

Research Statement

Summary

My overarching research interests revolve around marine microbial ecology and geomicrobiology, the interactions between microbial communities, their geochemical environment and the resulting modifications of the rock substrate and environmental chemistry. I use a combination of modern molecular microbiology (PCR, qPCR, next-generation sequencing, etc.), geochemical (wet chemistry and electrochemistry) and isotopic techniques (natural abundance, (radio)tracer-level, and isotope pairing) to answer the how, who, where and how fast questions of microbial ecology and geomicrobiology. More specifically I am interested in examining the direct and intimate relationship between the activity of the biological community, the geochemical signature of the activity and how that signature may translate between environments thus enhancing our understanding of the current and past processes that drive our planet.

Introduction

The cycles of the major elements on earth, carbon, nitrogen, sulfur, etc. are controlled by interaction between the biosphere, lithosphere and hydrosphere. The pathways or mechanisms that drive these processes, while ultimately thermodynamically favored, are often carried out and exploited by the microbiota. The result of the processes change the geochemical environment, often on a time-scale much more rapid than abiotic geochemistry could explain. For example, the oxygenation of the atmosphere and the accumulation of fixed carbon and nitrogen are direct results of microbial processes. My research interests center on examining the intimately linked interactions between the microbiological and geochemical spheres. I strive to use my novel training as both an environmental chemist and marine microbial ecologist, including tracer-level and natural abundance isotopic and molecular tools, to investigate environmental processes on a range of temporal and spatial scales. These tools directly apply to the central questions of geomicrobiology, *who* (molecular - 16S rRNA genes), *what process* and *where* (molecular - functional genes qPCR surveys, natural abundance isotopes) and *how fast* are these processes going (tracer incubations or natural abundance isotopes).

My interests focus on the cycling of both nitrogen and sulfur in the environment. Both elements are essential for life and because of their many oxidation states the resultant compounds can be used in alternative respiratory pathways in many environments ranging from common marine sediments to the deep-sea. Furthermore, these two elemental cycles have also been altered in the coastal setting due to anthropogenic inputs. For example, the Haber-Bosch process has doubled the worldwide inventory of fixed nitrogen (ammonia, nitrite and nitrate). This has resulted in our ability to feed our 7 billion mouths and keep our lawns green. However, fixed nitrogen are mobile species and when the rain falls, the fixed nitrogen follows the water to the local stream and ultimately to the coastal waters. Excess nitrogen in coastal waters is linked to many deleterious processes and events including phytoplankton blooms, hypoxia and anoxia, sea

grass and shellfish die off and human health concerns. The stimulation of biomass by the addition of nitrogen can produce local hypoxic or anoxic events and thus a greater proportion of the fixed carbon is available for anaerobic respiration. In the marine environment this respiration is dominated by sulfate reduction. This is but one way the nitrogen and sulfur cycle interact.

Research Settings

I envision my laboratory as having both local coastal waters and deep-sea field components. Many of the processes I study occur at many locations but the communities may change from location to location. Having two or more sites allows the evaluation of hypotheses about the mechanisms and the communities involved to be investigated separately.

I have worked in a lot of coastal systems such as salt marshes, salt ponds and coastal sediments, including my Ph.D. work examining the geomicrobiology of the nitrogen cycle in a coastal groundwater aquifer underlying the Waquoit Bay National Estuary Research Reserve (WBNERR). The local sites are easily accessed and can be used to generate long-term and/or high frequency monitoring data. Local sites are also excellent proving grounds for the development of techniques and instruments. Collaborations with other researchers within and outside of the department are also more easily forged in this small-scale setting. There are also advantages to working at sites and on environmental issues that have a more visceral impact on the general public. With half the world's population living within 50 miles of the coast, having a coastal research site is of great importance. For example, my work on nitrogen cycling in the groundwater under Waquoit Bay, MA has direct application for local decision makers and planners. These more politically minded people can use the primary research and interactions with the scientist involved to make decisions and judgments that improve the health of our coastal systems.

Remote environments offer an opportunity to examine geomicrobiological processes in more "pristine" settings that may serve as analogs for early earth history or provide ground truth data for extra-solar research efforts. Deep-sea systems such as hydrothermal vents have often been described as an analog to early earth. The chemical environment is extreme compared to our everyday experience. Organic carbon is limiting and toxic dissolved (up to mM level) and solid phase sulfur (H_2S , S_x^{2-} , pyrite, etc.) are the main sources of electrons driving primary production, as opposed to the light drive systems that support more familiar life. The major oxidants include the familiar oxygen but also extend rapidly through nitrate, metals and sulfate in a redox cascade similar to that observed in carbon rich coastal environments. Organisms that thrive in these environments must be able to tolerate high frequency changes in the chemical and physical

environment including dynamic redox potentials, pH and oxidants as well as severe changes in temperature in excess of 100°C.

Research Approach

Oceanography and geomicrobiology are interdisciplinary efforts. I strive to combine the use of isotopic, chemical, molecular and traditional microbiology to address the basic *who*, *what*, *where*, *how* and *how fast* questions that are the basis of microbial ecology and geomicrobiology. There are many advantages to using an interdisciplinary approach such as described here. First, each technique approaches the question from a different angle, different set of assumptions and the results may integrate over a different temporal or spatial scale. For example, combining the use of natural abundance isotopic and molecular data can yield overlapping views of the processes occurring in an environment. Isotopic measurements integrate over the history (temporal and spatial) of that solute pool. Changes in the $\delta^{15}\text{N}$ of the nitrate pool along a flow path from point A to point B reflect the processes that occurred in that pool during that transit time and within the matrix of that flow-path. Concurrent molecular data, 16S rRNA genes for example, provide evidence for which organisms are present, though 16S rRNA genes do not reveal what these organisms are doing. Furthermore, the DNA record has a lifetime of its own which may integrate of days, months, years or longer depending on the specific conditions of the environment. By combining these distinct techniques, inferences into the ecology of the system and rates of reaction can be estimated (ex. Rogers and Casciotti, 2010). Incubations with tracer-level isotopes (I have done this type of work with both radioactive ^{35}S , ^{14}C as well as stable ^{15}N tracer compounds) can be used to determine potential rates of specific processes including processes that are traditionally difficult to access such as anaerobic ammonia or methane oxidation (anammox and AOM respectively). I have employed ^{15}N stable isotope pairing techniques to assay these processes in both a groundwater aquifer (Ph.D.) and deep-sea massive sulfide (Postdoc) incubations. In the Casciotti laboratory, I used both natural abundance $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of the nitrite and nitrate pools and isotope pairing techniques with ^{15}N -labeled ammonium and nitrate to detect the presence and estimate rates of denitrification, nitrification and anammox processes. One of the results of this work shows that denitrification, in the nitrate rich groundwater, is limited to areas that experience mixing with reduced seawater (bay water) infiltrating through the sediment, however, the rates are similar to those reported for coastal sediments where the organic load is much higher. Molecular data from concurrent samples demonstrates a shift in the denitrifier community toward more marine-like nitrite reductase sequences as the profile transitions between the groundwater and the underlying reduced

seawater. In the Girguis laboratory, working with a graduate student, I am coupling ^{35}S tracer methods with functional gene abundance, through quantitative polymerase chain reaction, to examine the rate and locations of sulfate reduction in deep-sea massive sulfides from the Juan de Fuca Ridge. To our knowledge, this is the first study to provide estimates of sulfate reduction rates from deep-sea hydrothermal vent deposits across a range of relevant temperatures. I am also attempting to recover RNA from these difficult, metal rich samples in an effort to place constraints on metabolic rates per gene expression level.

Student Involvement

I have worked with and mentored students (high school, undergraduate and graduate), technicians and postdoctoral researchers. I can envision a role for researchers at each stage in my laboratory. For more the most junior, the high school and undergraduate students I prefer to employ them in small, term-length projects where techniques and fundamentals are heavily emphasized. As these students gain experience and technical skill they can be given more freedom and creativity in the laboratory. Graduate students initially (first year or two) will be trained on both technique and research philosophy, gaining greater independence as experience grows. I see Master's degree students as trained, skilled researchers but they ought to have concise projects that are mainly driven by my research interests. I expect Ph.D. students to have a greater role in the development of the project rationale, methodology and perhaps funding effort. The goal is to foster their development into independent and talented researchers. Postdoctoral researchers join my lab to either gain experience in a new field or in a new technique, adding to their professional toolbox. While I will strive to include postdoctoral funding in my research proposals I also believe it is important for young Ph.D. level researchers to attempt to establish their own funding sources. This benefits the Postdoc as much if not more than the established laboratory. The postdoctoral period is also a time to develop mentoring skills by working closely with graduate students, perhaps on a joint project, in the laboratory.

Future Directions

In coastal systems, I am interested in asking: Where does nitrogen removal occur along the coast? What are the potential pathways and rates of nitrogen oxidation and ultimately removal for the system? How do other cycles, such as sulfur, interact with nitrogen cycling? What environmental factors (carbon content, flow rates, etc.) may enhance or abate the nitrogen removal process? Is nitrogen transport into the coastal waters a focused or distributed flow and

how might that affect how we engineer solutions (move from septic to sewer, housing development level treatment options, beach barriers, etc.)?

Larger-scale deep-sea expeditions will come through collaboration with colleagues who maintain large ocean-going efforts and as ship time becomes available. The key questions I am interested in addressing are: Nitrate is available ($5 \mu\text{M}$) in the deep-sea and crenarchaea are abundant, what is the role of these archaea in the deep-sea nitrogen cycle? What role does nitrate from seawater and ammonia from thermal fluids play in microbial communities at organic poor systems like the Eastern Pacific Rise versus organic and ammonium rich (as high as mM levels) settings like Guaymas Basin? How widespread is the anammox process at these vent environments and what does this process look like isotopically in the ammonium, nitrate and nitrite pools?

Conclusion

I have developed a unique skillset that encompasses traditional microbiology, modern molecular techniques, isotopes and geochemistry. I am excited about using this skillset and training to investigate how the microbial world effects and drives the elemental cycles of nitrogen, sulfur and more. I believe the union of techniques, knowledge and skill from a range of disciplines is necessary to address these questions in the environment. My background and training bring this approach and skillset to together.