer protein was identified in all three species to belong to a small highly conserved group of hypothetical proteins. They were shown to be present in multiple glycosylated forms by using SDS-PAGE coupled with glycoprotein-specific staining, and by interaction with the lectin, Concanavalin A. This family of related S-layer proteins/genes identified in all the sequenced *Methanosarcina* genomes exhibited similar features including a signal P sequence, tandem DUF1608 domains, and a C-terminal hydrophobic transmembrane helix. To address S-layer structure and function, crystallographic studies were performed whereby the *M. acetivorans* S-layer protein DUF1608 domain structure was determined at 2.3 A. This structure provides a model for S-layer protein assembly onto the cell surface to form a lattice. Finally, several pore types were revealed that would allow for movement of small molecules to the cytoplasmic membrane. In conclusion, these studies reveal a conserved protein signature within the *Methanosarcinaceae* having distinct protein features and implied architecture that is absent in other archaea.

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