Research overview

Microbial metabolism in the terrestrial biosphere catalyzes critical biogeochemical cycles, with quantifiable impacts on global scales. Understanding how these subterranean microbial communities' function can inform resource management, soil and water quality, and climate models. Examples from my own work include harnessing terrestrial microbiomes for improved greenhouse gas predictions, uranium and hydrocarbon bioremediation, and bioenergy applications. In my laboratory, computational systems biology approaches result in predictions of metabolic potential in both individual microorganisms and microbial communities. These insights underpin laboratory investigations targeting physical, chemical, and biological controllers on biogeochemical processes. I couple holistic and reductionist approaches to interrogate the interactions between organismal bioenergetics, interconnected community metabolism, and geochemical processes.

Previous research experience

My prior research has focused on understanding microbial catalyzed biogeochemical reactions in soil and subsurface systems. Prior to graduate school, I was employed by industry (Chevron), using cultivation and molecular tools to understand hydrocarbon bioremediation in soils. For my PhD research, under the guidance of Professor John Coates, I examined microbe-mineral interactions both at the organismal and community scale. This research used ferric iron minerals, humic acids (anthraquinones), and anodes from microbial fuel cell (MFC) reactors as model electron acceptors to understand biochemical, organismal, and community wide roles in extracellular respiration (Wrighton *et al*., 2008, Wrighton *et al*., 2011). In addition to my direct doctoral research, I also led microbiome analyses pertaining to identifying active members responsible for denitrification in engineered systems (Wrighton *et al*., 2010) and agricultural soils (Van Trump, Wrighton *et al*., 2011).

Anticipating the importance of linking *in situ* microbial processes to ecosystem processes using systems biology tools, I conducted my postdoctoral training with Professor Jillian Banfield at UC Berkeley. I linked geochemistry to data from meta-omics technologies (metagenomics, metaproteomics, metatranscriptomics) to reconstruct the metabolism of groundwater bacterial communities at a Department of Energy (DOE) field site in Rifle, CO. My analyses were the first to genomically sample 80 genomes from five uncultivated phyla, a finding published in Science (Wrighton *et al*., 2012*)*. Since this first discovery, my subsequent post-doctoral research expanded this genomic sampling to generate a whole candidate phyla radiation including over 800 genomes (Brown *et al*., 2014), showed that these bacteria were close to the size limit for life (Luef *et al*., 2015), and revealed that these bacteria drove important, yet previously unappreciated hydrogen, carbon, and sulfur cycles in the aquifer (Wrighton *et al*., 2014; Wrighton et al, 2016).

Current research

My research program at the Ohio State University ***focuses on microbiomes in soils and subsurface systems with special attention to microbially catalyzed biogeochemical processes occurring at the soil-water-atmosphere interface***. Initially, my laboratory focused on methane cycling, but now are expanding to other greenhouse gasses (e.g. carbon dioxide and nitrous oxide). At a very broad level, methane emission from soils is largely dependent on the bioavailability of organic carbon and the integration of fermentative, methanogen, and methanotroph metabolisms. It is my view that elucidating the genomic and realized physiology at the organismal level will provide insights into ecosystem function, with implications for management strategies relevant to issues of global warming and nutrient cycling. Below I outline soil-influenced projects that I would take with me to CSU: (I) examining microbial greenhouse gas production in soils, (II) plant-microbial interactions, as well as (III) other complementary research endeavors. My portfolio highlights my capacity to link microbial metabolism, carbon chemistry, and ecosystem response.

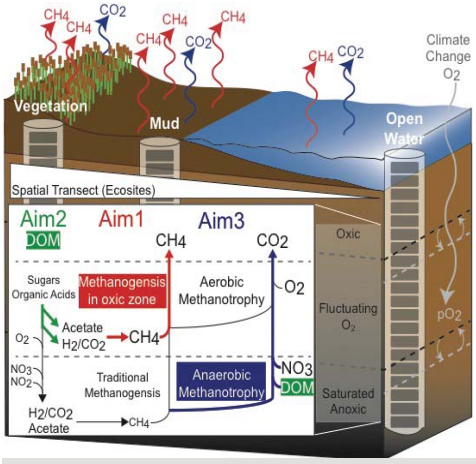
I. *Understanding microbial greenhouse gas production in soils and riparian systems*

Atmospheric methane is a significantly more potent greenhouse gas than carbon dioxide and is expected to increase from biogenic processes in response to climate change. Despite their relatively small land coverage, wetlands represent the largest source of atmospheric methane (20-40%), though uncertainty in emission budgets is high. Accurately predicting net methane fluxes from wetland soils depends on multiple interacting ecological

and geochemical constraints that are poorly understood, oversimplified, or missing in global biogeochemical models.

A key research focus in my laboratory is to identify the biogeochemical and genomic determinants impacting greenhouse gas emission, and the scale and physical distribution across which they operate. My group performs field research at the Old Woman Creek National Estuarine Research Reserve, located adjacent to Lake Erie, which is operated by the National Oceanic and Atmospheric Administration (NOAA). Diverse field data sets (spanning genomics to emissions) collected over two years and coupled laboratory physiological investigations have challenged widely held views on microbial methane metabolism.

Our first publication from this project examined microbial biogeography across the site and linked membership to microbial processes across ecological gradients (Narrowe *et al.*, 2016). Our most recent publication led by a graduate student in my laboratory, in press at *Nature Communications*, demonstrates that methanogens – normally considered to be obligate anaerobic microbes – persist in oxic, surface (0-5 cm) soils and contribute to 84% of methane emission site wide (Angle *et al.*). This research not only highlights our oversimplified notion of microbial metabolism in soils, but has serious ramifications for biogeochemical methane models. Work in progress in my laboratory is linking carbon and nitrogen cycles via nitrate driven methane oxidation (Smith *et al.*, submitted to *mBio*). This research was the basis for my recent Department of Energy (DOE) early career award, where I will explore relationships between dissolved organic matter composition, soil physical properties, and microbial activity on greenhouse gas emissions in surface soils. We have also received several state research grants (OWDA), DOE technology grants (from EMSL and JGI), as well as a graduate student GRFP (NSF) to support our laboratory's efforts.



Wetland soils project overview. We have demonstrated that methanogenesis can occur in oxic soils. Currently we are attempting to identify the genetic and environmental mechanisms supporting this unlikely metabolism (red). One factor that we hypothesize facilitates methanogenesis in oxic soils is dissolved organic matter (DOM) composition, which drives anoxic microsites and creates substrates necessary for methanogenesis (green). DOM concentration and composition is being characterized to better understand how carbon is decomposed in these systems. Lastly, methane emissions are largely controlled by activity of archaeal and bacterial methanotrophs, a metabolism we are beginning characterize across the wetland (blue).

Other environmental systems play a critical role in the cycling of microbially generated greenhouse gasses. Rivers dynamically exchange water and materials with surrounding soils and subsurface environments. In fact, 96% of the carbon dioxide produced within river systems occurs in hyporheic zones, or sediment regions beneath and alongside a river where groundwater and river water mix. My research group has recently begun to investigate the role of riparian corridors (and accompanying hyporheic environments) in driving greenhouse gas production through an award from the DOE Subsurface Biogeochemical Research Program. Despite this general knowledge, observational studies linking nitrogen and carbon gas production to microbial activity, and studies of overall gas emissions are limited across river systems, but especially absent from larger rivers. To identify microbial processes responsible for greenhouse gas emissions from large rivers, and to quantify their environmental drivers and dependencies, we selected the Columbia River as a representative field site. Here we are using microbial meta-omic tools, geochemistry, and paired emission data to better identify impacts of plant vegetation and river stage on microbial carbon and nitrogen cycling.

II. *Plant-microbial interactions mediating soil carbon cycling*

Through my career I have been interested in understanding how complex carbon can serve as an electron donor (Wrighton *et al.*, 2014; Solden *et al.*, 2017) and electron acceptor (Wrighton *et al.*, 2008). Expanding on this research, I have become interested in microbial interactions with plant tannins, which are the second most abundant plant phenolic after lignin, as these compounds have been demonstrated to alter soil carbon and nitrogen cycling. I am particularly interested in condensed tannins (CTs), as these compounds are prevalent in ecosystems from the gut to soils, and there is metabolite data suggesting they are degradable by microbes under

anoxic conditions. Understanding impacts of plant tannins on soil nutrient cycling is especially important for Arctic ecosystems, where climate-induced 'shrubification' and global warming is increasing the plant tannin content drastically at the landscape level. Increased plant tannins in these climatically vulnerable ecosystems negatively impacts microbial carbon cycling in wild herbivores gastrointestinal tracts, as well as belowground soil-carbon cycles with ramifications for greenhouse gas emissions.

In exploring CT degradation in soils, I realized that tracking an unknown CT metabolism in a complex physicochemical matrix like soil, with extremely high microbial diversity, made a difficult problem nearly impossible. I searched for a more tractable system for exploring anoxic polyphenolic metabolism, and surprisingly identified researchers at Alaska Fish and Wildlife using rumen fistulated moose to understand the impact of dietary polyphenolics like CT on microbial carbon cycling and animal health. On this project, we are developing methods and understanding microbial carbon cycling in the rumen system (Solden *et al*., 2016) and then scaling these findings to more complex soil systems. While this project is its infancy, we have already tracked CT metabolisms through rumen microbial communities using omics and metabolite data and isolated the first anaerobic CT degrading microorganism from any system. These findings will be used to identify enzymes involved in CT degradation across systems including soils and also resolve metabolic networks and impacts to soil nutrient cycling upon tannin amendment.

The project team I have assembled to better understand microbial interactions with plant tannins is highly interdisciplinary. It includes a soil microbiologist specializing in the Arctic (Dr. Vanessa Bailey, DOE PNNL), tannin biochemist (Professor Ann Hagerman, Miami University), Alaska wildlife physiologist (Dr. Bill Collins, Alaska Fish and Wildlife), and an analytical NMR chemist (Dr. David Hoyt, DOE PNNL). I have just recently submitted an NSF early career award on this project (submitted July 2017) and was awarded a DOE FICUS grant to support this project. Knowledge gleaned on this project will not only identify the currently unknown genes and metabolites involved in microbial condensed tannin degradation, but also better understand how soil microbiomes buffer against climate-induced changes in the plant biochemical landscape.

III. ***Other projects focused on microbial carbon cycling in soils, subsurface systems, and guts***

An area of expertise in my laboratory is identifying microbial carbon degradation metabolic networks from complex 'omics data (Solden et al, submitted; Daly et al, 2016; Wrighton et al, 2014). I leverage this knowledge with applications to soils, subsurface, and even host microbiome systems. Specific to soils, I was recently asked to contribute my knowledge of microbial metabolism to develop a computational approach to identify whether viruses in soil communities were capable of directly modulating biogeochemical cycling via auxiliary metabolic genes (AMGs). Here my laboratory analyzed metagenomes collected across an Arctic permafrost gradient to uncover 1,907 viral populations. While a majority of the genes lacked annotation, we identified viral genomes that encoded 24 glycoside hydrolase genes with the capacity for pectin, hemicellulose, starch and cellulose cleavage and demonstrated these viral proteins were biochemically active. The presence of virus-encoded GHs suggests that viruses may contribute to the degradation of complex carbon to release labile mono- and small oligosaccharides from plant-derived polymers into the environment, fueling microbial activity in soils. This research is currently in review at *Nature Microbiology* (Emerson *et al.*) and also is the foundation for a recent NSF Advances in Biological Information proposal submission.

My laboratory is also examining microbial carbon cycling in the deeper terrestrial biosphere, focusing on subsurface shale-rock systems before and after hydraulic fracturing. Microbial metabolism in these 2,500-meter-deep systems has detrimental (corrosion, fouling) or beneficial (methane production) economic and environmental impacts, yet we know little about life in this habitat. Using combined metabolite, metagenomics, and laboratory cultivation my laboratory determined that microorganisms persisting in fractured shales generate methylamine compounds in response to elevated salinity. These compounds are subsequently released into the extracellular environment following viral predation, fueling methylamine fermentation and subsequent methanogenesis that sustains microbial biomass long after initial hydraulic fracturing (Daly *et al.*, 2016). Current research in my laboratory is identifying biochemical and ecological interactions driving methylamine cycling and viral predation the laboratory (Booker *et al*, 2017; Borton *et al*, submitted). This research has recently expanded from Appalachian shales to also include shale plays in Colorado.

A key interest of mine is to study similar metabolisms across ecosystems, whether that be condensed tannin degradation or methylamine cycling. I believe that by using a cross-systems approach one can better elucidate the suite of abiotic and biotic controllers on microbial catalyzed reactions. Consistent with this objective, many of the shale methylamine metabolisms we identified are shared by microorganisms in the human gastrointestinal tract. The central hypothesis of this work is that microbial quaternary amine demethylation in the gut might serve to moderate microbially produced TMA production in the human intestine, providing therapeutic control of TMAO levels; and thus a means to decrease cardiovascular disease. To support this research, I have NIH R01 (NIDDK) support, where my laboratory is using omics tools to investigate integrated methylamine metabolism in human gastrointestinal tract, and linking this to host factors and serum metabolite levels.

Education and outreach

The same commitment to discovery and intellectual stimulation that drives my research, also motivates my teaching in and out of the classroom. I find the natural curiosity of students to be a constant source of new perspectives. My teaching philosophy centers upon sharing enthusiasm for microbial processes and trying to create life-long learners. Given that my research is multi-disciplinary, I use my research experiences to engage students from disparate disciplines. I incorporate bioinformatics tools directly into class exercises, or often use my own research results for in-class problem sets. For example, in my environmental microbiology class we spend time discussing microbial metabolism, then as a synthesis exercise I provide the class with unknown microbial genomes from my wetland soils. The goal of this exercise is to apply lecture material, expose students to new technology, and highlight the power and limitations of inferring metabolism from genomes.

I find active-based learning to be an effective way to engage students and encourage critical thinking of the lecture material. In my classroom, I try to establish an environment where all knowledge does not stem from me, and curiosity and intellectual probing are strongly encouraged. I use breakout sessions with varying group sizes to share and integrate perspectives. Collectively, I find these exercises instill a sense of team work (and accountability) and help students connect the course work to real-life examples. The ultimate goal of these activities is best summarized by a student on the course evaluation: "…the learning modules built camaraderie and helped us help each other learn the material." In my classroom, I actively stress that curiosity is integral to learning and is worth investing in.

As a graduate student instructor at California Polytechnic State University and UC Berkeley, I have been fortunate to teach both lab (General Microbiology Lab) and lecture classes (Microbial Ecology with Professor Rodrigo Almeida and Microbial Diversity with Professor John Coates). These classes helped prepare me for interacting with students in a classroom setting. Also, for the microbial ecology course, I developed the discussion section independently, which relied on creating interactive learning modules for students. The modules (lesson plans, spreadsheets, and grading rubric) that I designed are still used in the course today, and resulted in me earning the UC Berkeley-wide graduate teaching award.

Prior to my arrival at OSU, concepts of microbial ecology, metabolic diversity, and bioinformatics were largely missing from the curriculum across campus. Also, classes taught outside the department often failed to incorporate 'omic approaches that are common today. In response, I developed Micro 5155, Environmental Microbiology, an upper division and graduate student elective class. This course does not use a textbook, relying on primary literature. The first half is conceptual (biogeochemistry, meta-omics tools, phylogeny), while the second half we apply these concepts to real-world ecosystems, with more than half the content focused on the terrestrial biosphere. In the first year of this class I had 18 students. Three years later, I have 48 students (the class is at capacity for the room) and a waiting list of 10 students every semester. Class enrollment is no longer limited to microbiology, and in fact today the majority of my students come from outside the department (earth sciences, engineering, and natural resources). The student's appreciation for my teaching style and course material is reflected in my Student Evaluations, which are 4.9, 4.6, 4.7 on a 5.0 scale. Since starting my course, research in similar fields has grown on campus. In addition to my specific course, I have also given lectures in Geomicrobiology (Earth Sciences), Bioremediation (Environmental Engineering), and Bioinformatics

(Microbiology). In addition to any of the above topics, I would welcome the opportunity to develop soil microbiology lectures or a laboratory course, or a graduate course in microbiomes of terrestrial ecosystems.

Mentoring graduate students is integral to, and one of my primary motivators for a career in academia. Today I have 4 graduate students (from Environmental Science and Microbiology). In addition, I serve on the committees of 16 more, spanning a range of disciplines on campus (Engineering, Computation, Earth Sciences, Natural Resources, Environmental Sciences). While each of my student's research development is unique, I expect students to mature along a professional gradient that transitions from direct learning from me and their lab mates, to intellectual, and (hopefully) financial independence. This process instills strong technique, self-confidence, pride in research, and ultimately an environment where we both learn from one another. In the start of my fourth year at OSU, I am proud of the fact that all of my students have submitted a first author paper, two have fellowships (from OSU and NSF), and all four gave talks at the American Society of Microbiology general meeting this past year (two of which were on soils). Within my group, graduate students receive mentoring not only from myself, but also from two staff scientists, one who leads the computational biology and the other who leads the laboratory wet lab research. Building a culture in my research group did not happen overnight, but I am so pleased with the work ethic and scientific quality generated by my nascent research group. Ultimately, I am confident my natural leadership skills, teaching values, and multi-disciplinary basic and applied research will provide varied professional opportunities for CSU undergraduate, graduate, and post-doctoral researchers alike.

Given that my research is highly applied, with applications to terrestrial-water-atmospheric nutrient cycling, as well as bioremediation and bioenergy, I often find myself engaging with the public, enabling effective two-way communication between scientists and stakeholders. For instance, for the wetlands soil project, I am an active participant in the NOAA-supported visitor station adjacent to the wetland. Through collaboration with the OSU Journalism Department, we are together implementing a new component to the wetland visitor station/museum focusing on the importance of microbes to wetland services. This more formal collaboration resulted from brown bag seminars I have provided quarterly over the past two years. These lunchtime sessions are primarily attended by local science enthusiasts and retirees, and has addressed issues like microbial roles in climate change, agricultural run-off and eutrophication of Lake Erie, microbial plastic degradation in the Great Lakes. Other projects in my laboratory, like the shale-energy project, have involved stakeholders from federal and state government, academics, natural gas industry, and the public, while my recent NSF early career proposal provided authentic undergraduate research experiences in the form of a soil microbiology lab course.

Summary

My laboratory uses computational approaches to identify microbial metabolisms in soils and the subsurface, with a focus on anoxic carbon cycling. We validate these hypotheses using cultivation, (geo)chemical, emission, and molecular investigations. I enjoy working in interdisciplinary teams- including forging intellectual intersections with soil and earth sciences, microbial physiology and ecology, geochemistry, and mathematical sciences. As you can see from my recent publications at OSU, while collaborative in nature, research from my laboratory is independently led and trains early career scientists in a range of techniques and disciplines.

As someone currently housed in a microbiology department, I am particularly excited about the research opportunities that exist in the CSU Soil and Crop Sciences department. A thrust of my new DOE early career proposal is examining the impacts of soil chemistry and physical structure on microbial catalyzed methane production. I look forward to developing new collaborations in these research areas, as well as more specific cross-training for my students in biogeochemistry, isotope ecology, and soil sciences. In addition, transitioning my program also exposes me to new research opportunities, e.g., using my technology and intellectual framework developed for nutrient cycling in wetlands to interrogate other soil systems (e.g., agricultural), as well as projects associated with crop and animal sciences.