SBS '19 at Uof SC

6th Annual Southeastern Biogeochemistry Symposium University of South Carolina, Columbia, SC March 30 – March 31, 2019





Oceanography • Biology • Ecology • Geochemistry Astrobiology • Environmental Engineering • Geology

Locations

Friday Mixer (6:30-8 pm):Liberty Tap Room, 828 Gervais St., Columbia SCSymposium:Gambrell Hall, 817 Henderson Street, Columbia SCParking:On Saturdays and Sundays, parking is free on
campus. The Bull Street Parking garage is close by



Gambrell Hall



Student Organizing Committee

Brian Duggan Jessica Frankle Eilea Knotts Kelly McCabe Caitlyn Sarno Natalie Tyler

Faculty Organizing Committee

Dr. Claudia Benitez-Nelson (UofSC) Dr. Ronald Benner (UofSC) Dr. Carol Boggs (UofSC) Dr. Annie Bourbonais (UofSC) Dr. Jennifer Glass (GA Tech) Dr. Samantha Joye (UGA Athens) Dr. William Landing (FSU) Dr. Susan Lang (Co-chair, UofSC) Dr. Karen Lloyd (UT Knoxville) Dr. Jay Pinckney (UofSC) Dr. Tammi Richardson (UofSC) Dr. Lori Ziolkowski (Co-chair, UofSC)

Sponsors





<u>Keynote Speaker</u> Dr. Jennifer Glass

Georgia Institute of Technology, School of Earth & Atmospheric Sciences



Ironing Out Life's First Breaths

Respiration is the "ying" to photosynthesis' "yang". Broadly defined, respiration is the transport of electrons coupled to the pumping of protons to make ATP. Anaerobic respiration is fundamental to many biogeochemical cycles, and aerobic respiration was likely key to the evolution of complex multicellular life. But where did respiration come from? How did life "learn" to breathe? In this talk, I will: (i) present an evolutionary history of the respiratory electron transport chain; (ii) explain how the biochemistry of primitive electron transport complexes is broadly consistent with what is known about the availability of electron acceptors and metal cofactors through deep time; (iii) highlight how modern microbes with ancient forms of respiratory complexes may give glimpses of life's first breaths.

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Conference Agenda

Friday March 29, 2019

6:30 PM – 8:00 AM	Welcome Reception
	Liberty Tap Room, 828 Gervais St, Columbia

Saturday March 30, 2019

8:15 AM – 9:00 AM	Continental Breakfast
	Registration
9:00 AM – 9:15 AM	Introduction/Welcome
9:15 AM – 10:00 AM	Keynote Address: "Ironing Out Life's First Breath"
	Dr. Jennifer Glass, Georgia Institute of Technology
10:00 AM – 10:30 AM	Coffee Break
10:30 AM – 12:00 PM	Oral Session I
10:30 AM – 10:45 AM	Katie Sipes, <i>Marine-associated functional genes within metagenomic assembled genomes (MAGs) from freshwater permafrost</i>
10:45 AM – 11:00 AM	Taylor Royalty, <i>Partitioning of microbial function among taxonomic ranks across the tree of life</i>
11:00 AM – 11:15 AM	Samuel Putnam, Detection and monitoring of toxins in Lyngbya wollei algae in Lake Wateree, SC
11:15 AM – 11:30 AM	Eilea Knotts, <i>Effects of removed carbonic anhydrase</i> <i>activity on biomass and primary production of estuarine</i> <i>benthic microalgal communities</i>
11:30 AM – 11:45 AM	Gwen Miller, Estimating above ground biomass within a salt marsh utilizing Planet multispectral satellite data
11:45 AM – 12:00 PM	Isaac Keohane, Sensitivity of oyster filtration models to environmental limitation functions
12:00 PM – 1:00 PM	Lunch

1:00 PM – 2:30 PM	Oral Session II
1:00 PM – 1:15 PM	Dahae Seong, Elucidating the governing mechanisms of the fate and transport of microbes in indoor environments: effect of surface type
1:15 PM – 1:30 PM	Tito David Peña-Montenegro, Functional scanning approaches reveal early responses of extreme microbial communities to oil and dispersant exposure
1:30 PM – 1:45 PM	Sarah Brown, Enzymatic activities of microbial communities in the Indian and Southern Oceans exhibit distinct spatial and depth-related patterns
1:45 PM – 2:00 PM	Michael Philben, <i>Hillslope controls on anaerobic soil organic matter decomposition in a tundra watershed</i>
2:00 PM – 2:15 PM	Layla Ghazi, Enrichment and Isolation of Iron-Oxidizing Bacteria from an Ancient Earth Analogue
2:15 PM – 2:30 PM	Nan Xie, Dark production of ROS in microbially-driven Fenton reaction with solid phase Fe(III)
2:30 PM – 3:00 PM	Coffee Break
3:00 PM – 4:15 PM	Oral Session III
3:00 PM – 3:15 PM	Amanda Cavazos, Simultaneous Imaging of Microbial Cells and Manganese Oxide Minerals
3:15 PM – 3:30 PM	Katrina Godsey, Identifying Mn oxidizing microbes in acid mine drainage
3:30 PM – 3:45 PM	Abigail Johnson, Potential life strategies in gas clathrates
3:45 PM – 4:00 PM	Timothy Rogers, <i>Life under pressure: Exploring the variation in microbial communities across a volcanic arc</i>
4:00 PM – 4:15 PM	Natalie Tyler, Endolithic microbial carbon cycling in Dronning Maud Land, East Antarctica
4:15 PM – 4:30 PM	Coffee Break
4:30 PM – 6:00 PM	Poster Session

Sunday March 31, 2019

8:15 AM – 9:00 AM	Continental Breakfast
9:00 AM – 9:15 AM	Welcome
9:15 AM – 10:30 AM	Oral Session IV
9:15 AM – 9:30 AM	Kelly McCabe, <i>Effects of stormwater management practices</i> <i>on nonpoint source BOD loading to coastal waters in South</i> <i>Carolina</i>
9:30 AM – 9:45 AM	Fan Wang, Polymerization in place: Conversion of mobile contaminants to fixed polymers
9:45 AM – 10:00 AM	Ibraheem Hafiz, 3D seismic attributes analysis of subsurface fault and fracture systems in Llanos Foothills Colombia
10:00 AM – 10:15 AM	Brian Duggan, <i>Surface distribution of Nd in the Amerasian</i> <i>Basin of the Arctic</i>
10:15 AM – 10:30 AM	Brady Ferster, <i>Diagnosing biases in estimates of the Antarctic</i> <i>Polar Front location and variability</i>
10:30 AM – 10:50 AM	Coffee Break/Point Tallying
10:50 AM – 11:30 AM	Award Presentation/Closing Remarks
11:30 AM – 12:00 PM	Optional Campus and Facilities Tour

Oral Presentation Abstracts

* - Presenting Author

Oral Session I

Saturday 10:30 AM – 10:45 AM **Marine-associated functional genes within metagenomic assembled genomes** (**MAGs**) from freshwater permafrost Katie Sipes*, Tatiana Vishnivetskaya, Karen Lloyd

University of Tennessee, Knoxville

The Kolyma region of Northeastern Siberia Russia is home to the oldest undisturbed permafrost in the world. The permafrost was deposited within a freshwater environment, which makes the presence of marine-associated genetic capabilities within 30 metagenome assembled genomes (MAGs) a curious result. Further, there are many saline adapted genes within these metagenome genomes that may suggest that these organisms are from a brine-adapted community within the permafrost. These MAGs also contain genes that suggest that they may utilize NADH-consuming deacetylases to maintain DNA integrity during long term subsistence. Many of these MAGs also contain genes that may allow these organisms to live within this low energy environment. When compared to open access genomes and environmentally related taxa, it seems that these permafrost MAGs have a unique way of energy acquisition and could actually be alive in the liquid water present in small brine veins in the unique permafrost environment. With the use of bioinformatics and open access data mining, we make a case that these permafrost organisms have the ability to be alive in this ancient permafrost ecosystem.

Saturday 10:45 AM – 11:00 AM Partitioning of microbial function among taxonomic ranks across the tree of life

Taylor M. Royalty*, Andrew D. Steen University of Tennessee

Microbiomes influence their environments via the collective action of microbial metabolisms. Recent studies have demonstrated that microbial lineages preferentially occupy certain environments. Thus, a quantitative understanding of the extent that taxonomy influences microbial function is critical for better understanding both the global distribution of microbial traits and the influence that these traits have on their respective environments. However, the overwhelming majority of microbial lineages are resistant to culturing. This leads to challenges when characterizing microbial function with traditional experimental methods. One approach to address this problem is analyzing functional annotations associated with putative genes. Here we quantify the enrichment of clusters of orthologous gene functional categories (COG-FCs), a proxy for metabolic potential, within the genomes of 3207 microbe lineages in a custom-curated genome database. The custom-curated genome database consists of genomes from RefSeq v83 and Uncultivated Bacteria and Archaea (UBA). Genomes were only included when a microbial lineage had a minimum of 10 representatives for a given genus. Results show that, on average, 36.7% of the variation in COG-FC enrichment is explained by taxonomic rank, with domain, phylum, class, order, family, and genus explaining, on average, 2.3%, 12.9%, 2.6%, 9.4%, 6.1%, and 3.5% of the variance, respectively (p<0.001 for all). Furthermore, taxonomic rank explained 81.5% of the variance when a only 18 of the 23 COG-FC categories were considered for analysis. To our knowledge, this is the first work which quantifies the variance in metabolic potential from individual taxonomic ranks. A qualitative comparison between the COG-FC enrichments and order-level phylogenies, generated from concatenated protein sequence alignments, further supports the idea that metabolic potential is taxonomically coherent at higher taxonomic ranks. The coherence in metabolic potential at higher taxonomic ranks provides support to hypotheses which suggest that microbial lineages may be biased to occupy specific ecological or metabolic niches.

Saturday 11:00 AM - 11:15 AM

Detection and monitoring of toxins in Lyngbya wollei algae in Lake Wateree, SC

Samuel Putnam*, Meagan Smith, Tryston Metz, Geoff Scott, John Ferry University of South Carolina

Harmful algal blooms are an increasing human health risk that is poorly understood; blooms can produce a wide array of toxic materials that are often difficult to detect. The harmful algae Lyngbya wollei colonized Lake Wateree (South Carolina) several years ago and has been spreading throughout favorable areas of the lake. Lake Wateree, located in Ridgeway South Carolina, is a source of water for both recreation and drinking for the surrounding communities. It is a man-made impoundment at the confluence of the Catawba and Wateree Rivers that joins in with the Santee watershed. Lyngbya wollei is a filamentous benthic algae that is known to produce several compounds that can have dermatological or neurotoxic effects. Here we report molecular analysis of Lyngbya wollei obtained from multiple sites on the lake from June 2018 until February 2019. Samples were collected both over time and over a variety of locations to give an overall estimate of presence and potential toxin accumulation in algal biomass over the course of the season. Samples were lyophilized, extracted, and then analyzed by hydrophilic interaction liquid chromatography/mass spectrometry. Initial results show the presence of several toxins in the Lyngbya wollei toxin (LWT) family, specifically LWTs 1, 4, 5, and 6 (all saxitoxin derivatives). Extraction and instrumental methods were developed for the analysis of this toxin and initial quantification was performed. Correlation between nutrients and other anthropogenic stressors and the LWTs is presented. An estimate of the total potential toxin inventory of the lake is presented for the 8 month sampling period.

Saturday 11:15 AM – 11:30 AM **Effects of removed carbonic anhydrase activity on biomass and primary production of estuarine benthic microalgal communities** Eilea Knotts^{*}, Jay Pinckney University of South Carolina

Recent studies have focused on carbon concentrating mechanisms and the associated enzyme, carbonic anhydrase, to better understand the efficiency of CO2 uptake rates and carbon fixation in photoautotrophs. Some benthic microalgae (BMA) may be limited by inorganic carbon availability because high photosynthetic rates withdraw a large amount of CO2 and HCO3¬- in the top layer of sediment. The potential impacts of increasing atmospheric CO2 concentrations which may affect CO2 acquisition strategies have received limited study for BMA communities. The purpose of this research was to examine BMA community responses to induced carbon limitation through the removal of carbonic anhydrase (CA) activity using an inhibitor, ethoxyzolamide. Microcosm experiments were performed on intertidal muddy sediments from North Inlet Estuary, SC. Exposure to ethoxyzolamide resulted in a reduction of gross primary productivity (GPP) without a reduction in total BMA biomass. Furthermore, removed CA activity caused BMA cumulative GPP maxima to shift upward toward the surface in the sediment column. Active CA was necessary to maintain high GPP rates in these communities and allowed motile BMA to use a wider portion of the sediment column. Available HCO3- at lower depths could still be dehydrated into CO2 by microalgae with CA. Global carbon changes leading to higher CO2 availability at the atmosphere-sediment interface may alter the structure and function of these BMA systems, and the vertical distribution of GPP. These consequences may have important implications for the biogeochemical cycling occurring in estuaries.

Saturday 11:30 AM – 11:45 AM Estimating above ground biomass within a salt marsh utilizing Planet multispectral satellite data Gwen Miller*, James T. Morris

University of South Carolina

Understanding how biomass spatially changes within a salt marsh may lead to a better understanding of carbon stored within this ecosystem and improve estimates of "blue carbon." Collecting biomass data across an entire landscape in the field is infeasible due to the difficulty of the terrain, and the amount of time required to cover large areas. Satellite data therefore is a more realistic method for estimating landscape scale aboveground biomass. Traditionally free data such as Landsat is utilized for biomass calculations, but Landsat has 30 meter spatial resolution. The 30 meter resolution smooths out the spatial variability within the marsh, and makes it difficult to pick up fine scale changes over the landscape. A United States company Planet, recently established a free data sharing program for student researchers. The company provides students and researchers with free daily 3.7 meter resolution multispectral imagery, which allows for high spatial and temporal analysis of landscapes.

Utilizing multispectral data from Planet, I developed a model to accurately predict above ground biomass within the North Inlet Winyah Bay National Estuarine Research Reserve. I created a series of generalized linear regressions in R and compared their Akaike Information Criterion (AIC) scores, to determine which model best fit the data. From the AIC analysis, logged normalized difference vegetation index values best predicted salt marsh biomass. Using the best fit model, I then created a high resolution (3.7 meters) estimate of above ground biomass within North Inlet, which highlights regions with high and low biomass density. Saturday 11:45 AM – 12:00 PM Sensitivity of oyster filtration models to environmental limitation functions Isaac P. Keohane*, Jacqueline Tay, Rasika K. Gawde, Raleigh R. Hood University of South Carolina

Oysters (namely *Crassostrea virginica*) directly impact water quality through filter feeding and have been extensively studied due to the potential ecological benefits of population restoration. Numerical models have been developed that simulate their environmental impact for the purposes of both estimating current water quality conditions and evaluating prospective restoration strategies. Accurately representing oyster filtration in these models is essential to their value as management tools. Here we compare different formulations for filtration from several studies (Cerco and Noel, 2005; Fulford et al., 2007; North et al., 2010; Ehrich and Harris, 2015), which use environmental limitation functions of temperature, salinity, and suspended solids concentration (TSS) to determine a filtration rate. Using outputs from a water quality model with controlled forcing conditions we investigated how sensitive model output can be to the environmental limitation functions used. We found that due to feedback between TSS and filtration rate, the choice of TSS function caused significant variation in the model output beyond the scale of the differences in the functions themselves. We discuss what the observed variations in model outputs mean for the accuracy of larger water quality models with oyster filtration. Also, based on our observations and a theoretical understanding of model construction, we make recommendations for future modeling efforts to improve methods for representing the filtration.

Results from this study will give a better understanding of above ground biomass within salt marshes which is useful for blue carbon estimates. Landscape scale above ground biomass maps may also improve models such as the Marsh Equilibrium Model (MEM). MEM is a predictive model that estimates elevation changes within salt marshes in relation to sea level rise. One of the key inputs in the model is above ground biomass, and landscape scale biomass values will improve estimates obtained from landscape scale MEM analysis.

Oral Session II

Saturday 1:00 PM – 1:15 PM Elucidating the governing mechanisms of the fate and transport of microbes in indoor environments: effect of surface type Dahae Seong*, R. Sean Norman and Shamia Hoque University of South Carolina

Indoor microorganisms reflect the combined effects of the surrounding environments. Numerous studies have investigated the indoor environments to determine how they influence the microbes and vice-versa. Current literature gives a rich data on the diversity of the microbiome but lack details on the composition, quantity and concentration. The current investigation explores, identifies and quantifies the mechanisms that controls the microbiome characteristics in the indoor environment. The results presented here focus on bacterial species. The study incorporated a combination of field sampling and laboratory experiments to obtain data and greater insight. Statistical methods such as PCA/CCA (Principal Component Analysis/Canonical Correspondence Analysis), ANOVA and factorial design approaches were applied for analysis.

A survey of the literature showed that 24% of the predominant bacterial families, Bacillales was detected on surfaces and 22% were Actinomycetales. Frequently detected bacteria both in air and surface, were *Staphylococcaceae, Corynebacteriaceae* and *Micrococcaceae*, but their proportion is highly different depending on the indoor/sampling conditions. Results of sampling at schools in different age groups (elementary and high school) showed that the predominant bacterial species were *Staphylococcus, Micrococcus*, and *Bacillus* in agreement with the literature. Kindergarten showed higher bacterial concentration (293 CFU/m³) than high school (135 CFU/m³) due to higher activity. PCA/CCA analysis showed the bacterial concentration is significantly influenced by vent location (p<0.05). Lower levels of *Aerococcus* sp. (outdoor signature) was observed < 2 m distance from vents. The results showed that specific bacteria could be identified as a 'biofingerprint' that represents indoors. Factorial design analyzed that higher *Staphylococcaceae* level indicates higher human activity, *Lactobacillaceae* and *Corynebacteriaceae* have gender signature, and *Streptococcaceae* represents children occupants (p<0.05).

For assessing the influence of specific bacteria – surface interaction, experiments were conducted using centrifuge for calculating detachment caused by centrifugal force and QCM-D for assessing attachment/detachment behavior of different microbes. Eight experiments in triplicates have been designed based on two-level factorial design (2^3) . The three factors were centrifugal time, attachment time, and surface material. Factorial design analyzed that surface types impact on bacterial attachment/detachment (p<0.05). 58% of *Corynebacterium* attached on the hickory surface in 6 hours of exposure time, while attachment on metal and oak reached 28% and 21%, respectively. Detachment increased with centrifugal time until 15 minutes beyond which there was no further change. QCM-D experiments showed that detachment increased with flow rate and higher flow rate (200 µL/min) impeded attachment. More configurations of surface and bacteria combinations are being tested currently.

Saturday 1:15 PM – 1:30 PM **Functional Scanning Approaches Reveal Early Responses of Extreme Microbial Communities to Oil and Dispersant Exposure** Tito D. Peña-Montenegro*, S Kleindienst, AE Allen, J Arnold, SB Joye University of Georgia

The application of transcriptomic approaches to describe microbial signal from extreme environmental samples are frequently misplaced in terms of annotation and sensitivity. We aim to explore scanning strategies to uncover transcriptional signals of microbial communities under oil and dispersants exposure. The study is based on 2010 Deepwater Horizon spill simulation experiments as well as open field site comparisons where novel transcriptomic scanning methods have been applied to increase annotation resolution of microbiomes of extreme environments.

Saturday 1:30 PM – 1:45 PM Enzymatic activities of microbial communities in the Indian and Southern Oceans exhibit distinct spatial and depth-related patterns

Sarah Brown*, Adrienne Hoarfrost, J.P. Balmonte, Sherif Ghobrial, Carol Arnosti University of North Carolina

Heterotrophic microbial communities play a key role in the oceanic carbon cycle by processing substantial amounts of organic matter. Typically, to access high molecular weight organic matter, microbial communities must possess specific enzymes in order to hydrolyze substrates into small enough pieces that they can pass into the cell. The rate and location of specific microbial enzymatic activities can therefore determine patterns of carbon remineralization and sequestration in the ocean. Previous studies have found spatial and depth-related patterns in enzymatic activities in the Atlantic, Pacific, and Arctic Oceans, but few studies have investigated enzymatic activities in the Indian and Southern Oceans. To evaluate the enzymatic capabilities of microbial communities in the Indian and Southern Oceans, we collected water from 5 depths (including from the surface, deep chlorophyll max, Antarctic Intermediate Water, Upper Circumpolar Deep Water, and bottom) at each of 13 stations and examined the activities of polysaccharide hydrolases (analysis in progress), peptidases (leucine aminopeptidase, trypsin and chymotrypsin), and α - and β -glucosidases. The peptidase and glucosidase activities observed in the Indian and Southern Oceans exhibit patterns distinct from those previously observed in the Atlantic and Pacific Oceans. The spectrum and rates of peptidase activities in the Indian and Southern Oceans was broader and more even than in the Atlantic and Pacific Oceans, where leucine aminopeptidase activity typically made up the greatest proportion of enzymatic activities. Activities of α - and β -glucosidase also appear to be much lower in Atlantic and Pacific Ocean measurements than in those from the Indian and Southern Oceans. Furthermore, while the spectrum of enzymatic activities typically decreased substantially with depth in the Atlantic and Pacific Oceans, the spectrum of enzymatic activities did not change substantially with depth in the Indian and Southern Oceans. The differential hydrolysis of organic matter in different oceans may have consequences for atmospheric and oceanic carbon reservoirs. Future analysis of bacterial community composition will allow us to determine the extent to which enzymatic activities in the Indian and Southern Oceans are influenced by patterns in bacterial community composition.

Saturday 1:45 PM – 2:00 PM **Hillslope controls on anaerobic soil organic matter decomposition in a tundra watershed** Michael Philben*, Baohua Gu, Stan Wullschleger, Alexander Kholodov, David Graham Oak Ridge National Laboratory

We investigated rates and controls on greenhouse gas production in two contrasting water-saturated tundra soils within the same watershed, located near Nome, Alaska. Soil from a fen-like area at the base of the hillslope had higher pH and higher porewater ion concentrations than soil collected from a bog-like peat plateau at the top of the hillslope. We investigated how these contrasting geochemical environments affect CO₂ and CH₄ production by incubating soil microcosms anaerobically at -2°C and 8°C for 55 days. NH₄Cl was added to half of the microcosms to test for N limitation of microbial greenhouse gas production.

We found that total CO₂ and CH₄ production were higher in the soils from the bottom of the hillslope. We also observed that water-extractable organic C was higher in these soils, and fermentation of this C pool resulted in an increasing supply of low-molecular weight organic acids (e.g. acetate and propionate) throughout the incubations. Higher availability of labile DOC, in addition to higher pH, likely contributed to the more rapid greenhouse gas production at the bottom of the hillslope. Our incubations also demonstrated that decomposition in the peat plateau soils was more N-limited. Inorganic N concentrations were higher in the soils from the bottom of the hillslope, and these exhibited net N mineralization while the peat plateau soils had net N immobilization. N addition increased CO_2 production in the peat plateau soils but not the lowland soils, consistent with greater N limitation.

Our results suggest that the movement of water, ions, and nutrients down the tundra hillslope can increase the rate of anaerobic SOM decomposition by (1) increasing the pH of soil porewater; (2) providing bioavailable DOC; and (3) relieving microbial N limitation through nutrient runoff.

Saturday 2:00 PM – 2:15 PM Enrichment and Isolation of Iron-Oxidizing Bacteria from an Ancient Earth Analogue

Layla Ghazi*, Nadia Szeinbaum, Jennifer Glass Georgia Institute of Technology

On the surface of modern Earth, oxygen is the most widespread electron acceptor for respiration. However, in deeper anoxic environments, many microbes can use other electron acceptors to respire anaerobically. The goal of this study was to test whether anaerobic microbial growth could occur with Fe2+ as the electron donor. Enrichment cultures were inoculated with anoxic, Fe2+-rich sediments from Lake Matano, Indonesia, an ancient ocean analogue. Sediments were incubated with Fe2+ sulfide as the electron donor in a nitrogen:carbon dioxide (90/10%) atmosphere. Soluble manganese(III) or oxidized nitrogen were provided as electron acceptors. Control cultures were tested for aerobic Fe2+ oxidation. With Mn(III) as the electron acceptor, cultures showed some evidence of growth near the middle of the gradient tube. However, orange Fe(III) oxides were absent, suggesting that anaerobic Fe2+ oxidation had not occurred. A white precipitate indicated that the microbes were reducing Mn(III) to Mn(II), which precipitated as a white manganese carbonate mineral, which was not present in the uninoculated controls. Fe(III) oxides were also absent in tubes containing oxidized nitrogen. With oxygen as the electron donor, an layer of orange Fe(III) oxide minerals formed near the water-air interface, indicative of growth of microaerophilic Fe2+ oxidizing bacteria ; this layer did not form in uninoculated controls. Our preliminary results suggest that anaerobic Lake Matano enrichments are capable of Fe²⁺ oxidation using oxygen but not alternative electron acceptors.

Saturday 2:15 PM – 2:30 PM Dark production of ROS in microbially-driven Fenton reaction with solid phase Fe(III)

Nan Xie*, Yael J. Toporek, Thomas J. DiChristina, Martial Taillefert Georgia Institute of Technology

The "Fenton reaction" or the oxidation of ferrous iron (Fe(II)) by hydrogen peroxide (H_2O_2) to form reactive oxygen species (ROS) is well known to promote the degradation of organic substrates and is currently accepted as one of the most effective techniques for the degradation of organic pollutants. In recent studies, a microbially-driven Fenton reaction system was designed in batch reactors with the Fe(III)-reducing facultative anaerobe Shewanella oneidensis to produce Fe(II) and H₂O₂ via oscillation between aerobic and anaerobic conditions in the presence of soluble ferric ion. The Fenton reaction generated ROS to degrade source zone levels of TCE, PCE, and 1,4-dioxane as single contaminants or as binary and ternary mixtures. In comparison to conventional (purely abiotic) Fenton reaction, the microbially-driven Fenton reaction operates at circumneutral pH and does not require addition of exogenous H₂O₂ or UV irradiation to regenerate Fe(II). As iron oxyhydroxides are ubiquitous reactive constituents of soils and sediments, the metabolic activity of iron reducing bacteria may alternate with aerobic respiration during rain events and, thus, promote the microbially-driven Fenton degradation of various organic contaminants in subsurface environments. The microbiallydriven Fenton reaction with solid phase Fe(III), however, has not been investigated thoroughly. In this study the main pathways involved in the dark production of ROS was investigated with S. oneidensis and 2L-ferrihydrite in both batch and flow-through reactors. Our findings suggest that the microbially-driven Fenton reaction with solid phase Fe(III) produces ROS via a complex interplay between microbial metabolism, aqueous chemistry, and mineral precipitation.

Oral Session III

Saturday 3:00 PM – 3:15 PM **Simultaneous Imaging of Microbial Cells and Manganese Oxide Minerals** Amanda R. Cavazos*, Jennifer B. Glass Georgia Institute of Technology

Microbes play a significant role in the environmental cycling of manganese oxides (Mn(III,IV)O₂), which are ubiquitous and highly reactive. For example, hydroxylamine leaked from nitrifying microbes will rapidly react with MnO_2 to produce the potent greenhouse gas nitrous oxide. Determining the spatial distribution of microbes and MnO₂ particles typically requires the use of X-ray or electron microscopy, costly techniques that are not available at all institutions. We present a simple, cost-efficient method of visualizing cells associated with MnO₂ particles using leucoberbelin blue (LBB), a dye that reacts selectively with Mn(III, IV), in tandem with the common fluorescent nucleic acid stain 4',6diamidino-2-phenylindole (DAPI). Microbial samples with MnO₂ were fixed with paraformaldehyde and filtered onto cellulose nitrate filters, after which 0.04% LBB solution was added. After reaction in the dark for 8 minutes, DAPI in $1\times$ PBS was applied, and a cover slip was added. Imaging was done with an epifluorescent microscope with a digital microscope camera. First, slides were imaged by differential interference contrast microscopy to locate MnO₂ particles by their characteristic blue stain from reaction with LBB. Then, cells were imaged using the DAPI filter set (excitation: 351 nm/emission: 461 nm). All images were analyzed using the free software ImageJ with Color Threshold and Merge Channels extensions. Images were overlain for co-location of MnO₂ particles and cells. This method has been successfully applied to both laboratory and natural samples and has the potential to be used with gene-specific fluorescent dyes.

Saturday 3:15 PM – 3:30 PM **Identifying Mn Oxidizing Microbes in Acid Mine Drainage** Katrina E. Godsey*, Sarah K. Carmichael, Sydney E. Bear, Morgan O. Smith, Suzanna L. Brauer Appalachian State University

Acid mine drainage (AMD) is formed when sulfide minerals, in the presence of oxygen and water, are converted into sulfuric acid, allowing metals present to go into solution. This drainage can be particularly harmful to the surrounding environment, including a majority of flora and fauna. Being that Manganese (Mn) is a highly ubiquitous metal, it can be found in most environments, including AMD. The oxidation of Mn to Mn oxides, hydroxides, and oxyhydroxides (commonly referred to as Mn oxides) is most commonly mediated by microorganisms. Although the majority of microorganisms with the ability to oxidize Mn are intolerant to acidic conditions, they can be found in AMD. Mn oxide coated rocks and pebbles, confirmed with leucoberbelin blue (LBB), were collected from Ore Knob, Jefferson, NC, Appalachia, VA and Pound, VA. Samples were serially diluted and allowed to grow on agarose plates supplemented with different carbon sources. Isolated organisms were identified via sanger sequencing. The bacteria identified belong to the genera *Brevibacillus*, Chryseobacterium, Burkholderia and Pseudomonas, while the fungi identified thus far has been identified as belonging to the genera *Plectosphaerella*. Samples were also taken for community-based analysis. Illumina sequences are currently being analyzed utilizing dada2 in R. Future work will entail developing culture conditions to promote AMD bioremediation in situ.

Saturday 3:30 PM – 3:45 PM **Potential Life Strategies in Gas Clathrates** Abigail M. Johnson*, Jongchan Kim, Priyam J

Abigail M. Johnson*, Jongchan Kim, Priyam Raut, Dustin Huard, Anton Petrov, Loren Dean Williams, Raquel Lieberman, Sheng Dai, Jennifer B. Glass Georgia Institute of Technology

Microbial communities have recently been found in gas clathrates, which naturally occur on continental shelves and in permafrost. Gas clathrates are a natural gas resource and can also serve as Earth analogs for habitability elsewhere in our solar system, such as Mars and Titan. Survival strategies used by gas clathrate-inhabiting microbes in these high-pressure, low-temperature environments could provide clues for searching for life beyond Earth. My research will test whether bacteria living in gas clathrates employ a strategy similar to that of cold-water fish that express ice-binding proteins to inhibit the growth of ice crystals. We have (1) identified putative clathrate-binding proteins from metagenomes from clathrate-containing marine sediments, (2) developed a computational workflow to predict ice-binding amino acids and model 3-D protein structure, and (3) developed methods to synthesize different types of gas clathrates in the presence of putative clathrate-binding recombinant proteins, which will allow us to visualize and characterize their effects on the thermodynamics of gas clathrates. These proteins may aid in maintaining habitable environments for clathrate-dwelling microbes.

Saturday 3:45 PM – 4:00 PM **Life Under Pressure: Exploring the variation in Microbial Communities across a volcanic arc** Timothy J. Rogers*, Karen Lloyd

University of Tennessee, Knoxville

Life on icy moons, such as Europa and Enceladus, might be found in the subsurface were possible dewatering processes would likely push fluids, saturated with nutrient-rich chemical species, through rock and sediments; possibly giving rise to ideal chemical habitats sufficient for life. The Costa Rican convergent margin is analogous to what we would expect to find on these icy bodies. Here, the subducting plate is subjected to dewatering processes that allow fluids rich in hydrogen, carbon, and sulfur inorganic chemical species to be released into the overriding plate. Samples were collected from 25 geochemically distinct sites from around Costa Rica in February 2017. These sites include mud pools, hot springs, and volcano crater lakes. Metagenomic libraries were prepared and run through a sample flow cell of an Illumina MiSeq sequencer. Raw sequence reads were then uploaded to KBase for processing through Trimmomatic, metaSPAdes, and then through MaxBin2 and Metabat. Optimized bins were annotated by Prokka, checked for completeness and bin heterogeneity with Checkm, and those containing 16S rRNA gene sequences were classified for taxonomy with Silva. Despite covering a range of pH values from 0 to 10, and temperatures from 20°C to 60°C, we found that tens of high-quality bins could be obtained from each sample of these deep subsurface fluids. These included some taxonomic groups closely related to cultures, some belonging to groups that are not cultured but have been described by genomes, and even some belonging to deeply branching groups that have never been described. We will continue to analyze these genomes to hypothesize microbial functions in relation to deep-subsurface fluid emanations across the arc.

Saturday 4:00 PM – 4:15 PM Endolithic Microbial Carbon Cycling in Dronning Maud Land, East Antarctica

Natalie A. Tyler*, Lori A. Ziolkowski University of South Carolina

Microbes can adapt to inhospitable conditions by colonizing niches that protect them from severe environmental conditions, making them model organisms in the search for life on other planets. Endoliths are globally ubiquitous microorganisms that colonize structural cavities within rocks, which protect them from environmental stressors while maintaining access to nutrition and light sources. Despite harsh katabatic winds, extreme temperature fluctuations, harmful UV radiation and exposure to desiccation, endolithic communities have been observed in Antarctica. Previous studies have found viable and abundant biomass through microscopy and the addition of isotopic labeling - but due to the slow metabolic activity of these microbes, very little is known about the in-situ activities of these communities.

The purpose of this research was to determine how quickly carbon is being cycled within Antarctic endoliths through radiocarbon (14C) analyses of phospholipid fatty acids (PLFA), a component of the viable microbial membrane that degrades quickly upon cell death. Coupling ¹⁴C and PLFA can provide an estimate of the insitu activity of microbes, while gaining a broad understanding of community composition through the distribution of observed PLFA. Weathered rock samples with visible colonization (as surficial pigmentation) were collected from the Sør Rondane Mountains, a coastal margin range comprised chiefly of high-grade metamorphic lithologies located within the Dronning Maud Land region of East Antarctica. Within these samples, PLFA concentrations ranged from 0.3 to 9.3 $\mu g/g$ - consistent with previous studies. The distribution of observed PLFAs was found to be related to the PLFA concentration, with more concentrated samples containing more polyunsaturated fatty acids. Radiocarbon of PLFA ranged from -110‰ to 62‰, where most of the samples reflected a modern carbon source. This work provides new insights into the potential variability of Antarctic endolith activities on a continental scale and contributes to the discussion that these extremophilic communities may likely be more active than previously thought.

Oral Session IV

Sunday 9:15 AM – 9:30 AM Effects of stormwater management practices on nonpoint source BOD loading to coastal waters in South Carolina Kelly McCabe*, Erik Smith, Claudia Benitez-Nelson University of South Carolina

Urbanization is a leading cause of water quality dissolved oxygen (DO) impairment. Located in one of the most rapidly urbanizing regions in the USA, South Carolina now recognizes low DO as the primary water quality issue impacting aquatic life within the state's coastal zone. In order to maintain sufficient DO in the state's coastal waters, effective management of both point and nonpoint source discharges of oxygen demanding substances is critical. The conventional measure of oxygen demanding substances is the five-day Biochemical Oxygen Demand (BOD5) defined as the concentration of DO consumed by microbial decomposition of organic matter and the conversion of nitrogen compounds to the stable forms of nitrite and nitrate over a five day incubation at 20°C. Much is known about the BOD loads of point sources, in particular wastewater, while non-point source BOD remains ambiguous as it has been combined into the natural BOD loads of receiving waters. To elucidate the contribution of nonpoint source stormwater runoff to DO impairment along the coastal zone of South Carolina, BOD5 was measured in samples collected during the summer of 2018 from seventeen different stormwater catchments in Georgetown and Horry counties along a hydrograph of six different magnitude storm events. Catchments ranged from stormwater management conveyances and best management practices (BMPs) with watersheds spanning undeveloped forested and wetlands to high density urban developments. The particulate and dissolved fractions of organic matter (organic carbon, nitrogen, and phosphorus) were quantified in addition to the dissolved inorganic nutrients to provide a mechanistic understanding of the controls on individual site BOD loading. Further, the dissolved organic carbon was characterized using the optical techniques of absorbance and fluorescence as proxies for organic matter source and composition. Preliminary results show BOD5 values varied greatly among sites with varying drainage basin land cover and land use as well as within an individual site between rain events. These findings suggest watershed landscape, stormwater management practice, storm intensity, and antecedent weather conditions all influence the magnitude and lability of organic matter exported from land to coastal waters. These data begin to address the information gap regarding non-point source pollution to the coastal waters of South Carolina to support improved local and state land and stormwater management policies to prevent future DO impairment.

Sunday 9:30 AM – 9:45 AM Polymerization in Place: Conversion of Mobile Contaminants to Fixed Polymers

Fan J. Wang*, Shay Frankenfield, Tomas M. Makris, John L. Ferry University of South Carolina

Dense non-aqueous phase liquids (DNAPLs) are a functionally defined class of groundwater contaminants that include halogenated aromatic chemicals. The accumulation of aromatic DNAPLs on bedrock surfaces results in the formation of standing pools of organic contaminants that may take years to disperse. During that time the passage of adjacent groundwater generates of contaminated water that can pose a significant risk to human health and restrict the usability of the water. Here we apply in-situ polymerization strategies to manage risk by converting mobile contaminants to immobile oligomers with low solubility. This process, analogous to humification, was achieved through a peroxidase-mediated oxidation process performed under hydrogen peroxide and peroxidase conditions similar to those found in coastal soils. Fluoroanilines and halophenols, alone and in mixtures, were used as model contaminants. Their transformation by oxidation to high molecular weight, insoluble oligomers was monitored for several hours. Measurements include starting materials, hydrogen peroxide, peroxidase enzyme, and resulting oligomers. The molecular weights of aromatic condensation products have been determined to be ranging from dimers to larger than hexamers, depending on the substrate/enzyme ratio. These products are capable of sequential oxidation to molecular weights approaching 1000 Da. The maximum solubility of these products was significantly reduced relative to the starting materials, resulting in their direct immobilization. Resulting polymers have been characterized with respect to their affinity for still-dissolved residual parent contaminants. Their function as adsorbents has been quantified relative to an equal mass of powdered activated carbon and their adsorption constant (K) has been determined to be approximately 6.9x10³, less than that of activated carbon but still quite capable of adsorptive removal of dissolved organic contaminants.

Sunday 9:45 AM – 10:00 AM 3D seismic attributes analysis of subsurface fault and fracture systems in Llanos Foothills Colombia

Ibraheem Hafiz*, James Kellogg, Essam Saeid, Ziyad Albesher University of South Carolina

This study was made of an area south of the giant Eocene Mirador Formation Cusiana oil field in the Llanos Foothills of Colombia. In general, the fault and fracture systems in the fold thrust belts is affected by fold geometry and mechanisms. Previous studies shows that the natural fractures are present at different stratigraphic intervals including the main reservoirs (Mirador, Barco and Guadalupe). In general, the Barco formation is more intensely fractured than the Mirador and Guadalupe Formations. The well reports indicates that the reservoirs porosities are relatively low with good permeability. However, the fractures are a modifier of the reservoirs enhanced permeability when they occur open and connected. In this research, we study the subsurface fracture systems by using 3D seismic attributes analysis to calculate the fracture intensities, orientation, and compare our result with fracture systems of Cusiana giant oil field. We have produced a new 3D faults model, fractures network model with fracture drivers, and seismic attribute analysis (Ant tracking) of 3D seismic volumes.

Sunday 10:00 AM – 10:15 AM **Surface distribution of Nd in the Amerasian Basin of the Arctic** Brian Duggan*, Brian Haley, Steve Goldstein, Howie Scher University of South Carolina

In the Arctic Ocean (AO), surface waters are primarily influenced by the surrounding land masses and the extensive shelf system accompany them. While the AO is the smallest of the ocean basin, composing 1% of the global ocean volume, it receives 10% of global river discharge (Aagaard and Carmack, 1989; McClelland et al., 2012). Additional, approximately 50% of the area of the AO is underlain by continental shelves (Jakobsson, 2012). These unique aspects of the AO are noteworthy for understanding the distribution of neodymium concentrations and isotopes and the processes controlling them. Additionally, AO dynamics are in flux due to the effects of climate change. As global temperatures are on the rise the Arctic is affected disproportionately, warming at twice the rate of the rest of the globe (Osborne et al., 2018).

We measured neodymium isotopes (Nd) from eighteen high-resolution profiles across the Bering Strait into the Amerasian Basin in an effort to further constrain the sources and sinks that control the distribution of Nd. Recent studies indicate rising temperatures in the Arctic have led to reduced ice cover, permafrost thawing, and increased river discharge, which can all alter the distribution of Nd throughout the basin. We observed a large gradient across the Bering Strait, from the radiogenic Pacific endmember of -1.6 to -4.4 upon entering the Amerasian Basin. We attribute this shift to the discharge of pore fluids from the shelf where sediments are derived from terrestrial sources, like those entering the strait from the Western Alaskan Yukon river (-8.4; Yoshihiro et al., 2011). Similarly, the Canada Basin we observe near surface water (~100 m) to be less radiogenic than expected based on the composition entering the basin. As in the Bering Strait, we suggest pore fluid flux from the sediments would lead to the less radiogenic composition observed for near-surface water in the Canada Basin, which is corroborated by radium isotopes (Kipp et al., 2018).

Sunday 10:15 AM – 10:30 AM Diagnosing Biases in Estimates of the Antarctic Polar Front Location and Variability

Brady S. Ferster*, B. Subrahmanyam, A. M. Macdonald University of South Carolina

Two methods for estimating Polar Front (PF) location, one based on sea surface temperature (SST), the other on sea surface height (SSH), are compared. Using the latest product from the Estimating the Circulation and Climate of the Ocean (ECCO) group, the PF locations are found to be similar to both climatology and a 24-year mean position. The SST approach produces larger estimates of seasonal and monthly variability. Both methods indicate geographically localized patterns in the location of the PF; a shift northward in the central Pacific and southward in the Atlantic and Indian Basins. SST-based zonal averages indicate a significant northward trend in the Pacific Basin $(1.15 \pm 0.73 \text{ km year})$, which is not observed in the SSH-based averages. On the other hand, the SSH approach suggests a southward drift in average position in all basins over time, a result not seen using SSTs. The seasonal and interannual variability of the PF is moderately-to-strongly correlated to decreasing temperatures and salinity within the ACC region. Combined with the northward trends of the PF in the central Pacific, the northward shift could be driving the observed decrease in central South Pacific temperatures. The interannual variability of the PF is weak-to-moderately correlated with the Antarctic and Southern Oscillations, but a large portion of the PF variability remains unexplained.

Poster Presentation Abstracts

* - Presenting Author

Poster

Varied Irradiance and Stable Isotopes in Siderastrean Corals

Erin Malsbury*, James Porter, Patricia Yager University of Georgia

The scleractinian corals found near the mouth of the Amazon River experience months of complete darkness annually due to their depth and seasonal coverage by the turbid Amazon River plume. Solar-irradiance determines whether habitat is hospitable for hermatypic corals, which provide spatial complexity necessary for tropical reef ecosystems. As scleractinian colonies grow, they deposit calciferous skeletons, and the d¹³C and d¹⁸O ratios in these skeletons can be used to assess the coral's dependence on photosynthetic zooxanthellae. In order to provide a baseline for assessing photosynthetic dependence in Siderastrea corals from the Amazon, I analyzed the stable isotope ratios in Caribbean Siderastrea siderea from a variety of depths, expecting $d^{13}C$ to decrease with depth and $d^{18}O$ to remain stable. d¹³C showed a negative correlation with depth, while the d¹⁸O remained constant. In addition to testing museum specimen, I am conducting an insolation experiment on live Siderastrea radians collected from the Florida Keys. An experimental group is receiving decreased irradiance for six weeks, while a control group is receiving normal levels of light. After six weeks, I will collect skeletal material for isotope analysis using the methodology applied to the museum specimen. I expect the corals exposed to lower levels of light to rely more heavily on heterotrophy and to contain greater proportions of $d^{13}C$ than the controls.

Poster **Methanogen Communities in Peatlands of North America** Sydney Bear[•], L Jamie Lamit, Nathan Basiliko, Suzanna Bräuer Appalachian State University

Peatlands are wetland environments that account for an estimated one third of the Earth's terrestrial carbon. Peatland soil contains communities of archaea, bacteria, and fungi that interact with each other through nutrient cycling and competition. The methanogenic archaea in these communities create methane gas as a product of their metabolism. A community analysis of these methanogen orders and their abundances in varying environments will give insight into their interactions and how the changing environment will affect them. Extracted DNA from peat samples of 19 sites in eastern North America was analyzed through Illumina amplicon sequencing of the mcrA gene to observe changes in methanogenic community assemblages in varying environments. It is expected that the community analysis will show preferences for depths near the anaerobic/aerobic transition zone as well as the substrate and pH preferences of certain orders of methanogen communities in peatland environments and are important to consider in the face of climate change.

Poster

Activities of extracellular enzymes in sediments of the White Oak River estuary and Kongsfjord, Svalbard

Lauren Mullen*, Carol Arnosti, Taylor M. Royalty, Andrew D. Steen, Zachary Stooksbury

University of Tennessee

Proteins are a rich source of organic carbon and nitrogen in aquatic environments. However, these proteins must be broken down into its constituent pieces before they become bioavailable. This breakdown is facilitated by microbially derived extracellular enzymes. Heterotrophic microorganisms in the subsurface face unique challenges, their systems are heavily limited by organic carbon availability. However, it is still unclear how these organisms are accessing what organic carbon is available. This availability is particularly important in systems that are nutrient limited. This nutrient limitation was tested in two distinct environments; the White Oak River, a blackwater river in North Carolina and Kongsfjord, a fjord located in Ny Alesund Svalbard. The White Oak River experiences very seasonal temperature differentiations while Kongsfjord maintains a relatively consistently cold temperature in both the water column and sediment. We conducted water column enzyme assays to assess the possible potential present in the systems as well as anoxic enzyme assays. These data will be compared to pre-existing metagenomic data to develop an idea of what microbes may be operating in these environments.

Poster

Chloroplast Response when PAHs Accumulation in Wheat Leaf Yu Shen*, Ruocheng Gu, Jinfeng Li, Xinhua Zhan University Of South Carolina

It was reported that the accumulated PAHs would lead chlorosis in wheat leaf, thence we hypothesized that the inner structure damaged was the main reason for that. Through transmission electron microscopy, it was first found that the chloroplast morphology turned round and began to dissolve under phenanthrene treatment. Herein, iTRAQ (Isobaric Tag for Relative and Absolute Quantification) differential liquid chromatography-tandem mass spectrometry on a LTQ-Orbitrap with high-energy collision dissociation was applied to analysis the changes in the protein profile in chloroplast under phenanthrene treatment. A total of 517 proteins were identified, and 261 of them presented up-regulation. Based on the database engine and Real-time PCR result, we suggested that the chloroplast deformation is the reason of thylakoid destroyed whose structure proteins down-regulation and related genes positive expression under phenanthrene treatment. Moreover, we also first defined that: 1) the weakness of electron transferring chain and photosystems were the main changes in light reaction in photosynthesis, together with heat diffusing; 2) the decline of glycolysis was the main source in leading energy conversion fall; 3) and proteins related with proline, tryptophan and glycine presented positive, while those about histidine, threonine and arginine metabolism got negative; 4) it was first found that the most ribosome proteins would turn up-regulation when plant treated with phenanthrene. In the end, this study is the meaningful report to discuss the carcinogenic, teratogenic and mutagenic effects of PAHs in plant cells, as well as offers important references about improving crops resistance in PAHs pollution.

Poster Trace Element Geochemistry of a Planktic Foraminifer Living within the Oxygen Minimum Zone

Catherine V. Davis*, Robert C. Thunell, Karen Wishner University of South Carolina

The geologic record of marine oxygenation has become a topic of increasing interest, but reconstructing low oxygen environments is hindered by a scarcity of appropriate proxies. The shells of planktic foraminifera are the targets of many traditional and emerging geochemical proxy approaches; however the suite of species most frequently used in paleoceanography tend to occupy shallow and well-oxygenated surface waters. This limits their utility in generating records of low oxygen environments. With this in mind, we have examined the foraminiferal assemblages from MOCNESS tows taken in 2017 in the Eastern Tropical North Pacific (21°N, 118°W). The site of the tow overlies the Oxygen Minimum Zone, with strong environmental depth gradients, including in oxygen. The living assemblage within the upper oxycline and through the oxygen minimum zone is dominated by the species Globorotaloides hexagonus. This supports previous observations of G. hexagonus living in deep and low oxygen waters elsewhere in the western and northern Pacific, and suggests specialization for a low oxygen environment. Here, we present these vertical assemblages through the Oxygen Minimum Zone and further explore the potential for the shells of G. hexagonus to act as geochemical proxies for conditions within low oxygen environments. The trace element (Mg, Sr, Ba, Mn, Cd, U) signatures of shells recovered by MOCNESS are compared to those of recent (Holocene) fossils from nearby sediment cores in the Eastern Equatorial Pacific. Results demonstrate the potential for expanding the scope of paleoceanographic proxies in planktic foraminifera by the application of traditional geochemical methods to new species and settings.

Poster

Extracellular enzyme activity from hot springs in southern Costa Rica and northern Panama

Andrew Lonergan*, Katherine Fullerton, Karen G. Lloyd, Andrew D. Steen University of Tennessee

Microbes found in subsurface water and sediment play an important role in biogeochemical cycling. Heterotrophic microbes require extracellular enzymes to cleave bonds of macromolecules in order to take up and ultimately metabolize organic carbon and nutrients. Geothermal features are characterized by a wide range of temperatures, pH, and other geochemical parameters, and can add to our understanding of microbial effects on fluxes in the carbon cycle. Here we perform enzyme assays to examine microbes from various hot springs in southern Costa Rica and northern Panama and their enzymatic activity on different substrates. Using temperatures similar to that of the hot springs (20-60°C), we use fluorogenic substrate proxies to assay the potential activities of diverse extracellular enzymes in 10 hydrothermal springs. We will compare extracellular enzyme activities to 16S libraries and metagenomes from the same sites to better understand microbial degradation of polymeric material in these geothermal environments.

Poster

Isolation of Microorganisms from Peatlands in the Southern Appalachians Huc Eban*, Daniel Yu*, Sydney Bear, Suzanna Brauer Appalachian State University

Peatlands are a type of wetlands that form from the accumulation of decomposed plants and organic matter, and from water-saturated, acidic, and oxygen poor environments. They form a unique environment that is home to many biological species, and they perform important ecological functions such as the water and carbon cycles. A particular community that plays a crucial role in those cycles are microbial organisms. They are diverse and abundant, yet little is known about their functions in peatlands. Specifically, a large portion of anaerobic organisms are uncultured nor understood because of the challenges faced by trying to mimic the natural environmental conditions for their growth. Therefore, the goal of this project is to culture and identify novel strains from collected peat samples. Peat samples will be collected from a bog located in Pineola, NC, a wet, sphagnumdominated area with an average pH of 5.6. The samples will be taken at around 30 cm in depth from sites of different compositions of peat. The samples are then individually stored in jars and relocated to an anaerobic hood. The samples will be subjected to serial dilution before streaking for isolation. Petri dishes will be used to provide a solid growth medium that contains agar and nutrients with different carbon sources and pH levels. Once the plates are streaked, they are re-streaked until isolated organisms are obtained. The DNA of the isolated organism will then be amplified using PCR and the amplification will be determined by gel electrophoresis. The DNA of the isolate can then be sequenced. NCBI Blast will be used to determine the identity of the isolated organisms from their DNA sequences. This information will then allow us to identify how the isolated strains are genetically related to other microorganisms and can determine if novel species have been cultured.
Poster Geological controls on biological carbon oxidation in the White Oak River Estuary, NC

Zachery Stooksbury*, Andrew D. Steen University of Tennessee, Knoxville

Estuarine and deltaic sediments are responsible for forty-four percent of Earth's organic carbon burial due in part to preservation of organic matter by sorption to sediments. Organic matter sorption is controlled by sediment grain size and mineralogy. Here, we report the grain size and minerology of sediments collected from the White Oak River Estuary in order to characterize their relationship to both the preservation of organic carbon and the utilization of this carbon by microorganisms. The White Oak River is an estuary with rapidly accumulating sediments producing an anoxic environment a few millimeters below the sediment-water interface. Within this anoxic sediment sulfate is reduced in the first 30 cmbsf and methanogenesis occurs from 30-80 cmbsf. Sampling of the White Oak River estuary consisted of collecting 80cm push cores that were then sub cored in 3cm increments in order to create a downcore profile analysis. Grain sizes of the White Oak sediment where characterized by removing all organic matter and analyzed by a Malvern 3000 Laser diffractometer. Determining the linkages between the preservation and utilization of this organic carbon with the geology and microbiology should provide a broader perspective in the understanding how this dynamic system operates and how the White Oak River Estuaries and other estuaries can contain a large proportion of Earths preserved organic carbon.

Poster Sand Surface Bioaggregation with Methanotrophically Produced Extracellular Polysaccharides Ann-Marie Harik*, Terry Hazen University of Tennessee, Knoxville

For the public's health and comfort, sand adjacent to transportation, agriculture and infrastructure sites is often treated with a stabilizing agent aiding in the prevention of airborne sand and sand dust issues. In recent years there has been a shift from potentially hazardous and non-reversible chemical aggregation methods towards bioaggregation - aggregation through microbial means. The main bacterial player in current bioaggregation studies seems to be *Sporosarcina pasteurii* (formerly *Bacillus pasteurii*); however we propose the use of methanotrophs as potential agents for aggregation. Methanotrophs are bacteria that can utilize methane as their carbon source and are known to produce copious amounts of extracellular polysaccharides (EPS). EPS are sugars produced and excreted by many types of bacteria, it is a main component in biofilms and is believed to be found in naturally forming sand crusts.

The goal of this work is to understand the potential for methanotrophs and their extracellular polysaccharides to be used in sand surface stability applications. Therefore, I want to test my hypothesis that specific methanotrophic cultures can be applied in situ to increase sand particle adhesion.

Preliminary data shows an increase in sand particle size after application of cultures and sparging with methane gas. Current studies will identify when the individual cultures achieve their maximum EPS production rates, and how EPS of differing species affects bioaggregation.

Washed sand of uniform size is to be mixed with methanotrophic cultures at their peak EPS production period - discovered through simultaneous EPS production and bacterial growth curves. The sand-culture mixture is then placed in a mold and incubated in a methane and air headspace mixture. Time points, starting at time zero, are taken periodically through the experiment. Sand particle adhesion will be determined by microscopy, change in mass, pore space analysis, carbohydrate analysis, and dry sieving analysis. The experiment will have two negative controls: sand treated with media but no culture in the anaerobic bags, and sand treated with culture but not placed in anaerobic bags.

Linking Nutrients to Microbial Metabolism in the Lost City Hydrothermal Vent System

Cameron Henderson*, Julia M. McGonigle, William J. Brazelton, Susan Q. Lang University of South Carolina

The Lost City Hydrothermal Vent Field is an area of exposed mantle crust on the mid-Atlantic Ridge that is driven by water-rock interactions and characterized by high temperature and high pH conditions. Lost City is an aphotic environment, making the dominant biological reactions chemosynthetic rather than photosynthetic. The water-rock interaction of serpentinization has the primary control over fluid chemistry, occurring when seawater percolates into the rocky subsurface and reacts with the exposed mantle rock (specifically peridotites), resulting in fluids rich in hydrogen, methane, and other reduced compounds. This hydrogen is then used in chemosynthetic organisms to form the basis of a complex food chain independent of photosynthesis. In the Fall of 2018, a 22-day research expedition AT42-01 "Return to Lost City" was conducted in a joint biological, chemical, and geological effort to discover more about serpentinization-driven environment and the microbes that dwell there.

The aim of this project is to identify the link between microbial-limiting nutrients and carbon utilizing metabolisms at Lost City. Hydrothermal fluid samples were collected from venting chimneys across the field and analyzed for silicate, nitrate, nitrite, phosphate and ammonium concentrations. In addition, formate incubation experiments were performed to determine if this compound, which is present in high concentrations in Lost City fluids, is oxidized by microorganisms. Hydrothermal fluids were treated with various conditions (filtering, acidification, filtering + acidification, and no treatment) and spiked with ¹³C-labled formate. The ¹³C-CO₂ content of these samples was then determined to identify formate oxidation. Finally, both data sets are compared to each other in order to identify any relationships between potential nutrient limitation and formate oxidation.

The incubation experiment demonstrated that the conversion of ¹³Cformate to ¹³C-CO₂ was higher in 'no treatment' and 'filtered' samples when compared to acidified treatments. Curiously, filtered and unfiltered non-acidified samples had similarly high levels of conversion, suggesting some of the activity may occur extracellularly. While the nutrient data is still being processed, the anticipated result is that extreme nutrient limitation may influence microbial activity and therefore, the rate of formate oxidation.

Resource-Based Niche Partitioning in Surface Ocean Heterotrophic Bacteria Growing on Diatom Derived Metabolites

Frank Ferrer-Gonzalez*, Mary Ann Moran University of Georgia

Diatoms contribute to roughly 20% of global net primary production. A considerable fraction of the carbon fixed is subsequently taken up by heterotrophic marine bacteria within days of production, representing a critical flux between autotrophic and heterotrophic components of the ocean microbiome. To determine which compounds are taken up by surface ocean heterotrophic bacteria, we studied bacterial gene expression in co-culture systems in which the diatom Thalassiosira pseudonana CCMP1335 was the sole source of carbon and energy for four different taxa of bacteria. Ruegeria pomerovi DSS-3, Stenotrophomonas sp. SKA14, Polaribacter dokdonenses MED152, and Dokdonia sp. MED134 were individually co-cultured with T. pseudonana, and transcriptional patterns of transport and carbohydrate catabolism genes were studied. Bacteria were grown to exponential phase and added to the axenic diatom cultures (grown in L1 medium with a light cycle of 16:8 h) at a concentration of 10⁶ cells/ml for a period of 8 h. Bacterial cells grown on 2.5 mM glucose served as a carbon source control. RNAseq analysis indicated there were notable differences in the diatom derived substrates selected for uptake by the different taxa, suggesting a substrate-based niche partitioning by the different bacteria. The alphaproteobacterium R. pomerovi displayed a preference for monomers, including osmolytes, sulfonates, and sugars; the Flavobacteriia members Polaribacter dokdoniensis MED152 and Dokdonia sp. MED134 showed preference for polysaccharides, including chitin, chrysolaminarin, xylan-like and alginate-like glycans; and the gammaprotobacterium Stenotrophomonas sp. SKA14 utilized sugars, Beta-1,4 glycans, and chitin-derived oligosaccharides. These findings demonstrate the diversity of labile diatom-derived organic compounds supporting heterotrophic growth in the surface ocean, and the distribution of metabolite usage among marine bacterioplankton taxa.

Poster Biodegradation of microcystin-LR by nonspecific pathways Christopher L. Cook*, Lauren E. Krausfeldt, Steven W. Wilhelm, Andrew D. Steen University of Tennessee, Knoxville

Microcystin (MC), a cyanotoxin produced by several genera of cyanobacteria, including Microcystis, Planktothrix, Anabaena, Oscillatoria, and Nostoc, can poison drinking water sources and damage ecosystems. High concentrations of MC typically follow the lysis of cyanobacterial cells present in high abundances, *i.e.* cyanobacterial blooms. MC is a cyclic heptapeptide, whose stable ring structure is resistant to common physical degradation mechanisms, such as high temperature, extreme pH, and sunlight. The biodegradation of MC is of high interest due to the ineffectiveness of abiotic degradation mechanisms. Break down by microcystinase, an enzyme that first cleaves the ring structure at the 3-amino-9-methyoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid (ADDA)-arginine bond, is the only well-characterized pathway regarding the biodegradation of MC-LR and some other structural variants of MC. Bacteria known to utilize this pathway include some species of Sphingomonas, Sphingopyxis, Bacillus, and Novosphingobium. However, diverse microbes that apparently lack microcystinase have been discovered to degrade MC-LR, including Lactobacillus rhamnosus GG. The pathways by which microbes that lack microcystinase degrade MC-LR are not known. We propose that MC-LR can be degraded through nonspecific pathways. Here, a nonspecific pathway is defined as one that may possess some ability to degrade MC-LR, but only as a secondary function. We test this hypothesis through a series of incubation experiments designed to characterize the pathway of L. rhamnosus GG. L. rhamnosus GG was exposed to various fluorescent enzyme substrates that are structurally similar to some of the amino acids comprising MC-LR with and without the presence of MC-LR. MC-LR and enzyme substrate degradation were measured using high performance liquid chromatography (HPLC) and fluorometry, respectively. Degradation of MC-LR resulting from the different enzyme substrate and MC-LR pairings were used to characterize the potential degradation pathway used by L. rhamnosus GG. Future work will characterize the pathways of other biodegraders that lack microcystinase.

Constraining the water flux through a serpentinite-hosted hydrothermal vent field

Jessica D. Frankle*, Willard S. Moore, Claudia R. Benitez-Nelson, Susan Q. Lang University of South Carolina

Basalt-hosted hydrothermal vents impact ocean chemistry as a sink of magnesium and as a source of trace metals such as iron. Fluids passing through a serpentinite subsurface have a distinctly different chemistry than basalt-hosted systems, but to date their impact on ocean chemistry is largely unconstrained due to a lack of information on the flow of water through such systems. Short and long lived radioisotopes are promising tracers for constraining the flux of water through hydrothermal vent systems. Similar to submarine groundwater discharge, surface-adsorbed parent isotopes decay to radium, which can desorb into the fluids. In September 2018, we carried out a 22-day expedition to the Lost City hydrothermal field, an iconic serpentinite-hosted hydrothermal system. We analyzed vent fluids, chimney carbonates, and rocks for the Radium Quartet (223,222,226,228Ra) and some of their parent radioisotopes (Thorium, Actinium, and Protactinium). Rarely reported, we found highly elevated activities of ²²³Ra (halflife = 11.4 d) in the fluids. By pairing short and long-lived radioisotopes (i.e. 226 Ra, half-life = 1600 y) and their parents, we hypothesize that we will be able to calculate the fluid flux and residence time. Understanding the fluid flux in this system could place some of much needed constraints on the impact of serpentinization systems on elemental cycles in the ocean.

Poster Using Heterotrophic Growth to Indicate Patterns of Phytoplankton Exudate Release

William F. Schroer*, JE Schreier, MA Moran University of Georgia

About 50% of global net primary production occurs in the surface ocean and about half of this marine photosynthate is rapidly mineralized to carbon dioxide by heterotrophic microbial communities. Despite the important role marine microbial communities play in mediating global carbon cycles, the specific factors impacting the transfer of carbon between phytoplankton and bacterioplankton remain poorly understood. One question facing the field revolves around the extent to which phytoplankton actively alter the quantity or quality of exudates in response to the presence of heterotrophic microbes. Here we use heterotroph growth as a proxy to assess differential phytoplankton exudate release. In this system bacterial growth is limited by the amount of available carbon provided by phytoplankton exudate, therefor, greater heterotrophic growth is indicative of greater labile exudate release. This approach allows us to assess variation in phytoplankton exudate release in response to the presence of a bacterial co-member. Our preliminary results demonstrate that the model marine heterotroph, Ruegeria pomeroyi, reaches greater cell densities when grown on spent media from axenic cultures of the diatom *Thalassiosira pseudonana* than is reached when grown in co-culture with the diatom. This observed suppression of *R. pomeroyi* growth suggests the presence of a mechanism by which the diatom inhibits R. pomeroyi. Re-inoculating R. pomeroyi into spent medium from active co-cultures, effectively removing diatoms, appears to alleviate this inhibition allowing growth of *R. pomeroyi*. Moving forward we will be employing 2D NMR to characterize the exometabolomes of spent media from axenic diatoms and diatoms co-cultured with R. pomeroyi to elucidate the nature of this inhibition.

Poster Reproducibility and sensitivity of apparent quantum yield determination using moderate dissolved inorganic carbon (DI¹¹C) isotope enrichment (MoDIE)

Kun Ma*, Jay Brandes University of Georgia

Dissolved inorganic carbon (DIC) is a major product of the photooxidation of dissolved organic carbon (DOC), and is thought to be a significant but poorly constrained flux in the global carbon cycle. Typical DIC photoproduction rates in sunlight are in the order of nM h⁻¹ to mM h⁻¹ in the ocean while the DIC background concentrations are ~2mM. This high DIC background poses analytical challenges, so that previous efforts to determine the DIC photochemical efficiency, defined using apparent quantum yield (AQY) spectra, used data from DIC-stripped samples or other proxies. An isotope dilution method, moderate dissolved inorganic carbon (DI¹³C) isotope enrichment (MoDIE) was developed to provide more sensitive quantification of DIC photoproduction rates in marine environments. To test for reproducibility and sensitivity in an estuarine environment, we conducted a series of experiments using a single ¹³C-enriched Skidaway River Estuary sample. We irradiated the sample for 16 h under a polychromatic irradiation system, and the resulting integrated AOY ranged from 138.0 ± 28.99 to 191.7 ± 39.22 mmol DIC per mol photon absorbed. Apparent quantum yield at 325 nm for the four replicates ranged from 461.8 to 577.3 mmol DIC per mol photon absorbed, corresponding to 0.83 %, 1.9 %, and 22 % differences between replicate 1 and the other replicates. Although variability was higher than expected, MoDIE provides a reliable method to determine the DIC photochemical efficiency when DIC photoproduction rates are very low, and requires minimal sample manipulation that could potentially affect the photoreactivity of DOC.

Investigating the Nitrate δ¹⁸O to δ¹⁵N Relationship of Consumption Among Aquatic Environments

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Stable isotopes of nitrate ($\delta_{15}N$ and $\delta_{18}O$) provide tracers to identify nitrate (NO₃) sources, and to distinguish NO₃⁻ consumption (assimilation and denitrification) from its production (nitrification/anammox) in the environment. In particular, NO₃⁻ consumption from assimilation and denitrification is associated with an empirical $\Delta\delta_{18}O:\Delta\delta_{15}N$ ratio of 1 in microbial cultures and in marine systems, providing a benchmark to identify biological nitrate consumption. However, in aquifers, rivers, and lakes NO₃⁻ consumption typically is associated with an apparent $\Delta\delta_{18}O:\Delta\delta_{15}N$ ratio less than one, commonly ~0.6⁻. The observed difference from cultures and marine systems has been attributed to (a) production of NO₃⁻ by nitrification and/or anammox, thus overprinting the isotopic signal of NO₃⁻ consumption² or (b) denitrification mediated by an alternate dissimilatory nitrate reductase, NAP, which is associated with a $\Delta\delta_{18}O:\Delta\delta_{15}N$ of ~0.6³, rather than by the respiratory NAR nitrate reductase. This project aims to differentiate between an overprinting from nitrate production and the use of NAP.

Poster Elucidating the AHL-Based Quorum Sensing Regulatory Network for the Antimicrobial Indigoidine in *Rhodobacterales* sp. Y4I April Armes*, Alison Buchan University of Tennessee

Biofilm formation on particulate organic matter (POM) is important to biogeochemical processes in the ocean. Contributing to the formation and development of biofilms is microbial communication via N-acetylated homoserine lactone (AHL) mediated quorum sensing (QS). In marine environments, our knowledge of underlying mechanisms that control biofilm formation is currently lacking. Early colonizers are hypothesized to determine biofilm structure, dynamics, and function via AHL-mediated QS. Production of AHLs, as well as antimicrobials, contributes to the colonization success of the Roseobacter Clade. One member, Rhodobacterales strain Y4I, possesses two QS systems, phaRI (QS1) and pgaRI (QS2). Prior work in our lab suggested both QS systems contribute to the success of Y4I by coordinately regulating the production of indigoidine, a blue-pigmented antimicrobial. Here we provide genetic evidence that these two QS systems work in hierarchical fashion to coordinate regulation of indigoidine and biofilm formation. To elucidate the regulatory QS network controlling indigoidine production, we generated an AHL-synthase mutant in OS1. Indigoidine synthase (*igiD*) expression was quantified using RT-qPCR. Exogenous AHL add-back assays and RT-qPCR were performed to identify key players in regulation of indigoidine. Crystal-violet assays revealed mutations in either QS pathway, or *igiD*, leads to increased biofilm formation. Collectively, these data suggest QS plays a key role in Roseobacter physiologies, including antimicrobial production and biofilm formation. Understanding how regulatory networks contribute to colonization success of the Roseobacter Clade will allow us to make advances in our knowledge of biofilm formation and ecology, progressing our comprehension of biogeochemistry in marine ecosystems.

Poster Contributions of Sediment Mineralogy to the Resilience of Seagrass Beds Andrew Stancil*, Alyson Carpenter, John Hart, M. Dennis Hanisak, Jordon Beckler Florida Atlantic University

Seagrass dominated submerged aquatic vegetation (SAV) communities provide numerous ecosystem services, including habitat provisioning for juvenile fish, sequestration of nutrients to mitigate coastal eutrophication, and prevention of shore erosion through wave attenuation. In recent years, seagrass decline has been driven globally by increasing external pressures such as ocean acidification and warming water temperatures. Most often, field and laboratory investigations have focused on understanding primary environmental controls such as light intensity and wave energy. However, the effects of sediment biogeochemistry on SAV health has received much less attention, despite its recognized importance by the scientific community. For example, the deposition of organic carbon fuels microbial respiration and the production of sulfides ($\Sigma H_2 S$), which have a demonstrated toxicity to SAV. However, reactive oxidized forms of Fe (III) (e.g. Fe(OH)₃) may be able to titrate sulfide from solution, forming reactive forms of iron-sulfide minerals (e.g $FeS_{(3)}$ and FeS_{2}). This reactive iron would thus serve as a buffer, decreasing $\Sigma H_2 S$ bioavailability and promoting SAV growth. We present results demonstrating that, with all other variables constant, natural distributions of reactive iron and organic carbon play a large role in SAV growth patterns. By constraining the acceptable concentrations of reactive iron required for healthy sediments, these findings provide valuable insights into scalable strategies for restoration management.

Biogeochemical characteristics and fluxes of suspended particulate organic matter in response to low-tide rainfall

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We evaluated the effects of low-tide rainfall on salt marsh particulate organic carbon (POC) cycling. Suspended sediment samples from intertidal creeks were collected during rain and no-rain conditions. Bulk (carbon, nitrogen) and molecular (e.g., lignin) compositions of organic constituents in particulates were analyzed. Temporal patterns of suspended sediment concentration mimic fluctuations in rainfall intensity, but all POC constituents do not necessarily follow these patterns. For example, particulate organic matter concentrations in water column covaried with rain intensity yet organic matter (OM) contents in the particles showed trivial correlations with rain. In general, rainfall effects raised the suspended sediment and organic constituent concentrations in the water column by up to three orders of magnitude. Multivariate statistical analysis revealed that the rainfall-entrained particulates were compositionally distinct from calm weather suspended particulates, from salt marsh surface sediment, and from creek bank surface sediment. Rainfall-entrained particulates are depleted in bulk organic carbon and phytoplankton-derived OM but enriched in lignin phenols. These observations indicate that rainfall-runoff processes preferentially mobilize POC derived from *Spartina alterniflora* from marsh platforms and effectively transport these materials to intertidal creeks following low-tide rainfall events. Moreover, average net transport rates by rainfall are 0.020 ± 0.002 gPOC m⁻² mmRain⁻¹, and this POC turnover rate amounts to $\sim 19\%$ of the annual POC export from estuary to ocean. Therefore, estuarine outwelling estimates typically made under calm weather conditions represent minimum levels of POC export. Hence, low-tide rainfall can be expected to substantially enhance the delivery of marsh-derived POC to the coastal ocean.