Conclusions

This thesis presents studies on two topics in environmental microbiology: formate dehydrogenase gene diversity in lignocellulose-feeding insect gut microbial communities and the biological determinants of hydrogen isotope composition in bacterial lipids. Studies made on formate dehydrogenase gene diversity have implications for autotroph microbiology, the global carbon budget, and the evolutionary biology of symbiotic gut microbes. Studies of the biology underlying bacterial lipid hydrogen isotope content enhance knowledge of the basic biology behind cell composition and the impacts microbes have on their surroundings. I discuss specific conclusions for the topics separately in the remainder of this section.

Topic I. Formate dehydrogenase gene diversity in lignocellulose-feeding insect gut microbial communities

My research on formate dehydrogenase genes aims to clarify the microbial ecology of symbiotic acetogenic spirochetes inhabiting the guts of lignocellulose-feeding insects (Chapters 2-4). These bacteria perform an activity that provides significant benefit to the nutrition of their insect host and, by way of insect abundance, also impact the global carbon cycle. Most acetogenic spirochetes in lignocellulose-feeding insects belong to the genus *Treponema*. They are genetically diverse and likely occupy different environmental niches in termite guts. However, the nature of the niches and metabolisms associated with uncultured acetogenic spirochetes are unclear.

The work presented herein aims at revealing novel aspects of acetogenic spirochete microbial ecology. In this thesis, I describe studies in which I used the gene sequence for hydrogenase-linked formate dehydrogenase (FDH_H), a key enzyme in the acetogenic metabolism of the termite gut isolate, *Treponema primitia*, to explore the diversity, evolution, and activity of uncultured acetogenic spirochetes.

In Chapter 2, I used novel degenerate primers to establish that FDH_H genes are diverse, are encoded by uncultured acetogenic spirochetes, and can be broadly classified into selenium-dependent (Sec) and selenium-independent (Cys) enzyme clades in evolutionarily primitive wood-feeding insects (i.e, lower termites and a wood-feeding roach). Phylogenetic patterns imply that acetogenic spirochete communities existed in the last common ancestor of wood-feeding termites and roaches, and, moreover, harbored genes for both Sec and Cys enzyme variants. These results provide the first wide-scale evidence suggesting that selenium, a trace nutrient, may play a long-term role in shaping the genetic and metabolic capacities of diverse acetogenic spirochetes, and, as a consequence, impact acetogenesis, the primary H_2 sink in lignocellulose-feeding insect guts.

In Chapter 3, I present the diversity of FDH_H genes in higher termites, the most species rich and numerically abundant group of termites on earth, and show evidence for major evolutionary shifts within gut communities during termite evolution. Phylogenetic analysis indicates only a single lineage of Sec FDH_H is present in higher termites; all Cys clade and most Sec clade FDH_H genes were apparently lost from the FDH_H gene pool in

higher termite gut microbial communities. I also discovered multiple instances of selenium-independent FDH_H reinvention in two higher termite species. Finally, I identified a novel FDH_H group specific to termites that have lifestyles characterized by high soil exposure. Taken together, FDH_H phylogeny shows strong evolutionary trends that are consistent with an evolutionary bottleneck, convergent evolution, and recent symbiont invasion/acquistion having occurred in gut communities during the evolution of higher termites. I hypothesize that the extinction of cellulolytic protists in a progenitor higher termite may be an important determinant of gut community structure and gene content in extant higher termites.

In Chapter 4, I present a study in which I utilize gene inventory, high-throughput sequencing, and microfluidic digital PCR techniques to identify spirochetes responsible for significant proportions of FDH_H gene transcription in the gut microbial community of a lower wood-feeding termite. Transcriptional assessments indicate two *fdhF* phylotypes account for the majority of FDH_H gene transcription in the termite gut. I then use microfluidic digital PCR to discover the specific 16S rRNA ribotypes of organisms encoding important *fdhF* phylotypes. The results from this study (i) imply acetogenesis in termite guts may be largely driven by a few species of acetogenic spirochete and (ii) provide a framework for more targeted environmental transcription and single cell analyses of important uncultured termite gut acetogens.

Studies of formate dehydrogenase gene diversity described in this thesis provide a platform for future investigations of the microbiology underlying termite gut acetogenesis, a globally relevant process. I outline two possible studies below:

(i) The results from Chapter 2 suggest that variations in selenium concentration may influence transcription and acetogenesis rates in termite gut microbial communities. This hypothesis can be tested with filter paper feeding experiments (paper is dosed with different amounts of selenium and fed to termites), traditional $^{14}CO_2$ fixation assays, and RNA-Seq or other transcriptomic techniques. As shown in Chapter 4, microfluidics can then be used to elucidate the ribotypes of the most transcriptionally active bacteria for each selenium treatment. 16S rRNA sequence data then enable further targeted studies that can include FISH and whole genome amplifications of important gut bacteria.

(ii) Studies of *T. primitia* and termite gut communities suggest selenium availability may be limited in termite guts. Possible influences of availability in guts include selenium concentration and redox state. With regard to concentration, the trace element content of woody biomass should be fairly depleted relative to other forms of plant biomass. This suggests that dietary selenium may pose a challenge for selenium utilizing termite gut microbes. Redox state may be another factor influencing availability. Selenium appears in nature in a variety of redox states (Se^{2-} to Se^{6+}), some of which have very low biologically availability (e.g., iron selenides, elemental Se). The presence of steep radial redox gradients in termite guts therefore implies selenium redox state, and thus bioavailability, may vary spatially and impact the activity (and genome content) of motile

microbes. A follow-up study could explore these hypotheses. Selenium levels and redox state in food substrates and termite hindguts could be determined with inductively coupled plasma mass spectroscopy and micro-X-ray absorption spectroscopy. These measurements should yield insight on why both Sec and Cys FDH gene variants are present in lower termite guts and why most higher termite guts only harbor Sec FDH variants.

Topic II. Hydrogen isotope content of bacterial lipids

The second topic of this thesis focuses on elucidating the biological basis for hydrogen isotope content in lipids. I show in Chapter 5 that lipid D/H varies systematically with different pathways of central metabolism in bacteria. I propose lipid D/H is controlled by NADPH, a key metabolite used for lipid biosynthesis, and the different pathways by which NADPH is synthesized in cells. This hypothesis can be tested with cultures of bacterial mutants in future studies. If such studies support NADPH production as a key determinant of lipid D/H, lipid D/H may constitute an isotopic marker for energy metabolism and prove as useful to microbiologists, geobiologists, and organic geochemists as ^{13}C-based indicators of carbon fixation.