MBL Summer Course 2015, Final Course Report
How do bacterial interactions influence ecosystem development?
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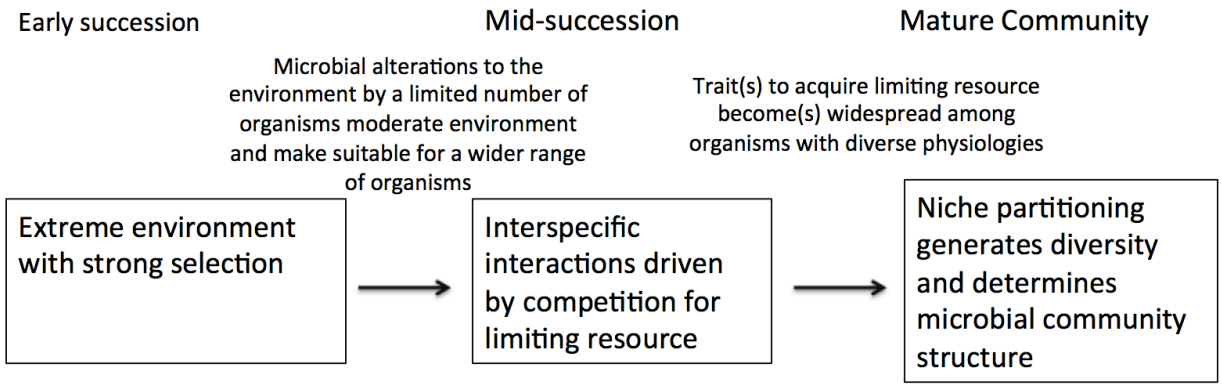
Introduction

Microorganisms are fundamental in regulating the earth's biogeochemical cycles, yet the processes that govern variation in microbial community structure across space and time remain poorly understood. Model systems drastically reduce ecosystem complexity and allow for high resolution examination into the processes that generate temporal variation in microbial community structure. Here, I attempt to address a central question in ecosystem ecology using a four species cheese rind system (Button and Dutton 2012, Wolfe et al 2014, Wolfe and Dutton 2015) undergoing succession, *How do microbial species interact to generate successional patterns in microbial community structure and ecosystem change?*

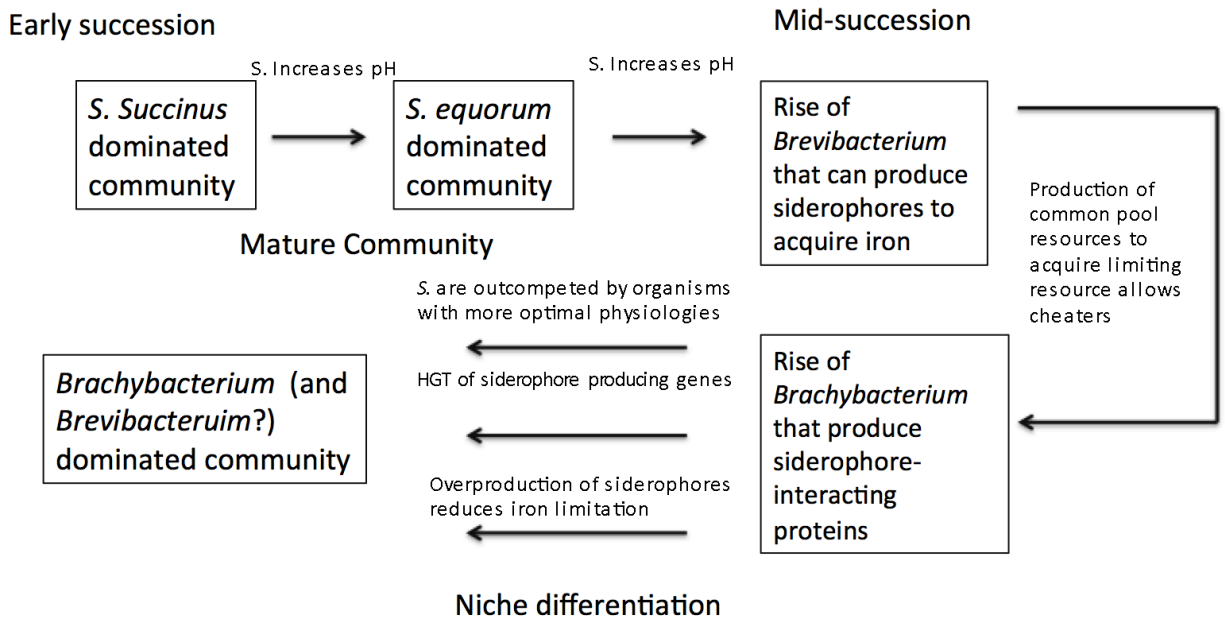
Using this dataset, I propose to evaluate a three-stage conceptual model that I have developed of microbial succession patterns in ecosystem development. The model suggests that the relative importance of interactions between microbial communities and the environment vs. interspecific interactions within microbial communities varies across time (Figure 1). I hypothesize that microbial alterations to the environment are crucial during early successional periods in which extreme environmental conditions persist. These microbial feedbacks on the environment are produced by a limited number of species and moderate the environment for later successional species. In a more temperate environment, microbial communities enter a second stage of succession in which a great number of species can exist, and interspecific competition is induced by growing population density and by strong resource limitation. During this stage, microorganisms with the ability to acquire limiting resources may be in direct competition for niche space and display a variety of life strategies including the use of quorum sensing or the exudation of compounds to physically interact with other organisms or to scavenge nutrients. Finally, as succession progresses, direct competition between organisms should lessen with increasing niche partitioning and community stability. Additionally, traits to acquire limiting resources should be selected for and become widespread in mature communities, either via immigration or horizontal gene transfer amongst phylogenetically distinct organisms. As such, microbial community composition in mature systems may be determined by physiological attributes of organisms and ability to occupy niche space rather than by interactions between organisms.

Figure 1. Ecological framework for organismal interactions across succession



Within cheese communities, pH and iron availability are thought to be key environmental variables governing the structure and function of microbial communities through time (Lawrence et al 1987, Monnet et al 2012, Watkinson et al 2001). Microbial succession of cheese rind communities is well-defined, with *Staphylococcus Succinus* and then *S. Equorum* as the primary bacterial early colonizers, followed by *Brevibacterium aurantiacum* and then *Brachybacterium alimentarium* (Wolfe et al 2014). Yet, the mechanisms by which this progression occurs remains unexplored, and I expect succession in this system to follow the ecological framework detailed above (Figure 2). As such, early colonizing *Staphylococcus* species should impact microbial community structure via raising the pH of cheese curd and creating more neutral and favorable pH conditions for later colonizing organisms. Consequently, *Staphylococcus* species should exhibit a negative feedback on their own abundances while positively impacting the abundance of other species that is preceded by changes in the environment. *B. aurantiacum* should then able to thrive at more neutral pH, as they produce siderophores that allow them to acquire iron, a putative limitation on growth in this system. However, siderophores are able to be utilized by a wide range of organisms, and *B. alimentarium* may increase in abundance through its ability to scavenge iron from siderophores produced by *B. aurantiacum*. As succession progresses, I expect of for interactions between *B. aurantiacum* and *B. alimentarium* to reduce iron limitation and/or generate sufficient functional redundancy for a more stable community structure that is dominant by *B. alimentarium*, who may be more physiologically suited for environmental conditions. This process may be mediated either by horizontal gene transfer of the genetic basis for siderophore production from *B. aurantiacum* to *B. alimentarium* or by an excess production of siderophores by *B. aurantiacum* that reduces iron limitation.

Figure 2. Predicted interactions in cheese rind communities across succession



Together these effects would aid in informing expectations of the importance of microbial community structure and function during ecosystem development (Wolfe et al 2014). For example, if microbial feedbacks are critical to shaping ecosystem parameters during early succession, the identity and physiology of early arriving organisms could help predict changes in ecosystem function. As such, stochastic variations in early colonizers generated by dispersal and/or ecological drift may create persistent effects on ecosystem development. Moreover, if interactions between organisms are most important during middle succession, high resolution data on microbial activity may be most valuable in explaining ecosystem function during this period. Lastly, during late succession, the phylogenetic spread of functional attributes (i.e., distribution across species) rather the abundance of such traits may be reflective of niche space and correlate with rates of ecosystem functioning. Understanding such dynamics is imperative to predicting ecosystem responses to disturbances in a rapidly changing planet.

Methods

To examine this dynamic, I conducted a temporally explicit experiment to monitor the abundance and activity of bacterial species through cheese rind succession as well as corresponding changes in pH, oxygen, and redox conditions within the cheese environment. Model communities were constructed on sterile cheese agar plates by inoculating with four strains of bacterial isolates present in fully developed cheese rind communities (*S. Succinus, S. Equorum, B. aurantiacum, B. alimentarium*). Cheese media was made using 100 g dried Bayley Hazen Blue cheese curd, 5 g xantham gum, 30 g NaCl, 17 g agar, and 1 L water then autoclaved for 15 minutes at 121 degrees C. Each species was inoculated at a concentration of 50,000 CFU/plate for a total of 200,000 CFU/plate. Plates were incubated at room temperature for 72 hr to allow biofilm formation. DNA and RNA were extracted from microbial communities every 24 hr for 10 days using Maxwell 16 LEV simplyRNA extraction kits (Promega Corp., Madison, WI,

USA) and 300-500mg biofilm following manufacturers instructions. DNA extractions were eluted in 50 uL TE, and RNA extractions were eluted in 50 uL nuclease-free water. pH, oxygen, and redox conditions were measured at each time point using microelectrode profiling (probes: OX-50, pH-N, pH-100, and RD-50, Unisense A/S, Aarhus, Denmark). All samples were obtained from triplicate biological replicates as well as triplicate technical replicates.

qPCR primers for each bacterial species were designed using Primer3web (http://primer3.ut.ee/) and the 16S rRNA gene sequences of each strain. Primers were designed for an annealing temperature of approximately 60 degrees C, and primer sequences were verified for specificity against other species using nBLAST (NCBI Database). Temperature gradient PCR using each primer set and each strain type was performed to verify primer specificity at annealing temperatures ranging from 57-62 degrees C. qPCR was performed in triplicate with one primer set that yielded specificity for both *S.* species using 2 uL of sample and 48 uL of master mix recipe listed in the microbial diversity handbook with an annealing temperature of 58 degrees C. Thermocycling regimes for both PCR and qPCR reactions are listed in the microbial diversity handbook. Transcriptomic sequences in two samples were obtained using Illumina MiSeq high throughput sequencing but sequences were not returned before the end of the class.

Results

Only pH substantially changed in cheese rind systems through time (Figure 3 and 4). However, several calibration curves were re-constructed at the end of the microprofiling time points, and these samples were excluded from analysis for the purpose of this report. Redox profiles (not shown) were variable and biofilm location was unclear within each profile. pH changed most drastically during early succession and stabilized during later succession, while oxygen appears stable during late succession. Oxygen profiles depicted microbial cheese rind communities becoming more anoxic towards the agar base, suggesting spatial structuring of cheese rind microbial communities, with stronger trends during late succession (Figure 5).

Figure 3. Changes in pH across succession.

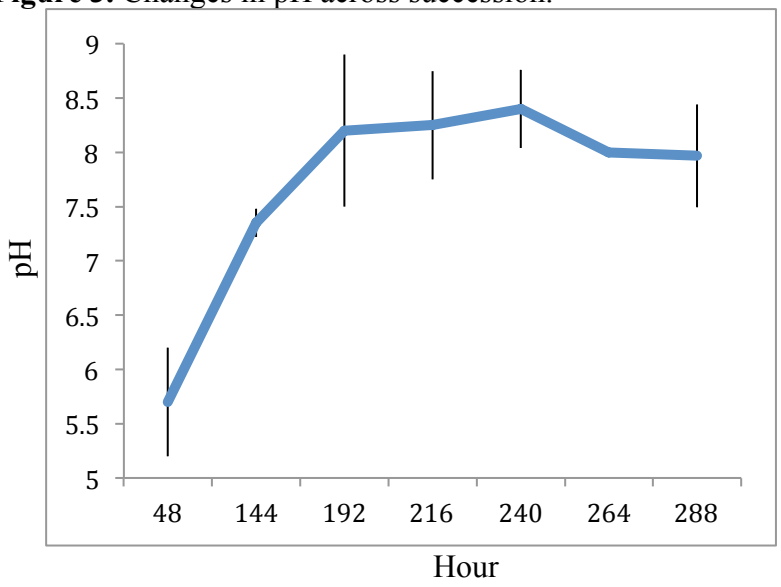


Figure 4. Changes in oxygen concentration across succession.

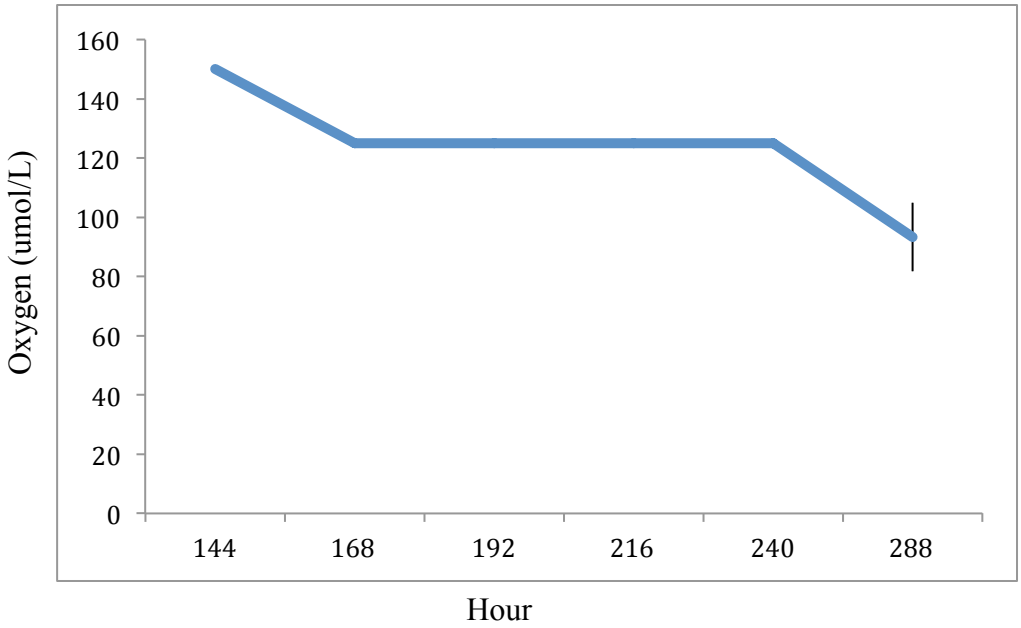
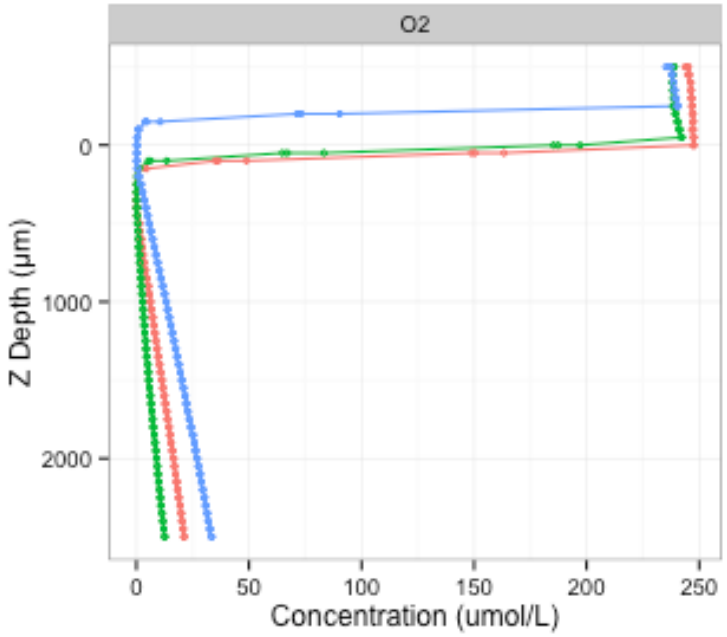


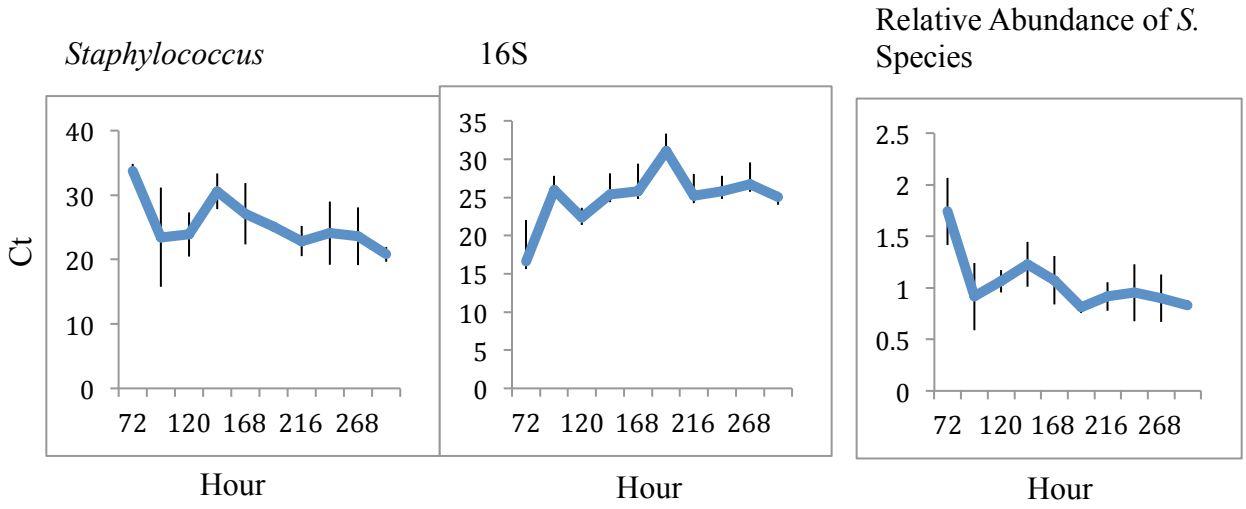
Figure 5. Oxygen profile at 168 hr.



PCR primers for each species were mostly non-specific and not sensitive to temperature gradients (Figure 6). However, the primers designed for S. Equorum were specific to both S. species but not actinobacteria at an annealing temperature of 58 degrees C. This primer set, as well as 16S universal primers, were used for qPCR (Figure 7). Although the standard curves, did not amplify for either primer set, *S.* species appeared to decrease in relative abundance across succession (Figure 7).

Figure 6. Representation of Temperature Gradient PCR gel in which primers for each species tended to anneal to all species at all temperatures.

Figure 7. qPCR of Staphylococcus species.



Conclusions and Future Work

Although my current results are incomplete, my post-course research will center on finishing molecular work for the samples collected during this course and analyzing data across the full time series. I will prep RNA and DNA libraries for metatranscriptomic and 16S rRNA gene sequencing, and then analyze correlations between changes in the environment, microbial community structure, and microbial gene expression using emerging pipelines for processing high throughput sequence data in combination with multivariate statistics. Importantly, the genomes for all organisms in this system have been fully sequenced, allowing gene transcripts to be assigned to the organisms from which they were produced. This approach will serve to

determine the temporal relationships between environmental conditions and microbial community structure and uniquely generate robust and data-driven inferences on the mechanisms producing these changes at the level of individual species. My project represents an unusual opportunity to apply my expertise in environmental and community ecology at an orgnanismal level with the assistance of an established molecular geneticist. My goal is to use extend this into a post-course project to produce a paper that integrates metatranscriptomics with ecological theory and furthers Dr. Dutton's attempts to to bridge the gap between traditional *in situ* and *in vitro* scientific approaches.

Acknowledgements

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