Molecular and geochemical insights into microbial life centimeters to kilometers below the seafloor

Thesis by

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ABSTRACT

At the broadest scale, this thesis is an investigation of how life modulates the movement of essential elements (carbon, sulfur, nitrogen, and silicon) on modern and geologic timescales. Chapters 1 and 2 explore carbon and sulfur cycling microbial communities found centimeters below the seafloor in hydrocarbon-rich methane seep ecosystems. At the Hydrate Ridge methane seep, we investigated how microbial partnerships direct the flow of methane and sulfide in these benthic oases by using identity-based physical separation methods developed in our lab (Magneto-FISH) in conjunction with community profiling and metagenomic sequencing. This method explores the middle ground between single cell and bulk sediment analysis by separating target microbes and their physically associated community for downstream sequencing applications. Magneto-FISH captures were done at a range of microbial taxonomic group specificities and sequenced with both clone library and next-gen iTag 16S rRNA gene methods. Chapter 1 provides a demonstration of how FISH probe taxonomic specificity correlates to resultant Archaeal taxonomic diversity in Magneto-FISHed seep sediments, with specific attention to preparation of Archaea-enriched samples for downstream metagenomic sequencing. In Chapter 2, a Bacteria-focused parallel environmental isolation and sequencing effort was subjected to co-occurrence analyses which suggested there may be far more microbial associations in methane seep systems than are currently appreciated, including partnerships that do not involve the canonical anaerobic methane oxidizing archaea and sulfate reducing bacteria. With samples from IODP Expedition 337 Shimokita coalbed biosphere, Chapter 3 provides evidence for an active microbial assemblage kilometers below the sea floor in the deepest samples ever collected by marine scientific ocean drilling. Using in situ temperature Stable Isotope Probing (SIP) incubations and NanoSIMS, we investigated whole community activity (with the passive tracer D_2O) and substrate specific activity with C1-carbon compounds methylamine and methanol. We found deuterium-based turnover times to be faster (years) than previous deep biosphere estimates (hundreds to thousands of years), but methylotrophy rates to be slower than previous carbon metabolic rates.

PUBLISHED CONTENT AND CONTRIBUTIONS

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E.T.R participated in conception of project, designed study, prepared data and analysis, and wrote the manuscript.

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E.T.R participated in conception of project, designed study, prepared data and analysis, and wrote the manuscript.

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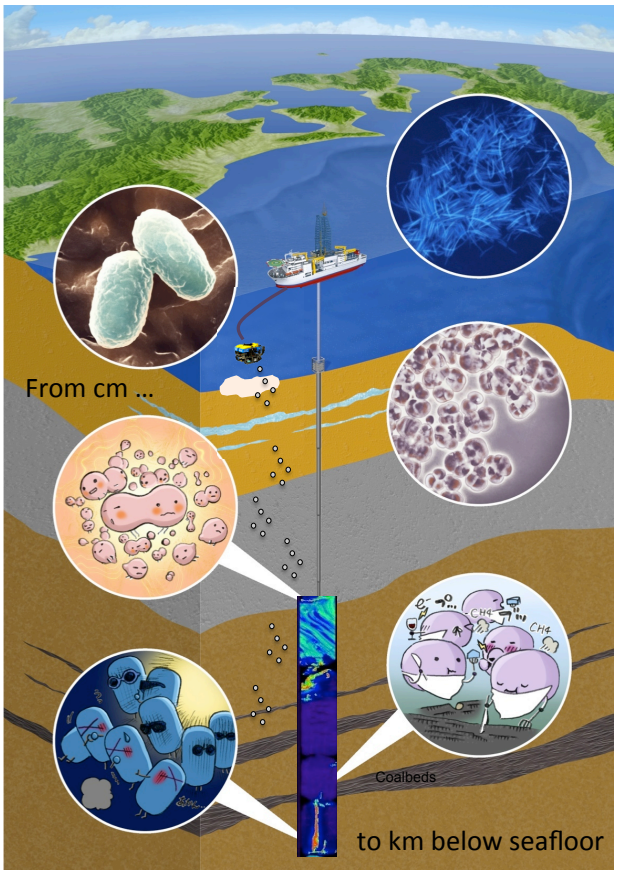

While not unified by a single method or study location, this thesis provides four examples of how targeted methods are uniquely able to resolve the character of biologically-mediated carbon, sulfur, silicon, and nitrogen cycling. Discerning the biological component of the systems explored herein is fraught with difficulty stemming from their complexity (Chapters 1 and 2), age (Appendix A and B), or metabolic reticence (Chapter 3).

Chapters 1 and 2 provide a method (Magneto-FISH) and an application (modern methane seep sediments) for dealing with complex microbial communities where multiple species may have, at least superficially, similar roles, such as sulfate reducing bacteria and anaerobic methane-oxidizing archaea, but yet certain partnerships appear preferred over others. By a phylogenetically-selective mechanism, we are able to enrich for target microbes and their physically associated microbial partners to explore spatial arrangement and sequence space in tandem. It is an attractive method for any environment with physically associated microorganisms that can bridge work done at the single-cell and bulk microbial community levels to provide a more holistic framework for microbial interactions.

The Appendices address more historical geobiological questions of how the evolution of land plants may have affected global silicon and carbon cycling (Appendix A) and if microorganisms may be responsible for the structures preserved in microbial mats from the rock record (Appendix B). In both of these systems the original biomaterial is a palimpsest – no longer present or too altered to

directly address our research questions. To overcome the effects of time, we utilized comparative biology methods to determine how extant plants (early evolving land plant lineages) and mats (modern carbonate platform analogs) create the biominerals and biostructures, respectively, that we may see preserved. The applications herein provide application and integration of modern biology to Earth history questions that exemplifies the toolbox of geobiology.

The final chapter (3) interrogates a unique deep biosphere sedimentary environment where terrestrial organic matter from a paleo-swamp has been buried for millions of years under what has now transitioned to an open marine environment. Initial genetic and geochemical results from IDOP Expedition 337 indicate an active assemblage of microbes similar to a modern swamp community (Inagaki et al. 2015), but cell abundances lower than retrieved from any other IODP cruise (1-100 cells/cm^3), despite the extremely high cell abundances at the sediment surface (10^9 cells/cm^3). This extremely low biomass provides a technical challenge to both measuring activity and ensuring the measured activity reflects that of the *in situ* community, rather than any of the myriad contamination sources from drilling to sequencing or an overprinting abiotic process. One could even argue that deep sea drilling is even harder than detecting life in Martian samples, as the contamination on the Earth's surface is so much higher.

As we abut the limit of cell detection, we can no longer hope that the in situ cell concentration will be above the background contamination signal. One of the biggest sources of contamination, drilling mud, is also required for the riser drilling technology that allows deep core recovery. Stringent contamination control, such as identification of samples with high porosity and fracture planes via onboard tomography (CAT scan), can aid in determining the most pristine samples in real-time to avoid using them for stable isotope probing (SIP) incubations. However, it was not possible to remove all sources of contamination from all samples. Therefore, tracking contamination is a more viable pursuit than attempting to remove it completely. This can be done onboard by adding chemical tracers like perfluorocarbon (PFC) to drilling mud and monitoring its concentration, or performing sequencing assays for known microbial contaminants such as water column marine organisms for all downstream biological samples. In addition to these microstructural, chemical, and genetic contamination identification methods, hydrogenase enzymatic and SIP-NanoSIMS activity-based controls showed that when putative contaminant cells did come into contact with samples, they were "dead on arrival," making our activity based measures robust even to contaminant cells for determining viability of in situ populations. While it

cannot be ignored that contaminant cells may provide a potential organic carbon source, we did not determine that any contaminant cells were present (based on expected size for deep biosphere cells) in the incubations discussed in this thesis.

In addition to tracking, technological advancements in sample collection were also used to reduce contamination. Cruises rely heavily on porewater data to determine potential metabolisms, activity profiles, effects of transition from in situ to incubation, or even simply concentrations to use for incubation conditions, but we were either unable to recover any porewater, or what was recovered was too contaminated with drilling mud, through traditional onboard squeezing methods from the 2 km below seafloor coalbeds. To overcome porewater exposure to drilling mud, Exp. 337 was able to use a specialized formation water-sampling device, Schlumberger's Quicksilver probe, for a few select horizons. This allowed us to recover more pristine interstitial water at formation pressures, which is extremely important for gaseous substrate concentration measurements. These Qucksilver probe samples allowed us to confirm the high (mM) levels of ammonium measured in our SIP incubations, highlighting a conundrum of the deep biosphere that has also been found in other studies: Why do deep biosphere cells show a clear preference for nitrogen incorporation over carbon, if they live an ammonium replete environment? The cause of this phenomenon should be a high priority for future deep biosphere research and emphasizes how much remains to be discovered about deep biosphere physiology.

Another approach to understanding deep life physiology has been through attempting to constrain metabolic rates and relating them to turnover of elements deep essential for life (i.e. hydrogen, nitrogen, and carbon). The deep biosphere literature has gravitated toward using a discussion of turnover time, as opposed to growth rate or doubling time, since production of new biomass cannot be predominantly attributed to production of new cells (doubling) over maintenance in energy limiting environments. There is also evidence that deep life is good at recycling biomaterials as a potential energy conservation technique (Takano et al. 2010), which provides an additional caveat to turnover calculations that requires further exploration. However, Morono et al. 2011 showed that most deep biosphere cells were viable, if not actively replicating. At its base level, turnover is the reciprocal rate of some process, be it sulfate reduction coupled to acetate oxidation or amino acid degradation. Converting these rates to turnover times enables comparison to geologic processes, such as sedimentation rate or thermal degradation of organic matter. Previously published deep biosphere biomass turnover times have all been upwards of thousands of years before SIP-

NanoSIMS based times, which appear to be months to years based on our findings that those of Morono et al. SIP-NanoSIMS provides powerful, single-cell resolution of minimal microbial activity that is not possible with bulk geochemical or SIP-RNA/DNA methods. Our study was also able to show that microbial assemblages appear to have different modes of activity within these viable populations and different biosynthesis rates between hydrogen, nitrogen, and carbon, as discussed in Chapter 3. The 2 kmbsf biosynthesis-based turnover times are slower than times for shallower samples that were provided more substrates (Morono et al. 2011), but we do not know if this is an effect of substrates provided and/or differences in the microbial potential for activity from 200 m to 2000 m below seafloor. As we continue to use SIP-NanoSIMS to study the deep biosphere, these distinctions may become more resolved. The continued application of deuterated water as a passive tracer can also provide a baseline metric for unamended, or minimally amended, activity conditions in each new system to connect all future SIP-NanoSIMS experiments, and better determine what is unique to a new environment and what may be universal to deep life.

Finally, the results from Exp. 337 have opened new avenues for conceptualizing the residence time of carbon in coals that have never reached sterilization conditions. With global lignite reserves estimated at 839 Gt (Killops & Killops 2013), understanding what portion of this carbon, assumed stabilized in the lithosphere, may be biologically mobilized and potentially returned to the surface biosphere is important for understanding both deep life and global carbon cycle regulation. Initial investigations into carbon isotopic composition of methoxy-groups in Exp. 337 coal samples are order 50 per mil enriched over the bulk coal carbon values, which provides the tantalizing prospect of a signal for microbial distillation over millions of years. While at the same time, other work suggests that high-pressure environments cause a significant (–20 ‰) depletion in biomass carbon from their carbon source (Fang et al. 2006).

Even with a high-resolution, single-cell activity technique, we must know more about microbial physiology under high pressure, high temperature, and slow growth conditions to be able to contextualize in situ deep biosphere measurements and tease apart what is physiology versus environmental in future deep biosphere SIP-NanoSIMS incubations. Target questions include: Why do microbial populations appear to show different physiological modes, even when in theoretically uniform conditions (Kopf et al. 2015)? How does high pressure affect both natural and labeled isotopic enrichments? What are reasonable water assimilation constants for slow growth conditions, and archaea in general? Can we overcome limitations of deep biosphere biomass and

develop methods to discern biosynthesis of new biomass from microbial maintenance and repair or recycling of necromass? These constraints on biomass turnover, along with technological advancements in three-dimensional imaging of deep biosphere spatial relationships, will then lay the groundwork for myriad other deep biosphere constraints, such as genetic exchange and evolution rates.

The more we know about how life thrives and survives in the present, the more we can plan for our future and interpret our past. The deep biosphere provides a unique environment that blends active biological processes operating in geologic time capsules that are isolated from solar primary production. Through the combination of additional microbially–motivated IODP cruises to more environments and carefully cultivated laboratory experiments, we have much to explore in the years to come that will advance our understanding of life's most extraordinary forms on our planet and maybe others.

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