

Oral Session I

10:15 AM-12:30 PM

Saturday March 12

McClung Auditorium

10:00 AM – Coffee Break

10:15 AM

1: Metabolic Pathways Of Hydrocarbon-degrading Bacteria From The Deepwater Horizon Oil Spill

Nina Dombrowski*, John A. Donaho, Tony Gutierrez, Kiley W. Seitz, Andreas P. Teske, Brett J. Baker

University of Texas Austin

The Deepwater Horizon (DWH) blowout in the northern Gulf of Mexico represents one of the largest marine oil spills. Significant shifts in bacterial community composition in the water column correlated to the microbial degradation and utilization of oil hydrocarbons. Nevertheless, the full genetic potential and taxon-specific metabolisms of bacterial hydrocarbon degraders enriched during the DWH spill remain largely unresolved. To address this gap, we reconstructed genomes of novel, marine bacteria enriched from sea surface and plume spill communities by coupling stable-isotope probing with genomic reconstruction. Thereby, we could identify bacterial community members that actively assimilate alkane and polycyclic aromatic hydrocarbons (PAHs) and resolve their physiological capabilities. We reconstructed a total of 7 genomes, including one genome from an alkane SIP-enrichment using n-hexadecane and three genomes each from PAH SIP-enrichments using naphthalene or phenanthrene. *Marinobacter* represented the active, alkane-degrading SIP-population, while uncultured Alpha- and Gammaproteobacteria, such as *Thalassospira* and *Alcanivorax*, characterized the PAH-degrading communities. Despite the observation that only *Marinobacter* actively degraded alkanes, metabolic pathways for alkane degradation were present in all of the 7 assembled high-quality genomes. Interestingly, the biochemical repertoire

of PAH utilization varied among the different bacterial species and the combined capabilities of the microbial community exceeded those of its individual components. Altogether, this implies that the degradation of a complex hydrocarbon mixture requires the non-redundant capabilities of a complex oil-degrading community.

10:30 AM

2: Microbial Community Response and Crude Oil Biodegradation in Different Deep Oceans

Jiang Liu*, Julian L. Fortney, Stephen M. Techtmann, Dominique C. Joyner, Terry C. Hazen

University of Tennessee Knoxville

Many studies have shown that microbial communities can play an important role in oil spill clean up. However, very limited information is available on the oil degradation potential and microbial community response to crude oil contamination in deep oceans. Therefore, we investigated the response of microbial communities to crude oil in various deep-sea basins from around the world where oil exploration is anticipated (Eastern and Central Mediterranean Sea, Great Australian Bight and Caspian Sea). In this study, microcosms were set up aerobically with three different treatments: seawater, seawater + oil and seawater + oil + oil dispersant. Samples were taken at three time points for the analysis of oil degradation by fluorescence and GC-MS, and microbial community changes by 16S rRNA sequencing. CO₂ evolution followed a similar pattern in all of the basins sampled. The treatment of seawater + oil + dispersant had the highest CO₂ production. The amendment of oil lead to a higher CO₂ accumulation than seawater treatment. However, they were all much lower than the data from the Gulf of Mexico (GOM). What's more, the dissolved organic mater revealed that a big portion of oil in the microcosms was degraded in the first several

*indicates presenting author

days, which was consistent with the GC-MS results. Oil biodegradation appears to occur rapidly in all of the sites. In addition, there was a clear succession of microbial communities during degradation of oil. The microbial diversity decreased in all of the microcosms over time. Oil amendment affected how quickly the diversity decreased. The relative abundance of Proteobacteria increased drastically while the relative abundance of archaea decreased. In particular, the percentage of Betaproteobacteria increased in samples from the Central Mediterranean Sea. However, Gammaproteobacteria increased in abundance in the microcosms from the Eastern Mediterranean Sea and Great Australian Bight, which was very similar to GOM.

10:45 AM

3: Microbial Nitrogen And Methane Sinks In The Water Column Of A Large Coastal Hypoxic Area, The Gulf Of Mexico “Dead Zone”

Mary Katherine Rogener*, Brian J. Roberts, Nancy N. Rabalais, Frank J. Stewart, Samantha B. Joye

University of Georgia

Excess nitrogen in coastal environments leads to eutrophication, harmful algal blooms, habitat loss, oxygen depletion and reductions in biodiversity. As such, biological nitrogen (N) removal through the microbially-mediated process of denitrification is a critical ecosystem function that can mitigate the negative consequences of excess nitrogen loading. However, denitrification can produce nitrous oxide, a potent greenhouse gas, as a byproduct under some environmental conditions. To understand how excess nitrogen loading impacts denitrification, we measured rates of this process in the water column of the Gulf of Mexico “Dead Zone” three times over the summer of 2015. The Dead Zone is generated by excessive nitrogen loading from the Mississippi River co-occurring with strong water column stratification, which leads to a large summer-time hypoxic/anoxic area at the mouth of the river and along the coast of Louisiana. Rates of denitrification ranged from 31 to 153 nmol L⁻¹ d⁻¹. Dead Zone waters are also enriched in methane and methane oxidation rates ranged from 0.009 to 0.4 nmol L⁻¹ d⁻¹. Maximal denitrification rates

were observed at stations with highest methane oxidation rates, suggesting a potential coupling between nitrate reduction and methane oxidation which both scrubs reactive N and methane from the system, thus performing a dual ecosystem service.

11:00 AM

4: Isolation and Characterization of Anaerobic Microbial Communities from Hydraulic Fracturing Fluids

Sheridan S. Brewer, Maria F. Campa, Amanda Garcia de Matos Amaral, Stephen M. Techtmann, Katie Fitzgerald, Julian L. Fortney, and Terry C. Hazen

University of Tennessee Knoxville

Hydrocarbon production from hydraulic fracturing of gas shale in the US has skyrocketed and is projected to keep growing. This water intensive drilling process creates toxic wastewater without an efficient disposal method. Because this method involves projecting fluid 1-3 km deep into the Earth, it is likely that microbial communities adapted to the extreme conditions of the subsurface have accumulated in the produced water. The goal of this study is to identify microorganisms that might have bioremediation capabilities for the toxic flowback water and characterize microbes isolated from fracking water samples in anaerobic conditions. Water samples were obtained from hydraulic fracturing locations in the Marcellus shale of Pennsylvania. These water samples include six different collections of flowback water, a flowback mix tank, and three different treatment tanks. Inoculations from the water samples were grown in anaerobic conditions in high salinity marine media and halotolerant hydrocarbon degradation dependent media. Samples were also grown at ambient temperature and at 37°C. DNA was extracted, and 16S rRNA gene Sanger sequencing was used to identify individually isolated microbes. Illumina sequencing was used to yield genetic information about the overall microbial communities. The Biolog Omnilog, a high-throughput phenotype microarray, was used to determine the genotype-phenotype characteristics of some of the most significant isolates. Early results show presence of numerous anaerobic microbes with metabolic variability and bioremediation potential including sulfate

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reducers and hydrocarbon degraders. There also have been a considerable number of human pathogens identified with the capability for antibiotic resistance from biocide exposure.

11:15 AM

5: Metagenomics Reveals The Response Of Regionally Pervasive Bacterial Populations To Warming In An Alaska Tundra Ecosystem

Eric R. Johnston*, Luis M. Rodriguez-R, Chengwei Luo, Mengting M. Yuan, Liyou Wu, Zhili He, Edward A.G. Schuur, Yiqi Luo, James M. Tiedje, Jizhong Zhou, and Konstantinos T. Konstantinidis

Georgia Institute of Technology

How soil microbial communities contrast with respect to taxonomic and functional composition within and between ecosystems remains an unresolved question that is central to predicting how global anthropogenic change will affect soil functioning and services. This is especially relevant for remote, northern latitude soils, which are challenging to sample and are also thought to vulnerable to climate change for greenhouse gas release. Here, we employed well-replicated shotgun metagenome sequencing, allowing for near-complete coverage of microbial community richness (~92% breadth), and the recovery of twenty-seven near-complete, high-quality population bins. These population bins, collectively, made up to ~10% of the total microbial community, and represented diverse taxonomic groups and metabolic lifestyles tuned toward sulfur cycling, hydrogen metabolism, methanotrophy, and organic matter oxidation. Several population bins were also present in geographically distant (~100-530 kilometers apart) tundra habitats (full genome representation and >99.6% genome-derived average nucleotide identity); thus, their relative contribution to various ecosystem functions is expected to be high. Our work demonstrates the response of these bacterial populations to in-situ warming of 2-5°C for 5 years and also reveals that these populations are highly dynamic, and can undergo rapid genomic alternations in gene content upon major environmental perturbations such as fire events.

11:30 AM

6: Activity and diversity of aerobic methanotrophs in a coastal marine oxygen minimum zone

Cory C. Padilla*, Laura A. Bristow, Neha Sarode, Emilio Garcia-Robledo, Peter R. Girguis, Bo Thamdrup, and Frank J. Stewart

Georgia Institute of Technology

The pelagic ocean is a sink for the greenhouse gas methane, with methane consumption regulated primarily by aerobic methane-oxidizing bacteria (MOB). Marine oxygen minimum zones (OMZs) contain the largest pool of pelagic methane in the oceans but remain largely unexplored for their potential to harbor MOB communities and contribute to methane cycling. Here, we present meta-omic and geochemical evidence that aerobic MOB are present and active in a coastal OMZ, in Golfo Dulce, Costa Rica. Oxygen concentrations were 5% of total 16S rRNA genes and >19% of 16S rRNA transcripts. This peak in ribosomal abundance and activity was affiliated with methane oxidation rates of 2.6 ± 0.7 nM d⁻¹, measured in seawater incubations with estimated O₂ concentrations of 50 nM. In contrast, methane oxidation was below detection at lower depths in the OMZ (100 m and 120 m). Metatranscriptome sequencing indicated a peak at 90 m in the expression of pathways essential to Methylococcales, including aerobic methanotrophy and the RuMP pathway of carbon assimilation, as well as the serine pathway of Type II methanotrophs. Preliminary analysis of metagenomic data suggests distinct adaptations by Methylococcales from the Golfo Dulce, helping explain the persistence of putative aerobic methanotrophs under very low oxygen in this OMZ. Taken together, these data suggest the boundary layers of OMZs, despite extreme oxygen depletion, are a niche for aerobic MOB and therefore potentially important zones of pelagic methane loss.

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11:45 AM

7: A novel isolate and widespread abundance of the poorly-described alphaproteobacterial order (Ellin 329), in southern Appalachian peatlands

Austin B. Harbison*, Michael A. Carson, Louis J. Lamit, Nathan Basiliko, Suzanna L Brauer

Appalachian State University

Peatlands of all latitudes play an integral role in global climate change by serving as a carbon sink and a primary source of atmospheric methane; however, the microbial ecology of mid-latitude peatlands are vastly understudied. Herein, next generation Illumina sequencing was utilized to elucidate the microbial assemblages in three southern Appalachian peatlands. In contrast to northern peatlands, Proteobacteria dominated over Acidobacteria according to relative abundance values in all three sites. An average of 11 bacterial phyla were detected at relative abundance values >1%, with three candidate divisions (OP3, WS3 and NC10) represented, indicating high bacterial diversity. Members of the Proteobacteria, including Alphaproteobacteria are thought to be primarily involved in the utilization of simple sugars. However, physiological traits of isolates of a novel alphaproteobacterial order, Ellin 329, obtained here and in previous studies, indicate that bacteria of this order may also be involved in poly- and di-saccharide hydrolysis. Sequencing data indicate that Ellin 329 is the third most abundant order and is most abundant near the surface layers where plant litter decomposition should be primarily occurring. In sum, members of the novel alphaproteobacterial order, Ellin 329 likely play important roles in organic matter decomposition, at least in these three southern Appalachian peatlands.

12:00 PM

8: Microalgae Cryopreservation: Your Experimental Backup Copy For Research

Estefania Paredes

University of Tennessee Knoxville

A backup copy is a duplicate instance of a data file, application, system or server that's created using backup software. It's used as a means to restore original data in case it's deleted, corrupted or lost. What if this could be applied this definition to our experimental organisms or community of organisms? It is possible. Cryopreservation has been successfully applied to numerous species of freshwater and marine microalgae. Among the advantages of applying cryopreservation to the test organisms of research are the possibility of maintaining an exact copy of your research microalgae in "suspended animation" without the need of time consuming maintenance, in less space, with less requirements and maintaining the exact same characteristics without the risk of cross contamination or the selective pressure of continuous reseedings. This is a quick overview of the methodologies available and of the successful cryopreserved species.

12:15 PM

9: Thorarchaeota: a Novel, Widespread Sediment Archaeal Phylum

Kiley W. Seitz*, Jimmy Saw, Eva Fernandez-Caceres, Lina Juzokaite, Kasia Zaremba-Niedzwiedzka, Anja Spang, Andreas P. Teske, Thijs J.G. Ettema, and Brett J. Baker

University of Texas Marine Science Institute

Marine and estuary sediments contain a variety of uncultured archaea whose metabolic and ecological roles are largely unknown. Unlike many other methods for environmental community analysis, the ability to construct unique genomes allows for insight into the ecological roles microbes play in the environment. De novo assembly and binning of high-throughput metagenomic sequences from estuarine sediments collected from the White Oak River in North Carolina revealed partial to near-complete (2.4-3.9Mb) genomes belonging to a previously

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unrecognized archaeal group. Phylogenetic analyses of concatenated ribosomal proteins and 16S rRNA genes revealed that these genomes represented a widespread and deeply-branched group that we proposed be named "Thorarchaeota". Recent community analysis of hydrothermal sediments of the Guaymas Basin in the Gulf of California (Mexico) indicate that Thorarchaea are a prominent member of the archaeal community. Reconstructed genomes from both sites were used to determine potential metabolic pathways for these archaea. The Thorarchaeota appear to be capable of acetate production from the degradation of proteins and carbon fixation via the Wood-Ljungdahl pathway. They have elemental sulfur and thiosulfate reduction genes suggesting they play an important role in intermediate sulfur cycling. Along with their metabolic potential, the deeply-branched phylogenetic position of these Thorarchaea indicates they may provide insights into the historical relationships between eukaryotes and archaea. The inference of the physiologies of this previously overlooked phylum enhances our understanding of biogeochemical cycling in sediments and evolution of eukaryotes.

Oral Session II
1:30 PM- 2:45PM
Saturday March 12
McClung Auditorium

1:30 PM

**10: Changes in Phytoplankton
Communities in Response to Oil and
Nutrients in the Northern Gulf of
Mexico: Correlating Experiments with
Field Observations**

Nigel D'souza*, Sarah Weber, Ajit Subramaniam,
Andrew Juhl, and Joseph Montoya

Georgia Institute of Technology

Natural hydrocarbon seeps account for around 47% of the oil released into the environment, with seeps in the Gulf of Mexico releasing up to 1.1×10^8 L oil year⁻¹. Our previous work in the Gulf of Mexico has linked natural hydrocarbon seepage occurring at depths exceeding 1000 meters to elevations in surface and sub-surface chlorophyll concentrations in the upper water column via plume-driven upwelling of nutrient rich waters from depth. Nutrients are also supplied episodically by the offshore extension of the Mississippi River Plume and upwelling events associated with loop-current eddies. We conducted a series of microcosm experiments across a range of offshore habitats to explore nutrient limitation of native phytoplankton communities in surface waters in the Northern Gulf of Mexico, including regions influenced by natural seeps. Phytoplankton appear to be co-limited by N and P, with noticeable differences in how different components within the phytoplankton assemblage respond to nutrient amendments. Furthermore, a change in the phytoplankton community structure was also observed upon oil inputs, with cryptophytes dominating the oil-amended microcosms. Field observations in the vicinity of natural seeps revealed similar shifts in phytoplankton composition.

1:45 PM

**11: Anaerobic Hydrocarbon
Degradation Under Iron- And Sulfate-
reducing Conditions By Sedimentary
Microorganisms From The Northern
Gulf Of Mexico**

Boryoung Shi*, Will A. Overholt, Kuk-jeong Chin,
Joel E. Kostka

Georgia Tech

This study investigates the rates and the microbial communities that catalyze anaerobic hydrocarbon degradation in areas of the Gulf of Mexico seafloor that were impacted by the Deepwater Horizon blowout. Sediment samples were collected on research cruises from 2012 to 2015. Enrichment cultures were initiated and pure cultures were obtained under iron- or sulfate-reducing conditions with hexadecane, or phenanthrene as the sole carbon source. Microbial activity was confirmed by quantification of electron acceptor utilization and microbial communities were characterized using next generation sequencing of 16S rRNA gene amplicons. Results indicate that sediment from shallower water depths stimulated faster growth of hydrocarbon degraders than sediment from the deeper sites. Members of the Deltaproteobacteria showed a high relative abundance in all enrichment cultures studied. Desulfobacteraceae were highly enriched, showing up to 50% relative abundance, in sediment-free cultures under sulfate-reducing conditions. Known sulfate-reducing groups such as Thermodesulfobacteriaceae and Desulfobulbaceae were enriched under iron-reducing conditions. Evidence suggested the potential for syntrophic hydrocarbon degradation under sulfate-reducing conditions.

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2:00 PM

**12: Assessing Hydrocarbon
Incorporation Into The Planktonic
Food Web At Cold Seeps**

Ana Clavère-Graciette*, Drake Lee-Patterson,
Joseph.P. Montoya

Georgia Institute of Technology

Oil and gas are constantly injected in the Gulf of Mexico (GoM) through natural seepage. While a large fraction of the hydrocarbons released by seeps are dissolved in the water column, accumulate at the surface, or are released to the atmosphere, a portion is incorporated directly into marine food webs. Over 20,000 cold seeps cover the sea floor in the GoM and probably account for significant incorporation of hydrocarbons into the food web. Chronic exposure to natural seepage could potentially result in hydrocarbon incorporation into the planktonic food web, so, baseline measurements are essential when evaluating impacts of accidental oil exposures like those seen during the Deep Water Horizon (DWH) incident. Low $\delta^{13}\text{C}$ values are characteristic of oil and methane derived carbon, while low $\delta^{15}\text{N}$ values are characteristic of organic nitrogen produced through nitrogen fixation. Here, we explore the effect of natural hydrocarbon seeps on the planktonic food web by assessing nitrogen and carbon stable isotopes in zooplankton collected over several years at three sites with different seepage activities. Our results reflect seasonal variations, with higher zooplankton biomass and lower $\delta^{15}\text{N}$ values in June than in September. This suggests higher incorporation of nitrogen derived from nitrogen-fixation during the late spring at all three stations, potentially fueling part of the increased biomass. Lower $\delta^{13}\text{C}$ values in June suggest the incorporation of an isotopically depleted source of carbon, potentially hydrocarbons. Zooplankton biomass and $\delta^{15}\text{N}$ values also show spatial variation, but only small differences in $\delta^{13}\text{C}$ values despite strong contrasts in seepage activity between sites. Our measurements thus provide a record of N and C sources supporting zooplankton biomass across a range of offshore habitats.

2:15 PM

**13: Comparative Genomics and
Heterologous Expression Studies
Reveal Cobamide Biosynthesis in
Geobacter Species**

Nannan Jiang*, Jun Yan and Frank E. Löffler

University of Tennessee Knoxville

Corrinoids such as vitamin B₁₂ and natural analogues serve as cofactors for reductive dehalogenases (RDases) that catalyze hydrogenolytic carbon-halogen bond cleavage. Previous studies have demonstrated that the lower bases attached to these corrinoid cofactors impact the activity of RDases differently in corrinoid auxotrophic *Dehalococcoides mccartyi* (Dhc) strains, which prefer a corrinoid with 5,6-dimethylbenzimidazole (DMB) as the lower base. In contrast, *Geobacter sulfurreducens* strain PCA produces a corrinoid with 5-hydroxybenzimidazole (5-OHBza) as the lower base. 5-OHBza is a DMB precursor but does not support RDase activity in Dhc strains. In this study, we cloned and heterologously expressed a unique *Geobacter lovleyi* O-methyltransferase gene implicated in the methylation of 5-OHBza to 5-methoxybenzimidazole (5-OMeBza). In contrast to 5-OHBza, 5-OMeBza supports Dhc reductive dechlorination activity. HPLC and LC-MS analyses demonstrated that the *Geobacter sulfurreducens* mutant expressed the O-methyltransferase and the native 5-OHBza-cobamide was converted to 5-OMeBza-cobamide. Co-cultivation of Dhc strain BAV1 and the recombinant *Geobacter sulfurreducens* in the absence of exogenous vitamin B₁₂ supported Dhc growth and the complete dechlorination of cis-1,2-dichloroethene to ethene. Collectively, these studies demonstrated that the O-methyltransferase catalyzes a critical step in DMB biosynthesis and provides a genetic basis for the observation that Dhc reductive dechlorination activity occurs in co-cultures with *Geobacter lovleyi* but not with *Geobacter sulfurreducens*. These findings emphasize the critical role of the community for sustaining the activity of corrinoid-auxotrophic organohalide-respiring Chloroflexi, including Dhc.

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2:30 PM

**14: Quantification of Deep Subseafloor
Bacteria and Archaea – An Inter-
Laboratory Comparison**

Joy Buongiorno*, Stephanie Turner, Gordon
Webster, Alexander Schumaker, Taylor Roy,
Andrew Weightman, Axel Schippers, Karen G.
Lloyd

University of Tennessee Knoxville

The marine subsurface biosphere is populated by abundant, diverse, and geographically distinct microorganisms. The two most common methods used for quantifying particular microbial taxa within subseafloor sediments are qPCR and CARD-FISH. Here, qPCR and CARD-FISH were performed on Baltic Sea Basin (IODP Exp. 347) sediment samples down to 85 mbsf by independent laboratories in three different institutions. Working with similar protocols, qPCR and CARD-FISH quantification data were reproducible across the laboratories, with total cell counts ranging from 1.39×10^7 to 1.87×10^9 cells/mL. The highest yields of qPCR were obtained with the use of an additional sediment slurry preparation step, resulting in 3.88×10^6 to 2.34×10^9 copies/cm³ combined bacterial and archaeal 16S rRNA gene copy numbers. Overall, the qPCR results consistently showed that bacteria are more abundant than archaea in these sub-seafloor sediments, and 88% of the samples contained archaea and bacteria above the qPCR quantification limit. Using the same bacterial and archaeal primers to amplify DNA extracted with similar protocols resulted in no significant difference (p -value > 0.05) in qPCR values across laboratories. Although total cell counts from direct counting were all above the quantification limit of 1.3×10^7 cells/mL (after accounting for the dilution to physically separate cells from sediment), only 45 out of 435 counts with CARD-FISH were above this quantification limit, with no interlaboratory differences. Proteinase K permeabilization of archaeal cell walls was not sufficient to bring these samples above the quantification limit. In addition, this study showed that quantification of particular microbial taxa gives inconsistent results when comparing CARD-FISH and qPCR, and that further improvement of methods is required for absolute quantifications. However, relative quantifications using similar methods are possible with qPCR, even when the

work is performed by different research groups.

2:45 PM- Coffee Break

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Oral Session III

3:15 PM- 4:15 PM

Saturday March 12

McClung Auditorium

3:15 PM

15: Microbial Diversity and Predictive Functions in Decadal Growth Layers of Large Conical Microbial Mats in Lake Untersee, Antarctica

Hyunmin Koo*, Dale T. Andersen, and Asim K. Bej

University of Alabama at Birmingham

Stromatolites are laminated accretionary sedimentary structures formed by microbial communities under sharply-defined fluctuating geochemical gradients. Biogenic, laminated conical stromatolites that rise to ~0.5 m above the lake floor have been discovered in the permanently ice-covered Lake Untersee (LU), the largest (11.4 km²) and deepest (~160 m) lake in East Antarctica. In this study, we have used the pyrosequencing approach and analyzed the data using bioinformatics tools in order to investigate the bacterial and cyanobacterial diversity associated with the three decadal growth layers (U1=upper; U2=middle, and U3=inner) of these microbial mats. The U1 was dominated by Cyanobacteria while U2 and U3 layers were dominated by Actinobacteria and Verrucomicrobia. Further, distinct taxa of each family and genus levels were identified using Oligotyping analysis; 20 oligotypes in Phormidium, 15 in Leptolyngbya, 12 in Cytophagaceae, 14 in Opiritaceae, and 84 in Xanthomonadaceae. PICRUSt analysis, which predicts the gene content of microbial community and compares the abundance of genes involved in KEGG metabolic pathways, revealed a high number of sequences related to energy metabolism in U1; whereas in U2 and U3 depicted amino acid metabolism, carbohydrate metabolism, and membrane transport. Interestingly, photosynthesis-antenna proteins were only found in Cyanobacteria, and phosphotransferase systems were only found in heterotrophs. In general, our study reveals an understanding of the microbial distribution and their functional

attributes for these mats, which are analogous structures of ancient stromatolites.

3:30 PM

16: Paired isotope records of carbonate and organic matter, Middle Ordovician (Darriwilian) of Argentina: Implications for global climate change

Miles A. Henderson*, Linda C. Kah

The University of Tennessee Knoxville

The Middle Darriwilian Isotope Carbon Excursion (MDICE), a globally recognized positive shift (+2 to +4‰) in the carbon- isotopic record of Darriwilian-Sandbian age marine carbonates, may represent the effect of changing ocean circulation patterns driven by long-term global cooling during the transition from greenhouse to icehouse climate conditions. The timing of this event is coincident with conodont thermometry suggesting global cooling in the Middle-Ordovician that may have ultimately led to the end Ordovician glaciation. The MDICE may thus represent ventilation of the Early Ordovician stratified ocean driven by invigorated thermohaline circulation, which led to upwelling of nutrient rich deep-waters, increased nutrient availability, enhanced organic productivity, and burial of organic carbon. The consequence of this event is recorded in the C-isotope record of authigenic marine carbonates, which are often used as a proxy for atmospheric pCO₂, since photosynthetic fractionation (p) dominates the C-isotopic composition of carbonate (¹³C_{carb}) and organic matter (¹³C_{org}). Recent biostratigraphic work on the Darriwilian-Sandbian aged Las Chacritas, Las Aguaditas, and Gualcamayo Formations in the Argentine Precordillera make them ideal for global correlation of our isotopic profiles with records documented elsewhere. Here we expand the carbon-isotopic record of the Middle-Ordovician of Argentina with new high- resolution ¹³C_{carb} and ¹³C_{org} profiles. The resulting Δ¹³C record of

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the Argentine Precordillera is utilized to approximate environmental changes during this interval of global climate change and oceanographic upheaval.

3:45 PM

**17: Iron mineral size controls
microbial methane dynamics in
ferruginous sediments**

Marcus S. Bray*, Jieying Wu, Benjamin C. Reed,
Cecilia B. Kretz, Frank J. Stewart, Thomas J.
DiChristina, Jay A. Brandes, David A. Fowle, Sean
A. Crowe, Jennifer B. Glass

Georgia Tech

Reactive Fe(III) minerals can reduce methane emissions by inhibiting microbial methanogenesis and stimulating anaerobic methane oxidation. Thus, it is puzzling how ferruginous Archean oceans supported high methane fluxes that warmed the early Earth under the Faint Young Sun. In this study, we examined the effects of Fe(III) on methane cycling in anoxic incubations of ferruginous sediment from Lake Matano, Indonesia. Bathyarchaeota were the most abundant microbial phylum, and increased with sediment depth from 28 to 47% total 16S rRNA sequences. Sediments were incubated with CH₄ or N₂ headspace in artificial freshwater amended with synthetic Fe(III) oxides as amorphous ferrihydrite or goethite. Initially, an endogenous crystalline Fe(III) mineral, likely nanogoethite, was reduced, and headspace methane concentrations declined. Stoichiometry was consistent with the predicted ratio of 1 CH₄ oxidized per 8 Fe(III) reduced. By day 4, the native reactive crystalline phase was depleted, and activity shifted to reduction of endogenous and/or synthetic ferrihydrite accompanied by methane production. Methane production was 20-fold greater during endogenous vs. synthetic ferrihydrite reduction. Bulk goethite was not reduced. ¹³C-labeled methane appeared in the DIC pool on days 6 and 8 for synthetic ferrihydrite and goethite amendments, respectively. The offset between the period of methane consumption and the subsequent appearance of the ¹³C label in DIC suggests that methane was initially assimilated into biomass and subsequently remineralized. 16S rRNA sequencing revealed differential enrichment of Deltaproteobacteria in the families

Desulfuromonadaceae and Geobacteraceae in ferrihydrite vs. goethite amendments, respectively. The rapid shift we observed, from methane consumption in the presence of nanogoethite, to methanogenesis in the presence of bulk goethite, suggests that Fe(III) oxide aggregate size should be integrated into future updates of Archean climate models.

4:00 PM

**18: Biogeochemical Controls over
Corrinoid Bioavailability to
Organohalide-Respiring Chloroflexi**

Burcu Şimşir*, Jun Yan, Allen K. Bourdon, Shawn
R. Campagna and Frank E. Löffler

University of Tennessee Knoxville

Chlorinated solvents such as tetrachloroethene (PCE) and trichloroethene (TCE) are toxic groundwater contaminants, probable human carcinogens. Dehalococcoides mccartyi (Dhc) is the keystone bacterial group involved in complete reductive dechlorination of chlorinated ethenes to benign ethene. The key catalysts are reductive dehalogenases, which require a corrinoid cofactor. Remarkably, Dhc strains lack ability for de novo corrinoid biosynthesis and not all corrinoid types support Dhc activity. To explore if the prevailing geochemical conditions determine the predominant type of corrinoid produced, microcosms were established using sediment collected from a chlorinated solvent-contaminated site in Knoxville, TN. Sulfate-reducing, iron-reducing, nitrate-reducing, methanogenic, acetogenic/fermentative conditions were established, and repeated transfers yielded sediment-free enrichment cultures while maintaining the respective redox conditions. The corrinoids produced in the cultures were extracted and characterized with a newly developed fluorescence-based HPLC method. The initial characterization efforts demonstrated that the electron acceptor available for growth affects the types of corrinoids produced, suggesting that the geochemical conditions influence the composition and concentrations of the bioavailable corrinoid pools. 16S rRNA gene amplicon sequencing and available genome information implicated specific community members as corrinoid producers under the different growth conditions. Apparently, the geochemical conditions and the associated

*indicates presenting author

microbiology determine the corrinoid pool, and consequently affect the dechlorinating activity of corrinoid-auxotrophic Dhc strains. Thus, refined decision-making at chlorinated solvent sites should take into consideration how remedial treatments will affect groundwater geochemistry and the corrinoid pool, and, in turn, support high Dhc reductive dechlorination activity and efficient contaminant detoxification.

Poster Session I
5:30 PM -7:30 PM (A-K)
Saturday March 12
Calhoun's on the River

**1: Microbial Niche Differentiation in
Enzymatic Activities Shaped By
Particle-Association and Salinity
Differences in a High Latitude Fjord**

John Paul Balmonte*, Karylle Abella, Carol Arnosti

University of North Carolina Chapel Hill

Glacial meltwater exports significant amounts of organic carbon and nutrients to fjords, efficiently fueling microbial heterotrophy and primary productivity. High organic carbon burial rates in fjords, however, imply that some glacial and autochthonous organic carbon evade complete remineralization in surface waters, and ultimately sink to the bottom of fjords. Hence, microbially-mediated organic carbon transformations largely determine the quantity and quality of carbon sources available to deep life. To investigate the manner in which fresh glacial meltwater influence microbial carbon processing in a high latitude fjord, we compare whole community versus particle-associated freshwater and marine microbial enzymatic activities from glacial-fed rivers (Tyroler River, Lerbugten River and Zackenbergriver), which connect to the Tyrolerfjord-Young Sound system in northeast Greenland. Using five structurally-varying peptide substrates, we show lowest enzymatic activities by freshwater microbes, and highest by their marine counterparts. In addition, the spectrum of hydrolyzed substrates do not differ between surface and subsurface waters at the same site, but vary along the salinity gradient. Finally, differences between whole-community versus particle-associated enzymatic activities indicate varying enzymatic strategies of particle-associated versus free-living heterotrophic microbes. Altogether, these results demonstrate an apparent microbial niche differentiation in carbon processing associated with salinity differences and particle-association.

**2: Ecological Niche Preferences for
Microbial Surface Mats in the Guaymas
Basin**

Evan Amber*, Howard Mendlovitz, Daniel Albert,
Karen Lloyd, Barbara MacGregor, Andreas Teske

The University of North Carolina at Chapel Hill

Hydrothermal vents provide new opportunities to examine niche partitioning in ecosystems founded on chemosynthetic microbes rather than on photosynthesis. In particular, the Guaymas Basin, a hydrothermal rift center in the Gulf of California that is uniquely characterized by a thick organic-rich sediment layer, has been proposed as a model evolutionary system where spatially and temporally fluctuating chemical and temperature regimes select for physiological adaptations of microbial groups, which in turn conduct chemical transformations of the hydrothermal fluids. Specifically, benthic sulfur-oxidizing bacterial mats, dominated by Beggiatoaceae or Epsilonproteobacteria, mediate chemical fluxes at the sediment-water interface of hydrothermal environments. The chemical and physical selection factors that define the niches of these two contrasting microbial mat communities remain to be identified. Niche definition based on oxygen availability has to be augmented since Guaymas Beggiatoaceae use nitrate as a terminal electron acceptor, while Guaymas Epsilonproteobacteria may be more versatile. This ambiguity displays a need for a clearer identification of the electron donor and acceptor regimes between these mats, as they define the organism's physiological niche. Here, we present contrasting geochemical regimes underlying these two mat types, along with microbial community compositions. The Beggiatoaceae mat (Cathedral Hill) was found to be hotter, higher in DIC and lower in acetate when compared to the Epsilonproteobacteria mat (Vinegar Mat). Further, Cathedral Hill displayed the presence of surface-compressed thermo and chemoclines that are absent in all but the hottest Vinegar Mat cores.

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These contrasts provide evidence of differential niche occupation by each mat community. Further work is needed to better quantify these differences, as well as to assess the influence of oxygen and nitrate on defining mat niche.

3: Bacterial Community Responses to Natural and Anthropogenic Stressors in Louisiana Marsh Soils, 2012-2014

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Microbial communities from salt marshes along Louisiana coastlines are impacted by natural and anthropogenic stressors. Natural stressors include tidal fluctuations and hurricanes. Stressors caused by anthropogenic activities include sea-level rise, changes in land use, and salinity fluctuations due to river diversion projects. Salt marsh microbial community responses to these natural and anthropogenic stressors are poorly constrained, which made it even more difficult to know how microbial communities would respond to oiling from the 2010 Deepwater Horizon oil spill. To begin to evaluate community responses, including oiling, bacterial diversity was examined with physicochemistry and hydrocarbon chemistry from inland marsh soils collected biannually from 18 geographically distinct marshes near Port Sulphur, Grand Isle, and Cocodrie, from 2012 - 2014. The marshes were moderately to heavily impacted by the 2010 Deepwater Horizon oil spill, with peak oiling occurring from fall 2011 through fall 2012 after Hurricane Isaac. After peak oiling, n-alkane biosignatures indicated hydrocarbon compounds were sourced locally from plant waxes. Statistical assessment of communities from nearly 5 million bacterial 16S rRNA pyrotags revealed that taxonomic abundances correlated to environmental gradients, including porewater sulfide concentrations, total organic carbon content, n-alkane composition, oiling history, vegetation height and coverage, tidal inundation frequency, and water depth on the marsh. Geography was not significant, as community diversity for any marsh site through time differed by no more than ~20% of the community at the phylum level or 45% at the class level. But, marsh communities from locations flooded more frequently were distinct taxonomically, suggesting that flooding history and not oiling has recently

controlled community compositions. The results from this study can be used to predict future changes in marsh microbiomes and marsh ecosystem dynamics.

4: The composition and extracellular enzymatic activities of pelagic, particle-associated and benthic bacterial communities in the Central Arctic Ocean

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The structure and function of Arctic bacterial communities have rarely been studied in concert, but are crucial to our understanding of biogeochemical cycles. As the Arctic transitions to become seasonally-ice free, a critical priority is to elucidate the present ecological role and environmental dependence of Arctic bacterial communities. We investigated the depth and regional variations in Central Arctic bacterial community composition (BCC) and extracellular enzymatic activities (EEA)—the initial step in organic matter breakdown—to explore links between community structure and function. Samples were collected across a gradient of sea-ice cover (open ocean, first year ice, multi-year ice) from 79°N to 88°N and from surface to bottom waters (~3.5 to 4.5 km). Pelagic BCC most strongly varies with hydrography and with particle-association, which likely selects for a specialized community of heterotrophic opportunists; benthic BCC show little regional variation. In contrast, EEA reveal significant depth and regional differences in hydrolysis rates as well as in the spectrum of substrates hydrolyzed. Particle-associated EEA reveal an equal or greater range of enzymatic capabilities than in bulk-seawater measurements, supporting previous findings that particles are hotspots of microbial heterotrophic activity. These patterns suggest a complex relationship between BCC, EEA, and the environment: while water mass characteristics consistently differentiate bacterial communities, additional local factors shape their capabilities to hydrolyze organic matter. Multivariate analyses will be used to further explore the relationships between composition and function as well as their correlations with environmental data. Our findings provide a baseline for future comparisons

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and initial insight into the functionality and biogeography of Arctic bacterial communities.

5: Microbial Degradation Of Agricultural Plastics

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Plastic mulches are commonly used in agriculture because they provide conservation of moisture, reduced weed growth and increased soil temperature. Conventional polyethylene black plastic mulches are generally preferred by farmers. However these plastics are environmentally unsafe as polyethylene is not biodegradable and remains in the soil for a long time with unknown fate. A shift to biodegradable plastics is thus required as an environmentally safe and sustainable choice. This work aims to identify the role of microorganisms in degrading commercially used biodegradable plastic mulches. To accomplish this, plastic-degrading microbes were enriched from soils collected from the East Tennessee Research and Educational Center (Knoxville, Tennessee) enriched on biodegradable plastics. Two commercially available biodegradable films were used: a polyhydroxybutyrate (PHB)/polylactic acid blend (produced by Metabolix) and a polybutylene adipate terephthalate (PBAT) based plastic (BioAgri, produced by BASF). Enrichment cultures were done in M9 minimal media with squares of films; initial cultures had 0.8% glucose minimal media and then weaned off of glucose with successive transfers. Five weekly transfers of soil inoculum were done. At each transfer, plastics were weighed to determine weight loss and cultures were stored at -80°C for future molecular and enzymatic characterization. Results indicate weight loss over time with bacterial and fungal colonies visibly growing on mulches. Preliminary microscopy show an increase in surface roughness index and thinning of plastic films as degradation occurs. Taken together, these results confirm that the enrichment has selected for soil microorganisms capable of degrading these commercially available biodegradable plastics. This work provides an important foundation for future studies on the mechanisms of plastic biodegradation in soil.

6: Host range study of a lysogenic phage in a roseophage-roseobacter system and characterization of lysogens in affecting marine biogeochemistry

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Viruses are abundant in the marine environment, where they actively infect marine bacteria and contribute to biogeochemical cycles. Bacterial viruses (phage) display two distinct lifestyles: host lysis or integration in their host's genome (where they are termed prophage). Prophages lie dormant until environmental conditions trigger their induction to a lytic cycle. In a limited number of well-studied phage-host systems, prophages have been able to provide their hosts immunity against infection by other phage. The extent to which this immunity is prevalent in marine bacteria and influences the occurrence of lytic events has not been well studied. We have isolated a marine bacterium, *Sulfitobacter* sp. CB2047, and its infecting phage (ϕ CB2047-A). The bacterium contains a prophage with high sequence similarity (85.65% identity) to ϕ CB2047-A. We have also isolated four additional *Sulfitobacter* strains, isolated from distinct environments and none of which contain a prophage. Integration experiments of *Sulfitobacter* CB2047 with ϕ CB2047-A results in a substitution of the prophage, yield the strain YM3A. We hypothesize that phage integration is possible in the additional *Sulfitobacter* strains and have data to support this for *Sulfitobacter* sp. EE-36. These data increase our understanding of the biotic factors that stimulate prophage integration. We also hypothesize that prophage in their lysogenic state will affect host physiology. Indeed CB2047 and YM3A have distinctly different phenotypes with respect to growth rates, cell size, and biofilm formation, suggesting that roseophage have the ability to dramatically alter bacterial physiology and marine biogeochemistry.

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7: Comparative Genomics Reveals Clues to the Evolutionary History of the Candidate Order Altiarchaeales

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Little is known about the recently proposed archaeal Order Altiarchaeales since they have never been grown in culture. 15 years ago the first member of this group was observed growing as a biofilm in sulfidic springs in Germany. Recent work has revealed that this biofilm-forming archaea, Altiarchaeum hamiconexium, possess two cell membranes, similar to cells of the ARMAN group and Methanomassiliococcus luminyensis, is likely autotrophic, and transcribes proteins associated with its unique, hook-like structure on single operon (Probst et al 2014, Perras et al, 2015). Here we combined previously published metagenome-based genome reconstructions (MGRs) (Probst et al., 2014, Rinke et al., 2013) with MGRs and a single-cell genome from three distantly related, non-biofilm associated members of this group inhabiting brackish, anoxic sediments in North Carolina to generate the first comparative genomic study across this distinct branch of the archaeal tree of life. Phylogenetic analyses of the conserved genes indicate that these organisms form a monophyletic group to include the lone uncultured member of a group previously designated pMC2A384, based on a single cell genome from Sakinaw Lake. Additionally, the Altiarchaeales genomes acquired a synapomorphic mutation in its single copy conserved phenylalanine tRNA synthetase β subunit, PheT, which causes the protein to be encoded as two subunits at distant loci. Comparative analyses suggests that the hook-like hami should not be considered a defining feature of the group, but are restricted to the biofilm forming Altiarchaeum hamiconexium. Lastly, several predicted genes including homologs to a Na⁺ transporter and membrane bound coenzyme A disulfide reductase are unique to the non-biofilm forming, brackish sediment genomes which could indicate adaptations to the marine, sulfur-rich environment where they are found.

8: Expression of Recombinant Orange Guaymas Beggiatoa Spp. Nitrite Reduction Pathway Proteins in E. coli.

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Members of the Beggiatoaceae family are generally characterized as sulfide-oxidizing bacteria that form large, filamentous microbial mats in freshwater or marine environments. Representative taxa contain a large central vacuole that accumulates nitrate that could be used to oxidize sulfide for energy. The Guaymas Basin in the Gulf of California is replete in hydrothermal vents and sulfide-rich seeps that support polymicrobial mat communities that volumetrically consist of mainly Beggiatoa Spp., and may reach several meters in diameter. The Orange Guaymas Beggiatoa Spp. (BOGUAY), like most marine Beggiatoaceae, remain unculturable and must be procured by submersible from the seafloor. Primary sample material subjected to physiologic and enzymatic procedures result in sample destruction, and therefore limits the amount of physiologic studies that can be performed. Amplified BOGUAY genomic DNA was used to create recombinant-proteins in E. coli for use in cell-free enzymatic studies, to reduce the initial amount of sample material used. Three genes currently targeted are BOGUAY_0691, BOGUAY_2386, and BOGUAY_2967, which putatively code for secreted orange nitrite reductase, periplasmic NirS-like nitrite reductase, and membrane associated octaheme nitrite reductase respectively. The classified enzymatic function of the named proteins is to reduce nitrite to either nitric oxide or ammonia. The enzymes represent parts of a proposed nitrate reduction pathway in BOGUAY. The project objective is to generate a cell-free physiologic characterization of Beggiatoa nitrogen metabolic pathways.

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9: Elucidation of Anaerobic Aromatic Hydrocarbon Degradation Pathways in *Geobacter daltonii* Strain FRC-32

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Fe(III) and U(VI)-Reducing bacterium *Geobacter daltonii* strain FRC-32 is capable of oxidizing various petroleum hydrocarbons anaerobically using a host of unique enzymatic mechanisms, all of which are pertinent to the degradation of crude oil and its components. Annotation of the *G. daltonii* genome revealed many duplicates and homologs of known genes from various anaerobic aromatic degradation pathways. One of these genes, *mbs*, encodes for *m*-hydroxybenzylsuccinyl-CoA synthase, while a homologous gene, *bss*, encodes for the radical forming, toluene activating enzyme benzylsuccinate synthase. Certain proteins integral to various degradation pathways utilized by other aromatic degrading bacteria are not present in *G. daltonii*, indicating the bottlenecking of multiple substrate pathways into a single, unified pathway. After growing *G. daltonii* anaerobically on various petroleum hydrocarbons including toluene and benzene as the sole electron donors, total cell RNA was isolated during the mid-log stage of growth. Quantitative real-time RT-PCR was used to measure the transcript level of the key functional genes *bamR*, *bamY*, *bss*, and *mbs*. The *bss* transcript level was higher during oxidation of toluene, a substituted aromatic, while the *mbs* transcript level was higher during oxidation of benzene, an unsubstituted aromatic. SDS-PAGE of total cell lysate analysis from cells grown on benzene and toluene confirmed the same gene product expression profiles, demonstrating expression of *Bbs*, an enzyme constituent of the toluene degradation pathway, in both. Our study aims to elucidate the novel pathways taken during anaerobic oxidation of a diverse array of aromatic carbon sources in *G. daltonii*. We hypothesize that *G. daltonii* methylates benzene to toluene to continue down the toluene degradation pathways via the *Mbs* activating subunit *MbsD*, and that the first step of unsubstituted aromatic degradation after methylation utilizes *Mbs*, while substituted aromatics utilize *Bss*.

10: Seasonal Effects On The Population Structure Of *Prochlorococcus* In The North Pacific Ocean

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Prochlorococcus is one of the most abundant marine phytoplankton and is responsible for a large portion of oceanic primary production. Basin-scale meridional transects and time series studies at fixed stations have established that the *Prochlorococcus* population in the surface mixed layer is partitioned with respect to temperature, with the eMIT9312 ecotype dominating the warmer, lower latitudes, and eMED4 dominating the colder, higher latitudes. Spatial and temporal resolution of this relationship is however not well characterized particularly in the higher latitudes where the transitions of ecotypic dominance occur. To improve our understanding of *Prochlorococcus* dynamics in these high latitudes, we performed a pair of research expeditions spanning the *Prochlorococcus* habitat range north of Hawaii. Winter and summer cruises along a similar transect allowed for assessment of seasonal succession in this region. For the winter transect, trends in ecotype abundances as a function of latitude were consistent with those found in prior studies. Surprisingly, ecotype abundances of the summer transect deviated from these trends, and indicate that seasonal progression in these high latitude waters is not simply a function of temperature dictating relative ecotype abundances. Potential reasons for this observation will be discussed.

11: Potential Emissions of Organic Chemical Additives from Liquid Storage Tanks in Hydraulic Fracturing in the Marcellus Shale play: A Modeling Approach

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Natural gas has become an important energy in the United States (U.S.) due to the development in hydraulic fracturing and horizontal drilling technologies. Hydraulic fracturing using large

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volume of water and some toxic chemicals poses adverse effects on natural environments and human health. This study used the AP-42 to investigate the organic emissions from liquid storage tanks during the operations of storing and emptying in the hydraulic fracturing in the Marcellus Shale play. The results showed the median value in well emissions was 0.038 kg•d-1 per well for 3,227 wells located in Pennsylvania, and 0.056 kg•d-1 per well for 468 wells in West Virginia. The median emission was 0.016 kg•d-1 per well in 2008, which increased into 0.042 kg•d-1 per well in 2014. Six organic chemicals founded in the hydraulic fracturing fluids are listed in the priority list of hazardous substances by the Agency for Toxic Substances & Disease Registry (ATSDR). More important, there are two organic compounds listed as “known to be a human carcinogen” while seven organic compounds are listed as “reasonably anticipated to be a human carcinogen” according to the 13th report on carcinogens by the U.S. Department of Health and Human Services.

12: In-Situ Low Temperature Neutron Powder Diffraction Study of Mixed CH4-CO2 Gas Hydrates

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Gas hydrates are found in deposits on the ocean floor and in sub-surface permafrost. They are composed of gas molecules trapped in a cage-structured host lattice. The cages are assembled from hydrogen bonded water molecules and can accommodate mixtures of various gases such as a mixture of CH₄ and CO₂. Methane hydrates are interesting structures to understand from a geologic perspective and as a potential energy source, while CO₂ hydrates are of interest for sequestering CO₂. In situ low temperature neutron powder diffraction was used to study the decomposition of mixed CH₄-CO₂ gas hydrates with compositions of 25, 50, and 75% CH₄. Neutron powder diffraction data were collected at the Spallation Neutron Source (SNS) on the POWGEN time-of-flight diffractometer at select temperatures between 10-240K. The Rietveld method for analyzing diffraction data was used to quantitatively track the decomposition of the

mixed gas hydrates to ice. Rigid bodies represented the gas molecules in the model to describe their interaction with the cage structure. The refinements show an increased weight fraction of ice with temperature, following the decomposition of the gas hydrate.

13: Seasonal Variability in Phytoplankton Responses to Water Accommodated Petroleum Hydrocarbons in the Northern Gulf of Mexico

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The Deepwater Horizon Oil spill in April 2010 highlighted the lack of regional baseline knowledge regarding phytoplankton succession as well as the effect of petrocarbon loading on different phytoplankton groups. Understanding whether there is seasonal variation in phytoplankton resilience to petrocarbon perturbation can inform models determining the extent of disruption to ecosystem services (e.g. passage of primary production to higher trophic levels) based on the timing of an oil spill. To understand the effect of petrocarbon loading on phytoplankton biomass and growth, we are conducting monthly experiments using water from the Alabama coast in the northern Gulf of Mexico. This region is highly dynamic due to influx from the Mobile Bay fresh-water plume; which carries high concentrations of inorganic nutrients, suspended sediment and dissolved organic matter. Multi-day grow out experiments are conducted using treatments of water accommodated fraction of crude oil (WAF, MC252 Surrogate Crude Oil) and chemically enhanced WAF (CEWAF, Crude + Dasic International Slickgone NS®) at concentrations of 1 ppm relative to controls. We are examining changes in photophysiology, productivity, biomass accumulation and community composition. Initial experiments indicate significant degradation of WAF and CEWAF concentration after 24 hours in the field and reductions in the rate of primary production, chlorophyll a biomass and variable fluorescence in the petrocarbon treatments relative to controls. Despite the initial decline observed in the petrocarbon treatments, multiple parameters indicate the onset of community

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recovery by 48 hours after the perturbation. Ongoing time-series results will be presented to examine whether similar sublethal effects are observed throughout the year and whether the magnitude of this variation is affected by the encountered community composition and hydrographic conditions.

14: Mississippi Canyon 118: What Stable C And N Isotopes In Plankton Tell Us

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8.9 nautical miles northwest of the Deep Water Horizon (DWH) wellhead, the Mississippi Canyon Federal Lease Block 118 (MC118) is a gas-hydrate and cold-seep field that has been used as a permanent observatory for natural gas hydrates in the Gulf of Mexico (GoM) for more than 10 years. Between 2010 and 2012, one month after the DWH spill, we started to collect samples in five compartments of plankton (i.e. suspended particles and zooplankton 200-500, 500-1000, 1000-2000, and >2000 μ m in size) for the characterization of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ on annual intervals. The $\delta^{13}\text{C}$ of suspended particles suggests that carbon derived from the DWH oil was incorporated in 2010 into the base of the food web, and was still recycled in subsequent years (2011-2012). However, this carbon does not seem to be transferred to upper trophic levels, as is not traceable in any of the zooplankton size-fractions. The $\delta^{15}\text{N}$ in our plankton compartments showed similar vertical distributions from 2010 to 2012. In the upper 100m of the water column, the $\delta^{15}\text{N}$ of suspended particles ($\delta^{15}\text{N}_{\text{sp}}$) reflects the influence of a mix of nitrogen sources (deep nitrate, atmospheric N_2 and/or riverine discharge). In deeper waters, $\delta^{15}\text{N}_{\text{sp}}$ appears to reflect primarily deep nitrate ($\delta^{15}\text{N}_{\text{sp}} > 5\text{‰}$), which is also the main contributor to organic nitrogen in zooplankton.

15: A Proposal to Profile Phosphorus Adsorption and the Influence of Organic Ligands on South Carolina Ultisols

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Ultisols are commonly used for agricultural production in the Southeastern USA, and represent 73% of the soil in SC. Ultisols are relatively infertile acidic soils with low cation exchange capacity, low buffering capacity, and kandic or argillic horizons. With appropriate management and cultural practices, such as liming and fertilizer application, Ultisols can be productive. Phosphorus is a limiting nutrient in SC agricultural soils due to its strong adsorption at low pH with Fe and Al oxides. Phosphate adsorption isotherms have been studied in several soil types in the presence of organic acids. These compounds occur naturally in the soil solution matrix in their anionic form as organic ligands. Some common examples include citrate, malate and oxalate. Along with phosphate these ligands can preferentially bind to anion exchange sites on colloidal surfaces as governed by the Hofmeister series. The goal of this study is to better understand the biogeochemistry of Ultisols by characterizing the sorption chemistry of P in SC Ultisols. The research objectives are 1) to compare the physiochemical characteristics of an Ultisol in a forested and agricultural setting and 2) to determine adsorption isotherms for phosphate in these soils in the presence of organic ligands. Results will be used to improve the understanding of phosphate and organic ligand sorption in agroecological systems. As cultural practices transition to improve soil health, the physiochemical effect of increasing OM in these systems warrants further study in order to understand how anionic plant macronutrients interact with the byproducts of decomposition in the soil.

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16: Exploring Hydrocarbon Degradation Along A Surface Transect Within A Persistent Oil Slick At The Taylor Energy Site

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Oil in surface slicks is subject to a variety of weathering processes, including photo-oxidation, evaporation, and biodegradation. The oil slick sourced from the site of the sunken Taylor Energy platform 23051, located 11 miles southeast of the mouth of the Mississippi River in the northern Gulf of Mexico, offers investigators a unique opportunity to explore the co-evolution of oil and microbial communities at the sea surface in an oil-impacted setting. We evaluated the ability of the surface water's microbial community to degrade oil by measuring turnover of radiolabeled hexadecane and naphthalene to carbon dioxide at four stations along a 5 nautical mile transect. We further evaluated the surface water at each station with cell and virus like particle counts, inorganic nutrient and dissolved organic carbon concentrations, and extractable hydrocarbon profiles. We observed that the in situ percent turnover/day of both hexadecane and naphthalene was highest at the site farthest away from the leaking point source, while cell counts were highest at the site closest to the source, yet considerably lower than the 1×10^6 cells/mL typical of seawater. The inorganic nutrient concentrations were consistent with a nutrient-rich, coastal setting, heavily influenced by the Mississippi River plume. Our current results indicate that this persistent oil slick is a dynamic system that exhibits varying responses to petroleum hydrocarbons.

17: An Investigation Of Anaerobic Methane Oxidation By Consortia Of Methanotrophic Archaea And Bacterial Partners Using nanoSIMS And Process-Based Modeling

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Consortia of anaerobic methanotrophic archaea and delta-proteobacteria have been shown to oxidize methane and reduce sulfate in marine sediments, thereby limiting the rise of methane into the water column and ultimately the atmosphere. However, the mechanism of the interactions between the two groups of microorganisms remains uncertain, with recent studies pointing towards potential direct electron transport (DIET). Here, we use reactive transport modeling to investigate potential mechanisms of methane oxidation. We consider (1) the exchange of electron donors such as molecular hydrogen produced by the archaea and used by the bacteria in sulfate reduction, (2) a pathway in which the archaea oxidize methane reduce sulfate to disulfide, which then gets reduced to sulfide by the bacteria, and (3) electron transport through nanowires. It was shown that reaction kinetics, transport intensities, and energetic considerations all could decisively impact the overall rate of methane consumption. Simulation results indicate that the exchange of chemical intermediates such as acetate, formate, and hydrogen lead to oxidation rates that fall short of measured ones. In contrast, simulations with disulfide as intermediate and via DIET can achieve methane oxidation rates that match or even exceed lab measured reaction rates. To distinguish between the two mechanisms, we compare the computed spatial distribution of reaction rates to the spatial patterns within the microbial consortia as measured by 15N uptake measured with nanoSIMS. These observations show that the activities are independent of the vicinity of archaea and bacteria. This is best reproduced in the simulations of DIET via nanowires, supporting the recent evidence for such a potential mechanism.

18: The fungal contribution to nitrous oxide formation: Are substrate induced respiration inhibition (SIRIN) studies misleading?

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Denitrification, the reduction of nitrate or nitrite to gaseous nitrogen, is mainly attributed to Bacteria1, but denitrifying fungi exist and are

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often neglected, even though they may significantly contribute to soil nitrous oxide (N₂O) production 2,3. Cultivation-based studies investigating fungal denitrification frequently utilize substrate induced respiration inhibition (SIRIN), in which broad spectrum antibiotics and fungicides are applied to investigate bacterial and fungal contributions to soil N₂O production 2,3. SIRIN experiments indicate fungi contribute 40-95% of the N₂O produced in soil ecosystems 4. Yet, the rates of N₂O formation by fungi in pure culture are known to be one to five-fold lower than denitrifying bacterial isolates 4. A potential bias of the SIRIN technique is the incomplete inhibition of nitrogen cycling bacteria. To investigate this bias, we inoculated medium containing 2.5 mM nitrite and 5 mM glucose with soil (2 g) from two distinct U.S. Midwest agricultural field sites. Microcosm treatments included the addition of bacterial inhibitors (streptomycin and ampicillin), a fungal inhibitor (cycloheximide), and appropriate controls. In 5-Day intervals, nitrite and N₂O concentrations were analyzed, and PCR and qPCR analyses performed to monitor relative abundance of fungal p450_{nor} and 18S rRNA genes, and bacterial nitrous oxide reductase (nosZ) and 16S rRNA genes. Nitrite concentration declined to zero by day 20 in all treatments with concomitant formation of N₂O observed. No increase in fungal 18S rRNA gene abundance was observed in cycloheximide-treated microcosms; however, fungal rRNA gene abundance increased by an order of magnitude in treatments without cycloheximide. Bacterial 16S rRNA gene and nosZ gene abundances increased in all incubations, including those that received streptomycin and ampicillin. Fluorescent staining of filtered samples confirmed the presence of bacteria in the antibacterial-treated samples. Since bacterial N₂O production rates are orders of magnitude higher than fungal denitrifiers, and bacterial relative abundance is high in bacterial inhibitor treatments, these findings suggest a pervasive bias in observations of the fungal contribution to N₂O production using the SIRIN approach.

19: Organic Geochemical Variability Of Dark Snow On Two Alaskan Glacier Surfaces

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Glacier surfaces are darkening, which has key implications for the rate of global sea level rise. Originally, albedo decreases were believed to be an effect of solely inorganic particles from surrounding rock erosion or combustion pollution. However, recently that has been proven untrue. Yet, the relative inputs of inorganic to organic particles found on glacier surfaces and their compositional variability is not well known. Therefore, samples of different types of supraglacial impurities were collected on Matanuska and Spencer Glaciers near Anchorage, Alaska in August 2015 to test for variability in the composition of these impurities. Supraglacial impurities can consist of many different surface-darkening materials such as biofilms, cryoconite, and inorganic substances. In order to study the chemical composition of these impurities, we quantified the n-alkanes to determine the relative contribution of terrestrial, algal, and geologic organic carbon inputs. Alkanes were isolated from samples using solvent extraction and then purified using silica gel chromatography before being quantified and identified using gas chromatography mass spectrometry. Overall, the higher relative abundance of odd to even alkanes indicated a higher abundance of organic material from recent biomass than aged geologic material. However, when comparing the two glaciers, there were large variations in the carbon preference index, n-alkane concentrations, and distributions. Combined, these results suggest that supraglacial impurities not only differ compositionally from glacier to glacier but also depending on the type of impurity. These results imply that biomass may play an important role in the albedo changes of glaciers, and that the type of supraglacial impurities must also be included to accurately model the increasing melting rates seen on the surface of glaciers.

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20: Nano-scale Elemental Imaging of Anaerobic Microbial Co-cultures and Environmental Consortia with Synchrotron X-ray Fluorescence Microscopy

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This study evaluated the potential of synchrotron x-ray fluorescence (SXRF) microscopy for imaging elemental distributions at the 100 nm scale in microbial pure cultures, co-cultures, and complex enrichments using the Bionanoprobe at sector 21 of the Advanced Photon Source. The benefit of SXRF over other bulk analyses such as inductively coupled plasma mass spectrometry (ICP-MS) is the ability to image individual cells in enrichment or co-cultures with multiple species. Pure and co-cultures of the methanogenic archaeon *Methanosarcina acetivorans* C2A and the sulfate-reducing bacterium *Desulfococcus multivorans* DSM 2059 were analyzed for bioessential transition metals (Mn, Fe, Co, Ni, Cu and Zn) in bulk and single cells using ICP-MS and SXRF. ICP-MS and SXRF results for pure cultures were broadly consistent. When grown in co-culture, *M. acetivorans* was enriched in Zn whereas *D. multivorans* was enriched in Co. Methane seep enrichment cultures contained microbial consortia (~40-530 μm^2 aggregates) comprised of anaerobic methanotrophic Euryarchaeota (2c) and Desulfobacteraceae sulfate-reducing bacteria that mediate anaerobic oxidation of methane coupled to sulfate reduction and are phylogenetically related to the species in the co-cultures. Four out of six of the aggregates were surrounded by a Si-K-Fe-Ti-rich ring, likely comprised of an aluminosilicate clay. Ongoing work is focused on comparing trace elemental composition of aggregates with and without a clay ring, in different regions of the aggregates, in comparison to the co-cultures, and incubated with and without methane.

21: Mn-oxidizing Isolates Affiliated with Genera not Previously Known to Contain Mn-oxidizers

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Manganese-oxidizing bacteria and fungi are ubiquitous in nature, however, only a few microbes have been isolated from cave environments. Isolating microbes has proven challenging, due to the fact that current culturing techniques generally retrieve only a few, high-nutrient adapted, fast-growing species. The purpose of this study was to isolate potentially novel Mn-oxidizing bacteria and fungi from caves in the southern Appalachian Mountains. Samples were collected and streaked onto AY and Citrate nutrient agars, supplemented with 100 μM MnCl_2 . Colonies that tested positive for Mn oxidation using leucoberbelin blue tests were streaked for isolation. DNA samples were then taken from the plate and amplified with the appropriate primers. Bacterial colonies were amplified using 27f and 1492r, while fungal colonies were amplified using ITS1f and ITS4r. Amplified DNA was then purified and sequenced at Beckman Coulter Genomics. Sequenced DNA was aligned and then BLAST searches were employed to find the closest representative sequences. Preliminary results from this study suggest that the two most commonly isolated Mn-oxidizing species included *Acremonium* sp. DCI1F and *Plectosphaerella cucumerina*, both of the Ascomycota phylum and both of which have both been previously isolated from caves. Potentially novel Mn-oxidizing isolates included a fungus with 99% ID to other cultured members of the *Dictyosporium* genus within the phylum Ascomycota, and a bacterium with 98% ID to other cultured members of the *Tahibacter* genus within the class Gammaproteobacteria. While members of these genera have been isolated before, neither of them have been shown to oxidize Mn in axenic cultures. This research is essential to further identifying the microbes responsible for the biogeochemical cycling of Mn and for determining the niches that particular organisms occupy in their environments.

*indicates presenting author

22: The Changing Microbial Community Along the Orca Basin Pycnocline

Andrew Hyde*, Lisa M. Nigro, Joseph Peter Montoya, Samantha Benton Joye, and Andreas Teske

University of North Carolina at Chapel Hill

Orca Basin in the Gulf of Mexico is the largest seafloor brine basin in the world, with a brine depth up to 220 m and an areal extent of 123 km². Within the chemocline and pycnocline of Orca Basin, salinity, temperature, oxygen concentration, porewater chemistry, and microbial community composition change within approx. 100 meters, from fully oxic and marine saline deepwater conditions at 2150 m to anoxic hypersaline brine at 2250 m depth. Previous surveys of Orca Basin have detected distinct peaks of metal-cycling bacteria, and of archaeal lipids in the Orca Basin chemocline. The steep pycnocline slows down the sinking speed and therefore concentrates organic matter and microbial populations from the water column; it also allows in-situ growth of microbial populations that can take advantage of coexisting electron donors and acceptors. To survey the microbial community structure and stratification in Orca Basin, we performed a high-throughput bacterial 16S rRNA gene sequencing of filter samples from the Orca Basin deep water, chemocline and brine, collected in April 2014 on RV Atlantis. Widely spaced 50 m sample intervals from 1800 to 2350 m depth were complemented with fine-scale sampling every ten meters between 2150 and 2250 m depth, centered on the pycno- and chemocline as evident from CTD data, and with additional samples taken at 2125, 2275, and 2375 m depth. While we expect abundant and diverse chemosynthetic interface bacteria and halophiles, we are also exploring the possibility that the Orca Basin pycnocline preserves and amplifies microbial hydrocarbon signatures in the Gulf of Mexico, as in a long-term particle trap.

23: Diversity And Abundance Of Alkane-Degrading Bacteria From Grand Isle, Louisiana, 2011-2014

Hunter Johnson*, Annette Summers Engel, Audrey Paterson

University of Tennessee Knoxville

After the Deepwater Horizon explosion and well blowout in April 2010, an estimated 779 million liters of oil were released into the Gulf of Mexico. Microorganisms in the Gulf waters degraded some of the hydrocarbon compounds, but oil reached coastlines along the Gulf in the months following the blowout. Salt marshes in Louisiana were significantly impacted by oil, with peak oiling (ranging from 8.5 to 38.2 mg/kg total n-alkanes) occurring in most marshes after Hurricane Isaac in 2012 redistributed buried oil in Barataria and Terrebonne bays. However, although some microbes can degrade hydrocarbon compounds if they possess specific enzyme pathways, including alkane hydroxylase (alkB), prior knowledge about oil biodegradation in the marshes was limited. Detection, quantification, and sequencing of alkB genes can provide information about the potential diversity of alkane-degraders. This study tested the hypothesis that the diversity of alkane-degrading bacteria in marsh sediments increased during peak oiling periods. Sediments were collected from a marsh at Grand Isle, Louisiana, in the spring and fall seasons, from 2011 through 2014. Total environmental DNA was extracted from 0-1 cm and 4-5 cm sediment depth. 16S rRNA and alkB genes were obtained from 454 tag pyrosequencing followed by analysis using mothur software. At the surface, from 16S rRNA genes, Gammaproteobacteria represented by Oceanospirillales and Thiotrichales dominate the sediments prior to Hurricane Isaac, then Alphaproteobacteria dominate through 2014, represented by Sphingomonadales and Rhodobacterales. At depth, gammaproteobacterial abundance increased. Quantitative PCR of alkB genes for each of these groups will be used to compare to pyrotag relative abundances. The results of this research are uncovering novel alkB diversity from understudied salt marshes, and the work has broad implications for understanding biodegradation of hydrocarbon compounds in coastal ecosystems.

*indicates presenting author

24: Quorum Sensing and Antimicrobial Production in Marine Biofilms

April Mitchell*, Drs. Nathan Cude and Alison Buchan

University of Tennessee Knoxville

Heterotrophic marine bacteria play a central role in the recycling of carbon in the ocean. One group of heterotrophic marine bacteria, the Roseobacter Clade, dominates marine biofilms in coastal environments. Species of roseobacter produce secondary metabolites with antimicrobial activities. These antimicrobial agents inhibit growth and metabolism of potential competitors, ultimately affecting carbon cycling within the biofilm. Density dependent cellular communication, in the form of quorum sensing (QS) molecules, play important roles in such relationships. However, the complex hierarchal regulation of antimicrobial production via QS in marine biofilms has not been fully explored. The roseobacter *Phaeobacter Y4I* produces a blue antimicrobial, indigoiodine, which has been shown to inhibit the growth of several marine bacteria, including *Vibrio fischeri*. In *Y4I*, indigoidine is under the regulation of two separate QS systems. Current genetic investigations of the contributions of each QS system in the genetic regulation of indigoidine biosynthesis and how these systems affect the colonization success of *Y4I* are underway. Elucidating the QS hierarchal regulatory network in relation to antimicrobial production will further our understanding of how antimicrobials are produced and how antimicrobial production alter marine biofilm structure and, therefore, function.

Oral Session IV

9:00 AM- 9:45 AM

Sunday March 13

McClung Auditorium

9:00 AM

19: Kinetics Of Abiotic Nitrous Oxide Production Via Hydroxylamine Oxidation By Iron And Manganese

Amanda Cavazos*, Martial Taillefert, Jennifer Glass

Georgia Institute of Technology

Nitrous oxide (N₂O) is a potent greenhouse gas that can be produced via enzymatic, abiotic, or coupled reactions. Bacteria and archaea produce NH₂OH enzymatically as the product of ammonia oxidation. This NH₂OH is further oxidized to nitrite, but can also leak out of cells and be oxidized abiotically by redox active metals. Thus far, the kinetics of NH₂OH oxidation have only been studied in low ionic strength and highly acidic solutions. In this study, we characterized the kinetics under more environmentally relevant conditions, in artificial seawater at pH 4-9. All experiments were performed with excess Fe(III)NH₄(SO₄)₂·12H₂O or acid birnessite (Mn(IV)O₂) and N₂O was measured using a Clark-type N₂O microsensor. Our preliminary results show that for NH₂OH oxidation by Fe(III) reduction, the order of reaction was pseudo 3.04 order with respect to Fe(III)NH₄(SO₄)₂·12H₂O, pseudo -2.24 order with respect to NH₂OH, and pseudo 0.23 order with respect to pH. By Mn(IV) reduction, the order of the reaction was pseudo -1.83 order with respect to Mn(IV)O₂, pseudo 0.78 order with respect to NH₂OH, and pseudo -0.11 order with respect to pH. The rate constant for NH₂OH oxidation via Fe(III) reduction was $6.62 \times 10^{-5} \pm 9.73 \times 10^{-5} \text{ M}^{-0.3}/\text{s}$ and $2.14 \times 10^{-10} \pm 1.98 \times 10^{-10} \text{ M}^{2.16}/\text{s}$ by Mn(IV) reduction. These rate constants will be applied to diverse ecosystems with varying Fe(III), Mn(IV), and NH₂OH concentrations to predict the fluxes and global importance of abiotic N₂O production.

9:15 AM

20: Carbon Sources Impact Microbial

Mn-Oxidizing Communities in Caves of the Southern Appalachian Mountains

Mara L. Cashay*, Sarah K. Carmichael, Michael A. Carson, Suzanna L. Bräuer

Appalachian State University

Microbial Mn oxidation is ubiquitous in nature and, yet, environmental parameters that stimulate biotic Mn oxidation have yet to be constrained. Cave and karst systems provide ideal natural laboratories for controlled field studies of Mn oxidation because they are nutrient-limited and therefore sensitive to exogenous nutrient sources. A variety of carbon sources were incubated in situ, Mn oxidation was monitored, and changes to bacterial and fungal Mn communities were determined using Illumina amplicon sequencing. α -diversity analyses based on Shannon's Diversity Index suggest that fungal communities were less evenly distributed (0.34-0.65) than bacterial communities (0.69-0.80). Fungal evenness was significantly lower than the bacterial evenness (P-value < 0.05) at two out of five sites. Sawdust and cotton seemed to have the greatest impact on the fungal community and fungal Mn oxidation, as there were significant differences (P-value < 0.05) in the α -diversity and β -diversity between the samples taken before and after stimulation at three of the 5 sites. There were also significant shifts (P-value < 0.05) in the relative abundance of fungal sequences at the phylum through genus level after stimulation with cotton and sawdust. At sites where fungal Mn oxidation was not stimulated, Zygomycota and Basidiomycota were in higher abundances when compared to sites where Mn oxidation was stimulated. Results of this study suggest that Ascomycota are the main contributors to Mn oxide deposition in cave ecosystems. It is important to note that unknown carbon sources may be able to stimulate Mn oxidation at sites where Zygomycota and Basidiomycota are more abundant. Overall, these data demonstrate that exogenous carbon impacts

*indicates presenting author

metal cycling and microbial communities. Further, anthropogenic impacts like cave litter may alter microbial ecology and biogeochemical cycling within karst ecosystems.

9:30 AM

21: Molecular Markers Suggest Differential Biogeochemical Activity Of Prochlorococcus Ecotypes Between Active Oligotrophic And Temperate Communities In The North Pacific Ocean

Alyse A. Larkin* and Zackary I. Johnson

Duke University

Due to its high abundance and primary production in the global ocean, the marine cyanobacteria *Prochlorococcus* sp. is one of the most biogeochemically important marine microbes, contributing a large proportion of energy, carbon, and other bioavailable nutrients to both marine food webs and elemental cycles. To examine patterns of *Prochlorococcus* clade-specific activity and its environmental drivers, we collected RNA and DNA from surface waters at 56 stations along four ~4000 km ocean transects crossing major environmental gradients in the North Pacific in October, March, January, and July. The 23S rRNA and rDNA regions, shown to distinguish between the major clades of *Prochlorococcus*, were amplified and sequenced (6,175,757 reads; 250 bp mean length). Non-metric multidimensional scaling of rank-ordered rRNA/rDNA ratios revealed a distinct separation between the active *Prochlorococcus* communities in the oligotrophic ocean versus those in temperate regions. This was confirmed by examining Kendall's tau-b for all pairwise station comparisons (median tau-b = 0.43, median $P < 0.001$). Bray-Curtis dissimilarities of rank-ordered rRNA/rDNA ratios were significantly correlated with latitude, temperature, salinity, day length, silicate and phosphate through permutational multivariate analysis of variance. However, differing trends were observed for the HL-I and HL-II ecotypes. Divergence in the active *Prochlorococcus* community between oligotrophic and temperate regions may have important implications for biogeochemical processes due to possible differences in cellular nutrient content as well as primary production.

9:45 AM

22: Interactions of Natural Organic Matter with Mercury Revealed by Ultrahigh Resolution Mass Spectrometry

Hongmei Chen*; Benjamin F. Mann; Rosalie K. Chu; Nikola Tolic; Baohua Gu

Oak Ridge National Laboratory

Dissolved organic matter (DOM) represents one of the dominant species affecting mercury (Hg) chemical speciation, transformation, and bioavailability in aquatic environments, owing to the high binding affinities between Hg and reduced sulfur functional groups in DOM. However, specific molecular species that react with Hg remain unclear because of the heterogeneous nature and complex composition of DOM. Here we demonstrate the use of ultrahigh resolution ESI-FTICR-MS to directly identify Hg-DOM complexes, and to characterize changes in molecular composition of DOM resulting from its interactions with Hg. Suwanee River DOM standard was obtained from the International Humic Substances Society. DOM from East Fork Poplar Creek, a Hg-contaminated stream in east Tennessee, was isolated using a solid-phase extraction procedure with styrene divinyl benzene polymer (PPL) cartridge. Hg²⁺ (as HgCl₂) was added to DOM solution at the final Hg concentration of 0.5 μ M and allowed to equilibrate for at least 24 hours before MS analysis. Molecular formulae assignment was conducted with the aid of isotope pattern recognition (e.g., 32S vs. 34S, 202Hg vs. 200Hg), resulting in approximately 1500 molecular formulae assigned for each DOM mass spectrum. Four Hg-containing DOM molecules were confidently identified with corresponding 200Hg isotope peaks confirmed in formulae assignment of Hg-spiked DOM samples. A number of Hg-containing molecular formulae were identified from newly present mass peaks after Hg addition, concurrent with the observation that their corresponding DOM molecular formulae (i.e., Hg-containing molecular formulae minus Hg, but plus 2H) disappeared or the peak intensity decreased after Hg addition. Most of the "lost" and "new" DOM molecular formulae after Hg addition were found to contain O, S, and/or N, thereby supporting current view that Hg(II) forms complexes with DOM through a two-fold coordination involving one reduced S and one O or N in natural organic matter.

*indicates presenting author

Poster Session II
10:00 AM -11:30 AM (K-Z)
Sunday March 13
McClung Reception Room

**25: Response Of Soil Microbial
Communities To Phosphorus In
Tropical Ecosystems**

Minjae Kim*, Eric R. Johnston, Terry C. Hazen,
Melanie A. Mayes, Chongle Pan, Qiuming Yao,
Konstantinos T. Konstantinidis

Georgia Institute of Technology

Microbial communities in soil exert significant control over release of greenhouse gases (GHGs) through degradation of soil organic matter (SOM). Previous studies have shown that phosphorus (P) availability limits both aboveground vegetative growth and soil microbial activities (including SOM degradation) in moist tropical forests systems. How microbial communities respond to P additions remains poorly understood however, limiting our predictive ability of how these ecosystems will respond to future climate change such as increased CO₂, increased temperature, and changes in the amount and seasonality of precipitation. In this study, soils were collected from the four different plots (valley and ridge at El Verde Field Station, and valley and ridge at Bisley Tower) within Luquillo Forest in Puerto Rico. Soils were incubated in the laboratory at 27°C under aerobic and anaerobic conditions with and without addition of exogenous phosphate. A Micro-Oxymax respirometer was used to continuously measure several gases, including CO₂, CH₄, H₂S, H₂, and O₂. Early results reveal that P addition stimulated SOM decomposition and CO₂ release in El Verde ridge and Bisley ridge soils, although mixed results were obtained for valley soils at both locations. Previous studies have demonstrated that total P concentrations in the valleys were higher than the ridge locations. This suggests that soil microbial communities from a P limited environment respond more dramatically to P addition. However, even within the same plot of soils, the soil microbial community showed different capacity and patterns of CO₂ emission due to the

heterogeneous characteristics of soils (including P availability). To provide further insights into these issues and a more detailed description of the microbial activities, metatranscriptomic analysis with mRNA of selected samples is currently undergoing.

**26: In Situ Chemical Oxidation of
Persistent Organic Molecules Found in
Hydraulic Fracturing Fluids**

Katherine Manz* and Dr. Kimberly Carter

The University of Tennessee Knoxville

As the United States continues to work towards energy independence and security goals, advancements in hydraulic fracturing have allowed natural gas to become a viable energy source via extraction of unconventional shale reserves. Hydraulic fracturing requires an enormous amount of water, two to ten million gallons of water per well fractured, to which a proprietary blend of chemical additives are added to ensure a productive fracture. The chemical slurry contains proppants, gelling agents, breaking agents, friction reducers, biocides, scale inhibitors, clay stabilizers, and corrosion inhibitors. In our research, we have identified several organic molecules used in a gelling agent, a biocide, a friction reducer, a breaking agent, and surface tension reducer using gas chromatography mass spectrometry (GC/MS). With the chemicals we have identified, it is our goal to determine the fate of these chemicals over the course a fracture and to evaluate the environmental threat that hydraulic fracturing poses. The research presented will discuss the reaction kinetics of a single organic molecule, furfural, which we identified in a hydraulic fracturing enzyme breaker. By-products of this reaction were also determined for these experiments.

*indicates presenting author

27: Further examining biogeochemical consequences of mesoscale eddies in the Sargasso Sea

Marquez, Israel A.*, Lomas, Michael W., Krause, Jeffrey W.

University of South Alabama

The Bermuda Atlantic Time Series (BATS) is an ongoing 25-year biogeochemical record in the North Atlantic subtropical gyre. Contemporaneous data on the particulate phases of four major bioreactive elements, C, N, P, and Si only exist during two years and also of a companion project (Trophic BATS, i.e. TBATS). A combined dataset from BATS and TBATS was used to better understand the coupling of C, N, P, and Si in the Sargasso Sea by analyzing particulate phases of each element in the water column and exported material. Three conclusions are inferred: N, Si, and particularly P were more efficiently recycled within the euphotic zones of eddies as elemental ratios in export material were greater than the corresponding ratios in the water column. This suggests that P may have a more critical biogeochemical role and its supply rate to the euphotic zone may control primary production in these closed systems. Second, despite apparent high diatom biomass, estimated to be primarily detritus, there was no observed increase in organic matter export. Third, the trends seen in these eddies do not support that export production was enhanced; instead these features had more efficient recycling of N, P, and Si relative to the BATS site. This decrease in export efficiency suggests a stimulation of export production above 'normal' BATS conditions within eddies would require significantly higher autotrophic standing stock and correspondingly high rates of organic matter production.

28: Characterization of microbial carbon on Alaskan supraglacial impurities

Drake McCrimmon*, Lori Ziolkowski

University of South Carolina

Albedo, or glacier reflectivity, has been steadily declining due to the increasing amount of dark-colored snow on glacier surfaces. Since the

increasing amount of dark snow causes glaciers to melt more rapidly, the characterization and source of dark material on glacier surfaces must be determined. Export from glaciers contains old carbon, which suggests a fossil carbon source, such as the combustion of fossil fuels. However, the chemical composition of exported material is microbial in nature. To reconcile this conflicting paradigm and to understand what carbon microbes on the surface of glaciers are using, biomarkers of the viable microbial community (phospholipid fatty acids or PLFA) have been quantified in dark supraglacial impurities collected from Matanuska and Spencer glaciers in southern Alaska in August 2015. Isolating PLFA through solvent extraction can allow for the identification of a broad class of microorganisms present in the supraglacial environment, as well as a cellular density of microbes present. Abundances of PLFA structures have been found to be consistent across both glaciers (around 40% saturated, 40% monounsaturated, 10% branched, and 10% polyunsaturated, cyclic, and other structures), but cellular density is more variable, with $5.0 \pm 0.8 \times 10^9$ cells per gram on Matanuska (n=11) and was over 50% higher ($8.5 \pm 1.7 \times 10^9$ cells per gram) on Spencer glacier (n=12). Both cellular densities are similar to what is found in surrounding soils. Additionally, the radiocarbon content of both bulk supraglacial impurity materials and extracted PLFA will be used to indicate carbon inputs into the supraglacial environment as well as the carbon source for microorganisms. Characterizing the microbial composition of supraglacial impurities and determining the source of carbon to microorganisms will help in investigating why dark material continues to populate glaciers, reducing albedo and increasing glacier melt.

29: Gene Content Does Not Always Predict Function; Denitrification In The Absence Of NO-Forming Nitrite Reductases

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University of Tennessee Knoxville

The two bacterial pathways for nitrate reduction are respiratory ammonification (RA) and denitrification. The reduction of nitrite (NO₂⁻) to nitric oxide (NO) is the first step in denitrification

*indicates presenting author

unique from RA, and the presence and abundance of nirS and nirK genes ($\text{NO}_2 \rightarrow \text{NO}$) are often used to estimate denitrification potential in the environment. The soil bacterium *Anaeromyxobacter dehalogenans* reduces nitrate to ammonium via RA and grows with N_2O as an electron acceptor, but is unable to denitrify. Past genomic studies identified nrfA ($\text{NO}_2 \rightarrow \text{NH}_4^+$) and nosZ ($\text{N}_2\text{O} \rightarrow \text{N}_2$) gene copies, but nirS and nirK genes were absent, confirming its characterization as a non-denitrifier. *A. dehalogenans* also reduces ferric iron to ferrous iron, which chemically reacts with nitrite. To determine how iron influences the final product of nitrate reduction by *A. dehalogenans*, pure cultures of *A. dehalogenans* were grown with ferric iron (as ferric citrate) and spiked with 100 μmoles nitrate or nitrite. The cultures produced $58 \pm 6 \mu\text{moles}$ and $112.8 \pm 2.2 \mu\text{moles}$ N_2O -N, respectively. The N_2O formed was subsequently consumed by *A. dehalogenans*. Abiotic controls containing ferrous iron and nitrite produced N_2O , confirming that a chemical reaction of nitrite with ferrous iron (i.e., chemodenitrification) caused the formation of N_2O . Cultures grown in the absence of iron reduced nitrate and nitrite to ammonium, with little to no ($\leq 1\%$) N_2O production. Genomes in the Kyoto Encyclopedia of Genes and Genomes (KEGG) were surveyed for nitrogen cycling genes and of the 230 genomes containing both a nitrate reductase gene and a nosZ gene, 21% do not have nirK or nirS. Despite the absence of nirK and nirS, *A. dehalogenans* contributes to denitrification, indicating that gene content alone is insufficient to predict microbial N transformation reactions. Like *A. dehalogenans*, the surveyed organisms may combine biotic and abiotic reactions to achieve complete denitrification.

30: Seasonal Denitrification Patterns In Restored NC Salt Marshes And Oyster Reefs

Kathleen M. Onorevole*, Suzanne P. Thompson, Michael F. Piehler

UNC Institute of Marine Sciences

Nitrogen loading is a concern for many coastal wetlands, as it can lead to eutrophication and a cascade of deleterious results. One way to manage for N loading is to maximize N removal. Previous research has shown that salt marshes and oyster reefs can enhance denitrification, the process by

which nitrate is converted to non-bioavailable dinitrogen gas. Restoration of these habitats can therefore be employed to increase overall N removal in a system. For restoration to be most effective, it is useful to predict how denitrification rates will change over seasons and years. This study used a space-for-time chronosequence to construct restoration trajectories for denitrification in North Carolina salt marshes and oyster reefs. Study sites were salt marshes with fringing oyster reefs that were restored 0-20 years ago. Sediment cores were collected from multiple elevations in each habitat and incubated in a continuous flow-through system. Water samples from each core were analyzed for N_2 gas flux, indicative of denitrification, using a Membrane Inlet Mass Spectrometer (MIMS). Sampling was conducted each season from summer 2014 to summer 2015. Denitrification rates were highest in the summer, during which they plateaued approximately 6 years following restoration. Average annual denitrification efficiency followed the same pattern. Denitrification rates for both habitats were more similar in older restored sites. Environmental parameters such as inundation time and sediment organic matter also interacted with the denitrification trajectory. The results of this research can inform management in regions where N cycling is a pertinent concern. With knowledge of denitrification trajectories, managers can better anticipate how restored system will compare to their natural analogues over time.

31: Sample Storage And Contamination Consideration For IODP Rock Cores

Hannah Park, Susan Lang, Emma Wanamaker

University of South Carolina

The International Ocean Discovery Program (IODP) drilled its first hard rock leg specifically devoted to identifying subsurface life in the Fall of 2015. The drilling target was the Atlantis Massif, where water passing through ultramafic rocks generates hydrogen and methane that can sustain microbial communities. A major goal of the expedition was to determine the relationship between rock type, fluid circulation, carbon cycling, and microbial activity. Biomass and carbon content is anticipated to be extremely low in these samples, requiring careful planning to store samples appropriately for multiple types of

*indicates presenting author

analysis, including maintaining anaerobic and sterile conditions for microbial incubation experiments, and minimizing the introduction of external organic carbon. In preparation for the cruise, storage materials were tested for their potential to transfer compounds such as lipid biomarkers and amino acids to samples. The presence of these compounds in the rock cores can be used to identify 'hotspots' of biological activity and, potentially, the synthesis of organic molecules in the absence of life. Minimizing the introduction of these compounds during sample handling is therefore essential. The sample bags preferred by microbiologists because they could maintain anaerobic conditions were found to contain high levels of fatty acids and n- alkanes. The Nylon bags recommended by trace metal geochemists had unacceptably high amino acid content. Only acid washed Teflon sheeting was found to be suitable to all researchers as it could be sterilized by autoclaving, did not transfer carbon compounds, and could be frozen at -80°C. These Teflon -wrapped samples could be placed in gas tight bags flushed with nitrogen to maintain anaerobic conditions. This recommended approach was employed for samples collected on Expedition 357.

32: Genomics and Transcriptomics Reveal the Response of Extreme Microbial Communities to Oil and Dispersant Exposure

Tito D. Peña-Montenegro*, Sara Kleindienst, and
Samantha Joye

University of Georgia

Understanding the microbial community response to environmental perturbation is particularly important. We simulated the deep water conditions that prevailed following the 2010 BP/Deepwater Horizon oil spill and evaluated the response of the microbial community to elevated levels of oil, chemical dispersants and dispersed oil using genomics and metatranscriptomics. Chemical dispersants are used commonly in response to oil spills. However, the impacts of dispersants on microbial community composition and activity are poorly understood. We documented dispersant-driven changes in microbial community composition and activity, shifting the dynamics from one dominated by oil biodegradation in oil-only treatments to one

dominated by dispersant biodegradation in dispersant-amended treatments. These results have important implications for understanding the impacts of chemical dispersants on the ability of microbial communities to efficiently degrade oil in the environment.

33: Toward Development of an Oil Aging Technique Using Radio-isotopic Analytes to Evaluate Environmental Exposure Time

Leigha Peterson*, Richard Peterson, Sarah
Harrison, Samantha Joye

Coastal Carolina University

Understanding the effects of oil release to the marine environment is inherently related to exposure time. Following the 2010 Deepwater Horizon oil spill, multiple lines of evidence were used to infer oil age since discharge (OASD) including distance from source, microbial community composition, and rates of oxygen removal. However, age estimates proved inconsistent between methods and this limitation remains nearly six years later, with little agreement among researchers as to the best utilized approach. Here, we present a developing method to estimate true OASD using radium isotopes, which new evidence suggests are released as oil is degraded. Samples were collected in 2015 along a transect covering distances 1 nm-5 nm SE of a failed oil platform, evidenced to supply the area with crude for over a decade. Surface water samples were homogenized and divided into three treatment classes: 1) analyzed immediately; 2) analyzed after ~24 hr, unamended; and 3) analyzed after ~24 hr, amended with 20:1 N:P. Notably, radium activities increased three-fold in nutrient amended samples closest to the wellhead relative to waters analyzed immediately following collection from the same location. In general, treatment effect decreased with increased distance from the wellhead and decreasing hydrocarbon concentrations. Preliminary estimates of OASD suggest sample ages ranged from 1.5 days-3.1 days since discharge across the 5 nm transect although distance from source and OASD were not uniformly correlated. Ages will then be used tangentially with measures of microbial community structure and activity toward development of an unambiguous oil aging tool.

*indicates presenting author

34: Diatom Frustule Morphology Effects on the Formation of Reverse Weathering Products

Rebecca A Pickering* and Jeffrey W. Krause

*Dauphin Island Sea Lab, University of South
Alabama*

The global rates for delivery of dissolved silicic acid (dSi) to the ocean and burial of biogenic silica (bSi) are dominated by processes occurring in land-sea interface systems. Previous work reporting the bSi content of low-latitude coastal sediments has been underestimated due to analysis methods failing to include authigenically altered bSi from reverse weathering. In the last decade this process has been shown to be an important sink for Si and other elements in these systems; however, the mechanisms regulating this process are still poorly understood. Here we report the introductory results of ongoing laboratory batch experiments (18-month duration), examining the formation of authigenic products using three morphologically distinct diatom genera (Thalassiosira, Navicula, Chaetoceros) as a bSi substrate. Cleaned bSi is incubated in a chamber which is separated from coastal clay (<2 μm) suspensions by 12-14 kDa dialysis membrane. Clay suspensions were made from northern Gulf of Mexico (GoM) sediments, collected on the 20 m isobaths, near the outflow of the Mobile Bay freshwater plume. All materials were suspended in HEPES buffered GoM seawater and placed in a rotating wheel (2 rpm) to keep particles in suspension. Preliminary results confirm increases in bSi dissolution between genera. Dissolution amounts for the first five months of sampling ranged from 330 to 530 $\mu\text{mol L}^{-1}$, 600 to 900 $\mu\text{mol L}^{-1}$ and 850 to 1150 $\mu\text{mol L}^{-1}$ for Chaetoceros, Thalassiosira, and Navicula respectively. The presence of clay suspensions reduces the bSi dissolution rate between 20 and 200 $\mu\text{mol L}^{-1}$ relative to controls with no sediments. Fluid chemistries are monitored over the course of the experiment and results from initial solid phase analyses after five-month incubations are presented.

35: The isotopic signature of fixed nitrogen removal in bioirrigated marine sediments

Jurjen Rooze*, Christof Meile

The University of Georgia

The reported extent of N isotope fractionation during benthic N_2 production has differed substantially between studies. To assess the range and identify mechanisms underlying such observations, we developed a reactive transport model and ran simulations evaluating the impact of nitrification, denitrification, and anaerobic ammonium oxidation on the isotopic composition of in-situ N_2 production. Different hydrodynamic regimes ranging from bioirrigation-dominated to purely diffusive transport were simulated, and the effects of the benthic mineralization rate and the composition of the overlying water on the isotopic signature of benthic N exchange fluxes were also quantified. Sediment redox conditions were found to control the N isotope effect, which under reducing conditions is driven by fractionation during nitrification and anaerobic ammonium oxidation and under oxidizing conditions by fractionation during denitrification. The mineralization rate, the bioirrigation intensity, and chemical composition of the overlying water affect the benthic redox zonation and therefore also the benthic N isotope effect. In shallow water environments, high mineralization rates leading to reducing conditions are often counteracted by active bioirrigating macrofauna injecting oxic water into the sediment. With increasing water-depth both sediment metabolism and bioirrigation intensity tend to decrease. Therefore, our simulations commonly lead to a relatively constant N isotope effect of approximately -3‰. This suggests that benthic N isotope effect of N_2 production is relatively small compared to water-column N_2 production, but due to the significant amount of benthic N_2 production nevertheless can have a large impact on the 15N:14N ratio of oceanic nitrate.

*indicates presenting author

36: Potential Activities of Organic Carbon Degrading Extracellular Enzymes in Baltic Sea Sediments

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Heterotrophic microorganisms in sediments produce extracellular enzymes to hydrolyze organic macromolecules so they can be transported inside the cell and used for energy and growth. Therefore, extracellular enzymes potentially act as mediators for the fate of organic carbon in sediments. The Baltic Sea Basin is a primarily depositional environment with high potential for organic matter preservation. Using core samples from the Lillebælt strait obtained by International Ocean Discovery Program (IODP) Expedition 347, the potential activities of multiple carbon degrading enzymes were measured using small substrate proxies containing the fluorescent compounds 4-methylumbelliferone (MUB) or 7-amino-4-methylcoumarin (AMC). The potential maximum hydrolysis rates (V_{max}) were measured at depths down to 77.92 mbsf for the following enzymes: alkaline phosphatase, β -D-xylosidase, β -D-cellobiohydrolase, N-acetyl- β -D-glucosaminidase, β -glucosidase, α -glucosidase, leucyl aminopeptidase, arginyl aminopeptidase, prolylaminopeptidase, gingipain and clostripain. Activities of extracellular peptidases were detectable at depths less than 54.95 mbsf and activity of alkaline phosphatase was detectable throughout the core, albeit against a relatively high activity in autoclaved sediments. β -glucosidase activities were detected above 30 mbsf, however activities of other polysaccharide hydrolases (β -D-xylosidase, β -D-cellobiohydrolase, N-acetyl- β -D-glucosaminidase and α -glucosidase) were generally indistinguishable from zero at all depths. These data suggest the microbial community is looking to acquire N and P via amino acids and phosphate. This is consistent with the behavior of a nutrient starved community, despite high nutrient concentrations in sediment porewater.

37: Anaerobic Oxidation Of Methane In A Deep Hypersaline Anoxic Basin (Orca Basin, Gulf Of Mexico)

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Seafloor hypersaline basins are intriguing extreme environments where deeply sourced fluids drive the distribution and availability of materials required for microbial metabolism. At Orca Basin, Gulf of Mexico, invading brines accumulate within two sub-basins (North and South) creating a dual-lobed, j-shaped hypersaline anoxic brine lake (123 km², 220 m maximum depth, and >250 PSU) separated by a shallow central saddle. Flowing brine sourced from breaching salt deposits enters the basin from above and mixes with the seawater to produce a chemocline characterized by a ~50 m meter gradient in salt, oxygen, nutrients, metals, and dissolved organic carbon concentrations. Decomposition of labile organic matter in the deep brine (> 200 PSU) generates CH₄ concentrations in excess of 600 μ M. There is substantial geochemical evidence for methane oxidation and sulfate reduction occurring in discrete lenses along the Orca Basin chemocline, but rates measurements have never been attempted. Here we compare anaerobic oxidation of methane (AOM) and sulfate reduction (SR) radiotracer rates between sub-basins. AOM rates vary from ~125 to 350 nmol CH₄ L⁻¹ d⁻¹ in the North basin to between ~780 and 1175 nmol CH₄ L⁻¹ d⁻¹ in the South basin. Sulfate reduction rates are generally below detection in the North basin, but reach a maximum of ~ 1 μ mol L⁻¹ d⁻¹ in the South basin. The variability of these data highlight the heterogeneous nature of microbial communities across the basin.

38: Nitrous Oxide Production In The Ferruginous Proterozoic Ocean

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Though the solar luminosity during the Precambrian was lower than today, evidence for liquid water throughout Earth's history is incontrovertible. This "Faint Young Sun problem"

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is often attributed to elevated carbon dioxide and methane. Nitrous oxide is much more potent than carbon dioxide and methane, but its contribution to the Precambrian greenhouse has largely been dismissed because it photodissociates at the low oxygen levels present in the Archean (10-5 times present atmospheric levels). Nitrous oxide may have become an important greenhouse gas in the anoxic and iron-rich (ferruginous) Proterozoic when oxygen was 0.0001-0.1 times present atmospheric levels. Only 1 ppm nitrous oxide (vs. 0.3 ppm modern concentrations) is needed to support surface temperatures above the freezing point with near-modern carbon dioxide (320 ppm) and methane (1.6 ppm). For our study, a 1-D photochemical model was used to determine steady-state atmospheric nitrous oxide as a function of oxygen and surface-atmosphere fluxes. To achieve 1 ppm nitrous oxide at 0.001-0.1 times present oxygen levels, a flux 10-500 times greater than modern is needed. We posit that the ferruginous Proterozoic ocean could have yielded the requisite higher nitrous oxide flux via reduction of nitric oxide to nitrous oxide by ferrous iron after microbial evolution of nitrite reductase. In this study, we measured rates of nitrous oxide production in artificial anoxic ferruginous seawater with 1 mM ferrous iron, 80 μ M ammonium, 7 μ M nitrite, and 0, 8, and 16 μ M nitric oxide. Minimal nitrous oxide was produced in the absence of nitric oxide. Doubling nitric oxide concentrations led to 50% higher nitrous oxide production. Experiments at lower ferrous iron concentrations, as well as flux calculations, are ongoing. We predict that coupled biotic-abiotic production of nitrous oxide in ferruginous seawater could have contributed to sustained habitability on early Earth.

39: Do mineralogy and methane matter for microbial manganese mobilization?

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Manganese (Mn) drives numerous redox transformations in the environment, including carbon remineralization via anaerobic heterotrophic Mn(IV/III) respiration. Mn(IV/III) respiration with inorganic electron donors, such

as methane (hereafter “Mn-AOM”) is on the “most-wanted” list of microbial metabolisms that are thermodynamically favorable but not yet found in nature. If Mn-AOM does in fact exist, it is likely ancient since life emerged under anoxic, methane-rich conditions, and geochemical evidence for Mn oxides dates back to the Archean eon. In this study, we examined the effects methane and oxidized Mn species on Mn(II) mobilization in anoxic incubations of sediments from Lake Matano, Indonesia, an early ocean analog where geochemical budgets suggest that Mn(IV/III) could be a missing electron acceptor for methane oxidation. Batch enrichments were established by amendment of sediment slurries with amorphous vs. colloidal Mn(IV)O₂ or soluble vs. solid Mn(III) (as Mn(III)₂(P₂O₇)₃ or CaMn(III)₂O₄, respectively), and methane or nitrogen headspace. Activity was monitored by production of extractable and soluble Mn(II). We hypothesized that methane would stimulate Mn(IV/III) reduction, and that reduction rates would show the following trend based on bioavailability, surface reactivity and oxidation state: soluble Mn(III) > solid Mn(III) > colloidal Mn(IV) > amorphous Mn(IV). Instead, we observed no effect of methane addition, and the exact opposite from the predicted trend, with highest Mn(II) production from amorphous Mn(IV) and least from soluble Mn(III). Autoclaved controls showed minimal activity with all substrates. Pure cultures reduce metals at rates directly proportional to surface area:volume ratio, and to solubility. Our results, in contrast, suggest that uncultivated sediment microbes are adapted to utilize the endogenous form of oxidized Mn in their environment.

40: Estuarine CO₂ flux: A comparison of two North Carolina estuaries

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High rates of organic matter delivery along the freshwater-marine gradient in estuaries make them sites of intense carbon remineralization. Previous studies have shown well-mixed, high latitude estuaries to be strong CO₂ sources to the atmosphere, roughly in balance with the continental shelf sink. In response to a combination of physical and chemical factors,

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autochthonous C fixation (photosynthesis, nitrification) may exceed respiration within temperate, microtidal systems common along the NC coast, forcing them towards autotrophy over daily to seasonal time scales. The Neuse (NRE) and New (NEW) River estuaries, NC are well-studied examples of this phenomenon. In order to constrain the magnitude of air-sea CO₂ exchanges in these systems, pCO₂ surveys were conducted (at bi-monthly to bi-weekly intervals) in the NEW beginning in July 2013, and in the NRE beginning in October 2014. pCO₂ was highly variable, both spatially and temporally. Excursions of over 1,000 uatm were observed over the course of a single day, while within-system variability was often thousands of uatms. In both of these systems, stratification often led to a spatial decoupling of autotrophic production and heterotrophic respiration, complicating interpretation of the potential relationship between ecosystem metabolism and CO₂ flux. Other environmental and climatic factors including temperature, nutrient supply, storms, and freshwater flushing time appear to play important roles in modulating both rates of biological activity as well as the physical factors responsible for air-sea gas exchange. Significant differences between these two estuarine systems were observed, potentially linked to anthropogenic impacts on watershed properties and hydrodynamics.

41: Characterizing Groundwater Residence Times And Associated Drivers Of Variability In Aquifers Around Coastal Stormwater Detention Ponds

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Groundwater residence time is an important characteristic that provides insight into water-rock and biogeochemical interactions that occur with the associated aquifer. This study used the natural radioisotopic tracer ²²²Rn to examine residence times of groundwater around two stormwater detention ponds. A technique involving equilibration of radon with aquifer materials provided an estimate of the radon that would be injected into groundwater from the sediments if residence times were greater than three weeks. Extracted groundwater was

measured for ²²²Rn activity so residence times of the groundwater could be calculated based on in-situ observations compared to the maximum ingrowth potential via standard radioactive ingrowth equations. Residence times of collected groundwaters ranged from 8 to 150 hours with variation within and between aquifers. We explore the potential controls of precipitation, pond depth, water table height, hydraulic conductivity, and groundwater discharge rate on the residence time variability, as well as resulting nutrient composition of those groundwaters. Knowledge of the residence times of groundwater is valuable to understanding material transport, as well as the various types of interactions occurring over time within the aquifer.

42: Quantifying The Importance Of The Rare Biosphere For Microbial Community Adaptation To Organic Pollutants In a Freshwater Ecosystem

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Microbial community analysis frequently focuses on abundant organisms, but natural communities commonly harbor thousands of low abundance, 'rare' organisms. The importance of this 'rare biosphere' for microbial community adaptation to environmental perturbations remains speculative. We tested whether rare species respond to changing environmental conditions by establishing 20-L, planktonic mesocosms with water from Lake Lanier (Georgia, USA), and perturbing them with organic compounds that are rarely detectable in the lake, such as 2,4-dichlorophenoxyacetic acid (2,4-D), 4-nitrophenol (4-NP), and caffeine. The degraders of these compounds were initially below detection limit of qPCR or metagenomic sequencing methods (i.e., <0.001% of total community), but increased substantially in abundance after perturbation. Almost all replicated mesocosms exhibited distinct degradation profiles, presumably due to the effect of the rare biosphere (e.g., not all species are present in all inocula due to low abundance) and/or stochastic processes in the activation of rare species and genes. To obtain further insights into the latter, we sequenced several 2,4D-degrading isolates, and assessed their genomes

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against time-series metagenomic datasets from the 2,4-D replicated mesocosms. We found distinct alleles of degradation genes, encoded frequently on transmissible plasmids, and distinct species dominating the post-enrichment datasets from each mesocosm. Collectively, these results support the hypothesis that the rare biosphere can serve as a genetic reservoir that enables community adaptation to changing environmental conditions via horizontal gene transfer, and provided quantitative insights into the size of the pool of rare genes and species as well as its role in degradation of important environmental pollutants.

43: An Anaerobic Betaproteobacterium Cultured From Pineola Bog, NC

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Peat-forming wetlands are one of the largest natural sources of atmospheric methane; and thus are important contributors to global climate change. In these wetlands, anaerobic bacteria slowly decompose plant material, producing H₂, CO₂, acetate and other methane precursors. Sequencing data suggests that a large proportion of the anaerobic organisms are novel and uncultured with very little known about their metabolic potential. Thus, the aim of this project was to culture and identify anaerobic microbes in a local peat bog and characterize their physiological traits. Peat samples were taken from Pineola Bog, a Sphagnum-dominated, acidic (pH ~5.5) poor fen located in Pineola, NC. The samples were anaerobically cultured in an N₂ rich headspace with glycerol as a carbon source. Cultures were found to have enriched for members of the phyla Actinobacteria and Proteobacteria. Herein, we present one culture of interest, denoted as strain CS3SC-2 that shares 97% identity to a cultured strain in the Rhodocyclaceae family of the Betaproteobacteria, *Uliginosibacterium gangwonense*. Betaproteobacteria represented approximately 16% and 18% (by relative abundance) of Illumina sequences in samples collected in 2013 and 2014 from Pineola Bog, although Betaproteobacteria

are rarely found in high abundance in the northern oligotrophic peatlands commonly studied. Overall, the study of strain CS3SC-2 may yield insight into the role of Betaproteobacteria in the environment.

44: Using High Throughput Sequencing Methods To Identify Keystone Bacterial Species In Recalcitrant Terrestrial Organic Matter Transformation

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The microbial transformation of terrestrial organic matter, particularly recalcitrant lignin and hemicellulose, is a significant but poorly understood phenomenon. 16S rRNA gene amplicon sequencing and metagenomic sequencing are relatively inexpensive molecular tools to quickly assess microbial diversity and functional genes in response to different carbon sources. Using sequencing and bioinformatics, we aim to elucidate the diversity and metabolic potential of bacterial communities subsisting on lignin and hemicellulose in laboratory cultures. Seawater microcosms were incubated with an added concentration of purified lignin or xylan. CO₂ respirometry and enzyme assays showed high microbial activity on both substrates. Using an Illumina MiSeq platform, both amended cultures and unamended controls were sequenced for 16S rRNA gene amplicons and metagenomics. Reads were annotated using Qiime and MG-Rast. Annotated data was compared in detail using phyloseq, vegan, and DESeq2 in R. Several species belonging to phylum of Proteobacteria, Flavobacteria, and Firmicutes significantly increased in abundance when lignin or hemicellulose was added. Groups of functional genes related to carbohydrates and aromatic catabolism significantly increased in abundance. Our methods may uncover important species that have been previously overlooked for terrestrial organics degradation.

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45: Metagenomic survey of sediment enrichments from an ancient ocean analog

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Lake Matano, Indonesia is unique ecosystem that possesses similar ferruginous and methane-rich conditions to the Archean ocean. We are studying microbial metabolisms of Lake Matano sediment that are possibly representative of ancient pathways. Two long-term sediment enrichments were established with inoculum from different years and water depths. Sediments were incubated under anoxic conditions with ferrihydrite and methane as the sole electron acceptor and donor, respectively. Microbial composition based on 16S rRNA amplicon sequencing differed between inocula, likely due to different storage conditions and durations. Despite those differences in inocula, similar microbes were enriched from both sediment samples, and microbial phylogeny from 16S rRNA gene amplicon and metagenomic datasets were broadly consistent. Deltaproteobacteria (Geobacteraceae) was enriched with and without methane, whereas Betaproteobacteria (Rhodocyclaceae) were only enriched in the presence of methane. Bathyarchaeota sequences were abundant in the fresher inoculum, but preliminary results identified solely euryarchaeotal *mcrA* genes in metagenomes. Binning produced nearly complete (>98%) genomes with <5% predicted contamination for several new species belonging to the Geobacteraceae and Rhodocyclaceae families. Ongoing work is focused on identifying and characterizing genes potentially involved in methane and iron cycling in these genomes.

46: Soil amino sugars in rice pot culture as affected by the addition of straw and ¹⁵N fertilizer

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Amino sugars are key compounds of microbial cell walls, which have been widely used as biomarker of microbial residues to investigate soil microbial communities and organic residue cycling processes. Soil micro-organisms could utilize inorganic N to synthesize amino sugar, but whether inorganic N is immobilized to amino sugars depends on microorganism's requirement. Crop residues provide resources for soil microbial metabolism thereby stimulating amino sugar buildup in soil. Recognizing the "new" amino sugars in the rice culture after the addition of straw and inorganic N may help develop sustainable agriculture and reduce the waster of inorganic nitrogen. We carried out an experiment to evaluate the effect of the addition of straw and inorganic nitrogen on soil amino sugars in rice pot culture. Our objectives were to 1) evaluate how the quantity of straw affect the accumulation and composition of amino sugars. 2) clarify the origin of amino sugars i.e. derived from fungi or bacteria. 3) make clear the synthesis ability of microorganisms to different "new" amino sugar. The experiment was conducted in Shenyang agricultural university in 2014. The soil was heavy clay of alluvial origin. The rice pot cultural experiment consisted of 6 treatments (CK, N, NC1, NC2, NC3, NC4) with the addition of ¹⁵(NH₄)₂SO₄(mg) and different amount of straw. We determined the content and composition of amino sugar. Then the microbial synthesis of amino sugars in soil was detected. Our investigation revealed that: 1) Compare to CK and ¹⁵(NH₄)₂SO₄ alone, the addition of straw can induced a relative increase in amino sugar in soil. And with the increase of straw, the contribution of fungi to SOM turnover was enhanced first, then the contribution of bacteria tended to increase. 2) The suitable input of carbon and nitrogen such as NC2 caused the most enrichment of ¹⁵N-amino sugar. It confirmed that the change of additional carbon can regulate the microbial synthesis of nitrogen.

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47: Methane Oxidation in Arctic Soils from High- and Flat-Centered Polygons

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The premise of global warming will cause deeper permafrost thawing, followed by increased carbon mineralization and CH₄ formation in saturated tundra soils. Arctic tundra soils also serve as potential sinks of atmospheric CH₄ in response to warming temperature, which might be a key process in the global CH₄ budget. Quantification of methane oxidation potential of Arctic tundra is an important component to constrain models assessing the C-climate feedback from high latitude soils. The signature polygonal ground of Arctic tundra generates high level of heterogeneity in soil hydrology and soil thermal regime. Thus, two distinct polygonal features were investigated in this study to evaluate CH₄ oxidation potentials under multiple biogeochemical controls. The drivers, magnitude, timing and location of methane oxidation were thoroughly compared between High- and Flat-Centered Polygons (HCP, and FCP, respectively). CO₂ production measured in microcosms provided an estimation of C mineralization rates with strong temperature dependency. A significant lag period of CO₂ production was observed in soil microcosms from HCP center but not from FCP center, which might be attributed to microbial biomass limitations in HCP center. CH₄ production was only observed from a transitional horizon of FCP, which also exhibited higher CH₄ oxidation potential measured via methane oxidation assays (MOA). CH₄ oxidation was observed from all horizons from both HCP and FCP, and prolonged thawing significantly accelerated CH₄ oxidation rates. The upcoming work will identify key variables controlling methane oxidation rate and develop parameterization that can be incorporated into terrestrial ecosystem models.

48: Biodiversity And Enrichment Of Organic Sulfur-Driven Iron-Respiring Microorganisms From Marine Sediment

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Anaerobic iron respiration may have been one of the very first forms of microbial metabolism to evolve early on in the history of life; yet up until recent years, little has been known about the mechanisms that drive such ancient respiration processes. The biodiversity of microbial communities capable of iron-reduction in anoxic environments has been previously overlooked and the significance of this activity is not fully understood. To better understand the phylogenetic and metabolic diversity of iron-reducing bacteria (FeRB), sediment samples from the salt marsh ecosystem of Skidaway Island (GA), were obtained and used to test the hypothesis that organic sulfur (S) compounds can act as electron shuttles for FeRB to extracellular insoluble Fe(III) oxides. The chemical composition of multiple core samples collected from Skidaway Island were analyzed, and core sediments exhibiting overlapping peaks of ferrous iron (Fe²⁺) and organic sulfur compounds (thiols) were used for subsequent microbial community analysis and enrichment of organic S-driven FeRB. Organic S-driven FeRB were enriched in cultures by providing an environment without oxygen (N₂ headspace) with the following conditions i) lactate or acetate as electron donors, ii) various thiols as electron shuttles and iii) solid Fe(III) oxide as electron acceptor. Enrichment of FeRB was also performed in the presence of rifamycin to select for Archaea. Microbial community structure in core sediments and enrichment cultures was analyzed via deep sequencing of the 16S rRNA hypervariable V4 region with bacterial and archaeal specific primer sets. Results indicate that thiols increase FeRB iron activity in enrichments and impact the community structure of enriched microorganisms. Molybdenum-containing controls suggest that sulfide-catalyzed electron shuttling is not a dominant mechanism for microbial Fe(III) reduction in marine sediments.

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