My research program addresses two grand challenges in science: antimicrobial resistance and quantifying global biogeochemical cycling. In the first, I am interested in how diversity and metabolic changes in biofilms contribute to the alarming and expanding problem of antibiotic resistance. In the second, I study anaerobic metabolisms associated with biogeochemical cycles as a biotechnological and ecological source of innovation. Traditionally these disciplines don't intersect. I would argue, however, that **microbial ecology and evolution mediated by metabolic feedback** lie at the core of each. Metabolic byproducts can open up new niches that influence eco-evolutionary dynamics and *vice versa*. Insights into diversification, resilience, resource competition, and cooperation in one system (environment) can inform another (host). Furthermore, the practical component uniting my research program is the study of ecology and evolution in anaerobic biofilms, particularly those that experience limiting resources (*e.g*. mucosal surfaces or natural environments). In addition, I am keenly interested in developing novel engineered devices and bioinformatic tools to address key scientific questions. I approach these questions from the perspective of an environmental microbiologist with expertise in anaerobic metabolism, evolution, and bioinformatics. My research program cuts across many aspects of microbiology and is supported by my track record for innovative (two patents), collaborative (clinicians, computational biologists, engineers), and interdisciplinary research. Below I outline two projects that exemplify the type of research I will pursue during the first 5-10 years of my career.

How do bacteria evolve resistance in biofilm communities? How do these lifestyle-dependent evolutionary dynamics impact survival in infections?

Antibiotic failure is a global health threat impacting millions of lives and billions of dollars in healthcare costs. One common, but understudied aspect of antibiotic resistance is the resistance of surface-attached communities called biofilms. The evolution of biofilm-adapted genotypes is prevalent in chronic infections yet how these adaptations can confer antibiotic and immune resistance is poorly understood. Biofilms become tolerant to antimicrobials due to slow (anaerobic) growth and/or by establishing a protective environment via charge interference or diffusion limitations. I am currently studying the evolutionary trajectory of resistance in biofilms, but **a major knowledge gap in the study of resistance mechanisms is the adaptation to oxygen-limited conditions**. My research aims to understand the interplay between tolerance and the evolutionary dynamics of resistance in low oxygen and low nutrient biofilms. To do this, my research program has three objectives, an *in vitro* system, *in vivo* samples, and a computational component that ties the two together:

1. **Experimentally evolve susceptible strains of Gram-negative pathogens in a biofilm model system under antibiotic pressure and varying degrees of oxygen and resource limitations**. This research objective exposes *in vitro* biofilms to antibiotics, oxygen, and resource limitations to mimic conditions in chronic infections. In order to survive these experiments, the microbial populations must evolve resistance or develop tolerance through biofilm-associated growth mechanisms. The result will be high-resolution evolutionary dynamics of resistance (antibiotic-specific genotypes) or tolerance (biofilm-adapted genotypes) using population resequencing and phenotyping. In addition to low oxygen conditions and nutrient limitations, other host stressors will be investigated to determine metabolic adaptations to clinically-relevant stressors (*e.g*. reactive oxygen species). These experiments can be automated in a high-throughput manner using a liquid handling robot. This objective will explore the possible molecular targets of resistance and the epistatic interactions between mutations selected across multiple environmental variables (*e.g*. biofilm and antibiotic).
2. **Sequence and phenotype longitudinal microbial populations from chronic infections (cystic fibrosis, burn wounds)**. This objective builds upon current work

to understand evolution of pathogen populations in a host. *In vivo* populations and isolates from clinical samples will be sequenced and phenotyped. Phenotypes (*e.g.* minimum inhibitory concentrations, biofilm formation, fitness, motility, other pathoadaptative phenotypes) will be associated with high resolution genotypes to determine the metabolic state and adaptations of the pathogen to the host. These samples will be accompanied by a rich database of patient metadata through collaborations with clinicians. (Preliminary data: Shields, Marshall *et al.* in prep, Gloag, Marshall *et al.* and Mustapha *et al.*).

3. **Use the genotypes from experimental evolution objective 1 and clinical evolution objective 2 to develop bioinformatic tools that predict clinical outcomes**. The question in this objective is similar to the famous Gould quote asking what would happen if we "replayed the tape of life". Specifically, based on understanding evolutionary dynamics, both *in vitro* and *in vivo*, can we predict the course of pathogen persistence in chronic infections? High resolution genomic signatures and flux balance analysis models will enable us to predict microbial lifestyle (*e.g.* biofilm, anaerobic, auxotrophies), compensatory mutations, and the susceptibility profiles that allow for better informed treatment courses. (Preliminary data: Dunlap, Marshall *et al*.).

Evolution and diversification in the early Earth - how did a single metabolic pathway diversify? And what does this tell us about life on other planets?

I am interested in understanding the diversification following the origins of life and the biogeochemical cycles that emerged as a result. Understanding the competition, cooperation, and diversification of life on early Earth is important to understand speciation, biogeochemical cycling, and the search for life elsewhere in the Universe. It is thought that the last universal common ancestor (LUCA) was an anaerobic thermophile capable of oxidizing hydrogen and fixing CO_2 by using the Wood-Ljungdahl pathway in an environment similar to modern hydrothermal vents (Weiss *et al*. 2016). Of the organisms living today, metabolisms basal to the phylogenetic branch of clostridia and methanogens align with this view on early Earth environments. I plan to **study the coevolution and diversification of microorganisms competing and cooperating for these limited resources, all employing the same backbone metabolic pathway**. There are three model organisms that fit the LUCA description but with slight variations on a single theme: 1. *Moorella thermoacetica* is a thermophilic acetogen capable of oxidizing H_2 and reducing CO_2 to acetate, 2. *Thermincola ferriacetica* is a thermophilic iron reducer that can oxidize either H_2 or acetate for anaerobic respiration and 3. *Methanothermobacter thermoautotrophicus* is a thermophilic methanogen that converts H_2 and CO_2 to methane. Thermodynamics dictate that methanogens should outcompete acetogens for H_2, but I and others have demonstrated coexistence of the two metabolisms and that increased CO_2 loading allows a competitive advantage for acetogens in what appears to be negative frequency dependent selection (Marshall *et al*. 2012). Furthermore, a mutualistic relationship between the acetogen with an acetate oxidizing partner (e.g. *T. ferriacetica*) may improve the kinetics of these reactions by reducing the inhibitory acidic end product of the acetogen (Preliminary data: Marshall et al. 2009, 2012, 2013, 2017). Therefore, the three organisms, all containing variations on the Wood-Ljungdahl pathway, can both cooperate and compete for the same resources. My research program will study the conditions in which all survive and alter the environment to establish new niches. I plan to do this in the following objectives:

1. **Combinatorial competition experiments to determine fitness of LUCA-relatives in H_2:CO_2 and iron oxides.** Thermodynamics and literature suggest an iron reducer will outcompete methanogens and acetogens when iron oxides are present. However, once available iron is reduced, methanogens and acetogens then become active. Furthermore, methanogens should outcompete acetogens, but spatial structure and

mutualism with acetoclastic iron reducers may allow for the selection of acetogens. This set of experiments will determine which conditions (with or without iron or spatial structure) contributed to the ecology following the diversification of the LUCA. (Preliminary data: Marshall and May 2009, Marshall *et al*. 2012)

2. **Co-evolution of LUCA-relatives in a common garden**. Since the LUCA was thought to arise in a hydrothermal vent-like environment, evolution of single, co- and tri-cultures will be carried out in thermophilic biofilms under iron-reducing conditions with H_2:CO_2 as the carbon and energy source. The first objective will inform the frequency trajectories of the cultures under different conditions and this objective will expand on that work to demonstrate the evolutionary adaptations to the environment and to competition around a single metabolic pathway. This work will demonstrate the eco-evolutionary feedback in a simulated early Earth environment and provide a framework for the diversification that took place when a small number of competitors existed.

Long-term Research Strategy

My research encompasses a wide range of disciplines including anaerobic microbiology and physiology, biogeochemical cycling, applied biotechnology, microbial ecology, and microbe-host interactions, while using state-of-the-art tools including metagenomics, metatranscriptomics, metabolic modeling, experimental evolution, and electrochemistry. This interdisciplinary focus is built upon being highly collaborative as exemplified by my manuscripts with engineers, biophysicists, clinicians, and computational scientists at the MUSC, Argonne National Laboratory, UChicago, and University of Pittsburgh. I have been awarded funding in the past from DOE, NSF, and industry and I have been key personnel on NIH and NASA funded projects, demonstrating that my background and collaborations position me to successfully acquire funding from many governmental and non-governmental sources.

As the fields of biology and microbiology advance over the next few decades, the use of genomics and informatics will be integrated into nearly every experimental design. Bioinformatics is an integral component of my research, but one thing that gets lost in this advancement in technology is the art of culturing and experimentation. One of the most valuable aspects of my research is my understanding of both worlds. Working with collaborators at PATRIC, KBase, RAST, and others I plan to experimentally test and validate metabolic modeling of simple and eventually complex communities across multiple scales. The value of this will eventually be in our ability to model community interactions and to predict treatment outcomes. I look forward to developing these capabilities and forging new relationships across campus.