Introduction

One of Darwin's great dilemmas when he published the Origin of Species was the sudden appearance of diverse animals at the beginning of the known fossil record, seemingly out of nowhere—what we now call the Cambrian Explosion, starting around 542 million years ago. He hypothesized that further investigation into the early rock record would reveal earlier, simpler forms that gave rise to the appearance of animals. However, it has only been in the past few decades that Darwin's suspicions have been proven absolutely correct. Not only was there an earlier history of simpler animals leading up to the Cambrian Explosion, but there is also a record of microbial life going back more than 3.7 billion years ago, almost to the beginning of the rock record on Earth.

The modern diversity of complex organisms like plants and animals is therefore a relatively recent innovation. The history of microbes like bacteria and archaea is much longer, spanning a period of time so long that the entire evolutionary history of animals—from simple sponge-like organisms to the entire diversity of jellyfish, bees, giant squid, and humans—could have occurred half a dozen times with hundreds of millions of years to spare.

It is therefore no wonder that the history of microbial evolution is a rich, complicated story, albeit a difficult one to read. While the history of animal life is recorded in fossils with characteristic morphologies, the bacterial fossil record is sparse and largely uninformative. Bacteria are not easily preserved, and those microfossils that do exist are nearly impossible to confidently classify because bacterial morphology is simple and similar forms can evolve convergently in distantly related taxa. If we are to understand the evolutionary history of bacteria and other microbes in deep time, we need to identify new records of past life and develop new strategies to investigate them.

The most important stories in microbial evolution are not based on morphology anyway, but on metabolism. One of the most significant innovations in the history of life was the invention of oxygenic photosynthesis by Cyanobacteria. Oxygenic photosynthesis, the biological ability to oxidize water using light in order to fix organic carbon, produces O_2 as a brproduct, and led to the oxygenation of Earth's atmosphere ~2.3 billion years ago. Oxygenic photosynthesis made complex, multicellular life possible, and revolutionized geochemical cycles and the metabolic opportunities available for life by providing molecular oxygen for oxidative weathering and aerobic respiration.

While animals all make a living by breathing oxygen and eating organic carbon, microbes can possess any number of a bewildering variety of metabolisms—from eating hydrogen to produce methane, to breathing heavy metals like uranium, to conserving energy by using light to rust iron. Many of these microbial metabolisms only exist in niche environments today, but are vestiges of earlier periods in Earth history—particularly before the oxygenation of the atmosphere ~2.3 billion years ago—when they were major parts of the biosphere. These metabolisms, and the organisms that possess them, present us with valuable targets for investigating the evolution of metabolism and ways to understand the ancient biosphere.

The nature of the biosphere before the rise of oxygen—the metabolisms that existed, the amount of productivity they supported, and the structure of biogeochemical cycles—is one of the largest and most exciting open questions in historical geobiology. However, there is much uncertainty in the metabolisms that were possible, what metabolisms had evolved by this time, how efficient they are at driving productivity, and how this played in to other Earth system characteristics such as the composition of the atmosphere. If these questions can be answered, we can better understand the history of our own planet and how it came to be the place we live today, and we can also make predictions about what biospheres on other planets may be like and how to detect them if they do exist. The first section of this dissertation therefore addresses the nature of the early Earth, before the evolution of oxygenic photosynthesis and the Rise of Oxygen. These chapters primarily utilize geochemical models of the flow of electrons between redox-active molecules utilized by biology, including electron donors like molecular hydrogen and ferrous iron supplied by geology but used by early life to support primary productivity. These box models are relatively simple, utilizing previous estimates of the fluxes of geological and atmospheric processes on the early Earth to make order of magnitude estimates of the scale of biological productivity. Because these models are relatively simple and grounded in the best available constraints from the rock record, they are relatively insensitive to uncertainties in poorly constrained aspects of the early Earth like volcanic outgassing rates or the size and distribution of continents. This is in contrast to other, much more complex models, which can have unexpected nonlinear dependencies on poorly constrained parameters. These models provide valuable predictions about the relative scales and rates of biological processes as related to geological ones, and introduce new ways of thinking about the relationship between early life and the Earth.

Chapter 1 considers the potential rates of primary productivity that could be supported by the early biosphere, dominated by methanogens and/or anoxygenic phototrophs, and concludes with estimates of productivity many orders of magnitude lower than are characteristic of the modern, oxygenic photosynthesis-fueled biosphere. Chapter 2 considers the timescales over which life and the Earth change in response to the evolution of oxygenic photosynthesis, with the result that oxygen accumulates rapidly and therefore the GOE should follow closely the evolution of oxygenic Cyanobacteria. Ultimately, this section paints a picture of the pre-oxygen biosphere as being much smaller and less productive than what we see today, and highlights the significance of oxygenic photosynthesis—and the ability to use water as a virtually unlimited electron donor—as the fundamental driver of the large, complex, productive biosphere we see today that allows life to develop geologically-relevant feedbacks for maintaining climate, atmospheric composition, and biogeochemical cycles.

Insights from the rock record, and geochemical models like those described in the first three chapters of this dissertation, are valuable, but are limited by the incompleteness of the rock record due to billions of years of gradual degradation due to weathering and tectonics. To understand how the early biosphere functioned, we need to develop novel techniques for supplementing the incomplete rock record with the other record of life on Earth: the biological record, as preserved in the diversity of extant organisms and their genomes. By developing and testing hypotheses using both the rock and biological records, we can develop a much more thorough understanding of how life functioned on the early Earth. In Chapter 3, I investigate the biological record of anoxygenic phototrophy in the Chloroflexi phylum of bacteria to address the antiquity of anoxygenic photosynthesis. I demonstrate from comparative phylogenetics that the distribution of phototrophy in this clade is the result of horizontal gene transfer, and therefore may be a relatively late evolutionary occurrence. This is an important result for understanding not only the history of this clade, but also the role of horizontal gene transfer in shaping the diversity and distribution of metabolic pathways across the tree of life. Furthermore, by demonstrating that anoxygenic photosynthesis in the Chloroflexi may be a relatively late innovation, it helps to demonstrate that in many cases it may not have been crown group playing a role in driving the early, pre-oxygen biosphere. Instead, the Archean biosphere was likely dominated by extinct stem group lineages, and that these may have been wiped out during adaptive radiations following the expansion of productivity and new metabolic and ecological opportunities following the GOE.

An alternative strategy for understanding the ancient Earth is by characterizing niche environments that support processes that are in some ways analogous to those that occurred on the ancient Earth. These process analog environments can reveal the interplay of environmental conditions and microbial communities that determine rates of primary productivity, the structure of geochemical cycles, and preservable geological and geochemical signatures. In Chapter 4 of this dissertation, I characterize a novel iron-rich hot spring microbial community in Japan that is in some ways analogous to periods in Earth history. This hot spring, Oku-okuhachikurou Onsen in Akita Prefecture, Japan, supports aerobic iron oxidizing bacteria and deposits highly oxidized, organic carbon-lean iron deposits similar to Banded Iron Formations deposited after the Rise of Oxygen. This system highlights the low metabolic yield per electron donor of lithotrophic bacteria relative to phototrophs, a largely underappreciated but potentially far-reaching trend that may have relevance for ancient Earth environments described in Chapter 1 and diverse planetary environments discussed in Chapter 5.

Stephen J. Gould once remarked that if one were to rewind the tape of life and play it back from some earlier point, there is no telling if it would play out the same or if the diversity of life would turn out wildly different. The biosphere we have today is the result of billions of years of stochastic phenomenon, contingent on the internal randomness of evolution as well as external influences from the environment. On other worlds, where the environment may provide very different metabolic opportunities for life, the trajectory of evolution may play out very differently. Understanding how Earth's biosphere evolved to reach its present state provides a framework for predicting how life may develop on other worlds with very different conditions. I conclude this dissertation with a consideration of how the history of oxygen on Earth differs from that on Mars, Europa, and other planets, and what these comparative histories may mean for the opportunities for life on other worlds. This chapter highlights the role that planetary context plays in shaping the metabolic opportunities available to life, and how this may relate to the evolutionary trajectories that life can follow. On the early Earth, life was limited to making use of limited geological fluxes of electron donors to drive low energy and low productivity metabolisms until the evolution of oxygenic photosynthesis freed the biosphere from these constraints. On other planets, the balance of electron donors and acceptors available to life may vary, resulting in some environments where less metabolic evolution is necessary to achieve highly energetic metabolisms than on Earth.

Finally, this dissertation includes several appendices, including projects on which I was a substantial contributor but not lead author and projects that are underway but not yet complete. Appendix 1 considers the nitrogen cycle on the early Earth, before the rise of oxygen, and demonstrates that in the absence of molecular oxygen it was likely simple, restricted to reduced compounds, and primarily driven by abiotic processes. This ties in to the first section of the dissertation in demonstrating that before the evolution of oxygenic photosynthesis, the biosphere may not have been capable of driving geochemical cycles as it does today. Appendix 2 discusses a hot spring ecosystem supporting cone-forming microbial mats analogous to those interpreted that produced conical stromatolites in the ancient rock record. Stromatolites are frequently interpreted as evidence of oxygenic Cyanobacteria. This analog system however supports cone-shaped mat morphologies developed by anoxygenic Chloroflexi, providing good evidence that diverse microbial taxa can lead to similar mat and stromatolite morphologies, and therefore ancient stromatolites may have formed in the absence of oxygenic Cyanobacteria. In Appendix 3, I describe Jinata Onsen, on Shikinejima Island, Japan, where an iron- and hydrogen-rich hot spring mixes with sulfate- and oxygen-rich seawater along its outflow, and so over short spatial scales recapitulates the range of conditions present in Earth's oceans over the Proterozoic eon, in which the oceans transitioned from oxygen-poor and iron-rich to modern conditions low in iron but rich in sulfate and oxygen. Appendices 2 and 3 tie in closely to Chapters 3 and 4; these appendices describe the field sites from which the metagenomes analyzed in

Chapter 3 were sampled, and the use of these field sites as analogs of ancient environments is complementary to that described in Chapter 4. Appendix 4 describes genomic sequencing efforts of diverse nonphototrophic Chloroflexi, expanding representation of sequenced members of this phylum to span all of the described classes, and uncovering previously unrecognized metabolic diversity. This ties in closely to Chapter 3, and together these two sections greatly improve our understanding of the metabolic diversity of previously understudied lineages of Chloroflexi. Appendix 5 discusses the evolution of oxygenic photosynthesis in Cyanobacteria, using cross-calibrated relaxed Bayesian molecular clocks to date the radiation of crown group oxygenic Cyanobacteria ("Oxyphotobacteria") and their divergence from their recently-discovered nonphototrophic sister clade Melainabacteria. This analysis demonstrates that oxygenic photosynthesis must be fairly young, less than ~2.5 billion years old, consistent with estimates for the timing of the GOE estimated in Chapter 1. This study also demonstrates that the crown group radiation of Oxphotobacteria occurred ~2 Gya, and that the GOE must therefore have been driven by stem group lineages, highlighting the importance of extinct and stem lineages in powering the ancient Earth. Appendix 6 is a companion to Chapter 5, and describes innovative modeling of oxygen solubility in low-temperature liquid brines on Mars, demonstrating that under a range of Martian conditions today and in the past oxygen may accumulate to substantial concentrations, high enough to support respiration by bacteria and potentially even simple multicellular animals like sponges.