From Genomes to Methane Emission: Targeting Critical Knowledge Gaps in Wetland Soils

Adrienne Narrowe[1*] (adrienne.narrowe@colostate.edu), Bridget McGivern, Tim Morin[4], Jorge Villa Betancur[2], Pengfei Liu, Rebecca Daly[1], Mikayla Borton[1], David Hoyt[3], Christopher Miller[6], William Riley[5], Trent Northen[5], Malak Tfaily, Gil Bohrer[2], **Kelly Wrighton**[1]

[1]Colorado State University, Fort Collins, CO; [2]The Ohio State University, Columbus, OH; Pacific Northwest National Laboratory, Richland, WA; [4]State University of New York College of Environmental Science and Forestry, Syracuse, NY; [5]Lawrence Berkeley National Laboratory, Berkeley, CA; [6]University of Colorado Denver, Denver, CO

Project Goals:

This early career research project interrogates carbon cycling in soils from a freshwater, coastal wetland adjacent to Lake Erie. Due to climatically driven increases in water levels in Lake Erie, the wetland water table has increased by ~3-6 ft over the sampling period, with hydrological inundation serving as a natural, model system for climatic induced shifts on soil biogeochemical cycling. With a sampling that incorporated time-series and highly spatially resolved sampling over this hydrological perturbation, we use multiple microbial genomics methods to resolve microbial community metabolic activity over years, across and within seasons, and along centimeter depth to meter land coverage gradients. Leveraging the multi-omics data in conjunction with geochemical, metabolomic, and greenhouse gas measurements, we provide unprecedented insight into how hydrological perturbations impact methane flux from a coastal, freshwater wetland.

Abstract:

Wetlands are the largest natural source of atmospheric methane, a potent greenhouse gas. Being able to predict the state and the changes microbial methane metabolism in wetland soils is critical to accurate modeling of methane flux. During our sampling over a three-year period, the wetland has experienced a shift in hydrologic state, with mud flats that were seasonally exposed now submerged under 3-6 ft underwater and where the water depth in prior open water channels increased by up to 2 ft. In this project we quantify how climatically induced hydrological disturbances change soil redox, organic carbon content and concentration, microbial decomposition, and methane cycling across these flooded conditions, offering a new chemical and microbial resolution of the controllers of the soil microbial methane cycle and how these are impacted by hydrologic perturbations caused by rising lake levels and modified storm events.

We first verified this hydrologic perturbation decreased the redox in historical season mud-flat and open water surface soils with bulk porewater dissolved oxygen levels dropping from oxygenic (>65 µM) to anoxic (< 2µM) in the first 0-5 cm of soil. Regardless of historical land coverage (open water, seasonal mud-flat), previously anoxic deeper soils (25-35 cm) remained anoxic. Consequently, we hypothesized that microbial carbon cycling in surface soils, especially those seasonally exposed, will be more impacted than in deeper soils which are better insulated from climatically driven perturbations. Here using multi-omics and in situ methane production data we address the impacts of shifting redox on microbial carbon cycling by initially targeting microbial metabolism at two critical stages of the carbon cycle that are historically thought to be regulated soil oxygen concentrations: plant polymer degradation and methanogenesis.

In oxygen-depleted soils, it is widely asserted that polyphenols inhibit microbial activity through cellular toxicity, substrate binding, and microbial enzyme inhibition, thus acting to "lock" soil carbon cycling. Given the need for a tractable substrate, we first used anoxic wetland soil reactors amended with and without a chemically defined polyphenol to test this hypothesis. Challenging the idea that polyphenols are not bioavailable under anoxia, we provided time-series metabolite and gene expression evidence for polyphenol depolymerization, resulting in monomer accumulation and catabolism, and further phenolic acid metabolism. Our findings also indicated that polyphenol amendment was not universally toxic, instead selectively stimulating a subset of microorganisms. Furthermore, this data provided a new view of microbial-polyphenol interactions, where these compounds did not restrict, but enhanced, overall soil carbon cycling increasing the production of methanogenic substrates (formate and acetate) relative non-amended controls. Collectively, our high-resolution multi-omic results provide the first demonstration that soil microbiota subverted the polyphenol lock, to sustain carbon depolymerization, and downstream carbon processing even in anoxic soils.

To better track carbon cycling and methane production/consumption in these soils in situ, we developed the MUCC (Meta'omics to Understand Climate Change) wetland genome database. This database was composed of over 17,000 genomes and contains a catalog annotated genomic content of over 2,500 dereplicated quality microbial genomes. Although soil methanogens and methanotrophs have been cultivated for decades, our genome recovery approach resulted in 88 methanogen genomes representing all methanogenic pathways. These include 3 novel families, 17 novel genera and for some taxa, the first genomes identified in a wetland environment, illuminating the phylogenetic and metabolic diversity harbored in terrestrial ecosystems. Over this three-year period, we have generated an unprecedented wetlands metatranscriptome of 115 spatially and temporally distributed samples which we have used better understand how soil microbial metabolism is impacted by flooding. We link this metabolic activity to *in situ* porewater methane measurements, methane chamber flux measurements, geochemistry, and NMR, LC-MS, and FT-ICR metabolite data to provide a highly resolved view of the methane cycling organisms and the upstream and downstream processes impacting the resulting methane emissions.

First, we used NMR-metabolites to survey the distribution of methanogenic substrates across the wetland, with flooding we observed increases in methanogen substrates of acetate and methanol only in the surface soils. Concomitant with increased availability of these substrates, we observed gene activity from methanogens utilizing acetate and methanol in these surfaces increased 4.9 and 1.6-fold respectively with flooding. This expanded substrate availability and concentrations is likely responsible for the nearly 5-fold increase in methane production reported in the surface soils with flooding. Surprisingly, while we expected the shift to anoxia would decrease aerobic methanotrophy in surface soils, gene expression data indicated aerobic methanotrophs metabolized methane using very low oxygen concentrations, and we also demonstrated first soil gene expression data suggesting nitrate enabled methanotrophy in soil systems. Given that methanol utilizing methanogens and anaerobic methanotrophs are currently not accounted for in climate models, our findings provide important data to be used in updating biogeochemical models of terrestrial methane emissions. Despite these redox and soil carbon changes, using co-expression gene network analysis we show the membership of carbon cycling communities does not change, but their activity is enhanced with flooding.

In summary, while our findings show redox shifts associated with flooding, the microbial communities and metabolic networks are largely maintained over years despite overwhelming environmental changes. We find that anoxia does not short cut microbial carbon cycling as thought, but instead previously underappreciated microbial carbon depolymerization increases the concentration of methanogen metabolites. This increased availability of substrates, coupled to anoxia, resulted in significant increases in methane production in surface soils with flooding, regardless of historical land coverage type. Our findings demonstrate methane metabolic circuitry encoded across microbial genomes may be resilient drying/rewetting/flooding pressure, but the flux through these networks may result in greater in situ production of methane. Collectively, our results highlight the soil microbial metabolisms influencing the terrestrial microbial methane cycle under climatic induced shifts, thereby offering direction for increased realism in predictive process-oriented models of methane flux in wetland soils.

References/Publications:
1. Angle JC, Morin TH, Solden LM, Narrowe AB, Smith GJ, Borton MA, Rey-Sanchez C, Daly RA, Mirfenderesgi G, Hoyt DW, Riley WJ, Miller CS, Bohrer G, Wrighton KC. 2017. Methanogenesis in oxygenated soils is a substantial fraction of wetland methane emissions. Nature Communications 8:1567.
2. Narrowe AB, Angle JC, Daly RA, Stefanik KC, Wrighton KC, Miller CS. 2017. High-resolution sequencing reveals unexplored archaeal diversity in freshwater wetland soils. Environmental Microbiology 19:2192-2209.
3. Narrowe AB, Borton MA, Hoyt DW, Smith GJ, Daly RA, Angle JC, Eder EK, Wong AR, Wolfe RA, Pappas A, Bohrer G, Miller CS, Wrighton KC. 2019. Uncovering the Diversity and Activity of Methylotrophic Methanogens in Freshwater Wetland Soils. mSystems 4:e00320-19.
4. Smith GJ, Angle JC, Solden LM, Borton MA, Morin TH, Daly RA, Johnston MD, Stefanik KC, Wolfe R, Gil B, Wrighton KC. 2018. Members of the Genus *Methylobacter* Are Inferred To Account for the Majority of Aerobic Methane Oxidation in Oxic Soils from a Freshwater Wetland. mBio 9:e00815-18.