

EFFECT OF COMMUNITY INTERACTIONS IN SCALING OF MICROBIAL PROCESSES

Overview: Understanding the mechanistic basis of how ecosystems respond to global environmental change is a pressing question in ecosystem ecology. Many emergent ecosystem functions are driven by microbial physiology, and models of ecosystem function are beginning to incorporate variation in phenotype and genotype of microbes at community and population scales^{1,2,3}. However, mechanistic models that predict ecosystem function from microbial physiological traits have largely ignored the influence of community interactions⁴. Community interactions may be an important predictor of ecosystem function if they result in community-average physiology that is non-additive, meaning that observed community-scale physiology is not equal to the average of individuals. My **research goals** are to determine whether community interactions lead to non-additivity in community-average traits of microbes and to quantify the influence of environmental conditions on the strength and direction of this

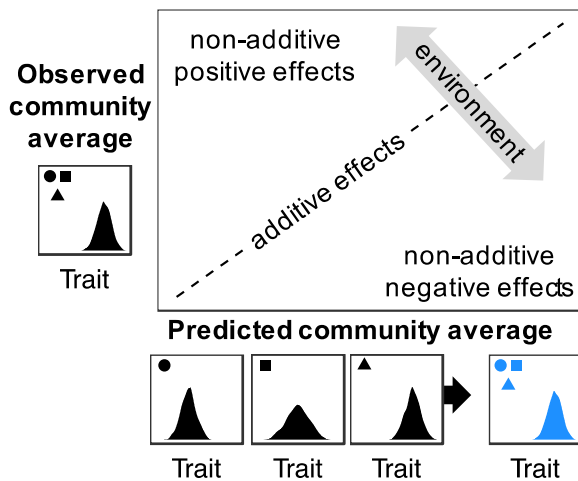


Figure 1. Framework for establishing Rule of Life, where black colored distributions represent data and blue colored distribution is model generated: Community interactions influence the scaling from individuals to ecosystems if community average traits are non-additive. The strength and direction of the non-additivity are dependent on the environment.

non-additivity (Fig. 1). Answering these questions will help to establish a **Rule of Life (RoL)** for how microbial responses at individual, population, and community scales influence ecosystem function and will provide a mechanistic basis for prediction under novel environmental change. To do so, I will investigate community interactions of leaf litter microbes (microbes in dead leaves and grass) in the context of drought as a model system. This system is ideal to develop this RoL because microbial isolates have been previously cultured in which we can observe physiological traits^{5,6}, there is theory for how community interactions may influence physiology under drought scenarios^{3,7}, and drought is expected to change in both frequency and intensity with global change⁸. I will use a combination of empirical and theoretical approaches in both the laboratory and the field to test **two specific hypotheses** about leaf litter community interactions and the role of drought: **Hypothesis 1)** Observed

community-average traits of leaf litter microbes (growth, extracellular enzyme activity, and stress tolerance) are not equal to the average of individuals. **Hypothesis 2)** Positive non-additive effects are more prevalent under moderate drought scenarios. This research will increase our understanding of hierarchical biological responses to environmental change and make specific predictions for how Mediterranean grassland ecosystems may respond to drought. Finally, training from Dr. Steven Allison at the University of California Irvine (UCI) will prepare me for a career as an independent scientist, teacher, and mentor. Through my **training plan**, I will expand my expertise in microbial ecology and carbon biogeochemistry, advance my computational and modeling skillset, and gain additional experience in inclusive teaching and mentoring.

RESEARCH PLAN

Background: Microorganisms affect ecosystem carbon cycling through resource assimilation into biomass during growth⁹. As microbes grow, they must also invest energy towards acquiring more resources and maintaining their cells against stress³. Finite resources mean that microbes must invest differentially in growth, resource acquisition, and stress tolerance, leading to physiological tradeoffs that

have consequences for ecosystem rates of decomposition^{10,11}. Previous work has provided robust frameworks for modeling microbial decomposition as a function of community composition (genotype) and physiological traits (phenotype)^{2,3,12}. However, data used to parameterize these models are often collected at the population scale, and the effect of community interactions on the expression of physiological traits is still uncertain. Shared use of extracellular enzyme products is one example of community interactions that may lead to changes in community-average traits and rates of leaf litter decomposition. To acquire resources, microbes often produce extracellular enzymes, which convert complex molecules into smaller components that can be used for growth and maintenance¹³. However, enzymes are energetically costly¹⁴ and access to products by all community members may lead to “cheating”, or using products without paying the costs¹⁵. Thus, interactions between producers and cheaters have the potential to alter resource acquisition strategies and influence the strength and direction of non-additivity in community-average traits.

Drought is hypothesized to affect community interactions, and thereby the non-additivity of community-average traits and ecosystem function, by influencing the spatial structure of leaf litter^{7,15}. In lab cultures, high moisture conditions result in resource pools that are well-mixed, and the availability of extracellular enzyme products promotes cheating to the point of producer collapse¹⁶. At the field scale, community interactions are difficult to observe, but community composition has been shown to influence decomposition responses to drought^{17,18,19}. Understanding the mechanistic basis of how community interactions influence ecosystem function is key to expanding microbial genotype-phenotype relationships to consider the expression of microbial traits across population, community, and ecosystem scales.

Hypotheses and Approaches: I will use empirical and theoretical approaches in both the laboratory and field to establish a RoL for how community interactions contribute to non-additivity in community-average traits and are influenced by environmental conditions (Fig. 1). My research will be organized into two specific hypotheses, each of which aims to link variation in microbial populations to physiological traits across multiple scales of biological organization.

Hypothesis 1 Observed community-average traits of leaf litter microbes (growth, extracellular enzyme activity, and stress tolerance) are not equal to the average of individuals. Interactions in the microbial community feedback on physiology by affecting the benefit of enzyme production^{15,16}. This feedback may result in differences in physiology when microbes grow in communities versus in isolation, leading to non-additivity in community-average traits. I will test this hypothesis using empirical and theoretical approaches (Fig. 2).

Approach 1.1 (empirical test): I will use data at the population scale to predict community-average traits and compare predictions to observations of experimental consortia. I will measure physiological traits of 50 ecologically important, abundant, and previously cultured leaf litter microbes^{15,16} in both monocultures and consortia. To mimic resource availability in natural ecosystems, all physiological measurements will be conducted in incubations of leaf litter substrate at non-limiting moisture conditions (50% leaf litter water-holding capacity) for Loma Ridge, CA, a Mediterranean grassland site. I will estimate microbial physiological traits as described in Malik et al. 2020³. To estimate growth, I will measure leaf litter mass loss, rates of respiration via headspace sampling and gas chromatography, and microbial biomass via cell counts and flow cytometry. To estimate investment in resource acquisition, I will measure extracellular enzyme activity via fluorometric and colorimetric assays. Lastly, to estimate stress tolerance, I will measure osmolytes using liquid chromatography-mass spectrometry. To test for non-additivity in observed community-average traits, I will randomly assign microbial isolates to 25 unique experimental consortia, each containing 10 isolates with every isolate represented in 5 consortia. Incubations will be inoculated with approximately equal relative abundances⁵. Consortia composition will be measured using 16S rRNA sequencing at the beginning and end of the incubations to determine any changes in relative abundance. Traits of both monocultures and consortia will be normalized by cell counts to be comparable. I will predict community-average traits of the 25 experimental consortia using the average of monocultures weighted by consortia composition. I will

compare predicted community-average traits to observed and determine non-additivity based on deviation from the 1:1 line using the root mean square error (RMSE), calculated from the difference between predicted and observed. All statistical analyses will be completed in R²⁰.

Approach 1.2 (theoretical test): To determine the effect of non-additivity in predictions from a trait-based model of microbial function, I will compare empirical results from Approach 1.1 to simulation results from a model of leaf litter decomposition, DEMENT^{2,21}. DEMENT is a spatially explicit process-based model that simulates the assembly and function of diverse microbial communities on a 100 X 100 lattice grid. DEMENT traces substrates, enzymes, products, and microbial membership through time in which each microbe has an assigned genotype and associated phenotype (physiological trait values). DEMENT incorporates community interactions implicitly via shared use of resource pools and can be initialized with different community compositions and different environmental forcings (moisture, temperature, and initial resource pools). As a test of the effects of community interactions on community-average traits, I will perform DEMENT simulations in two ways. First, I will predict community-average traits of the 25 consortia by initializing the model to simulate dynamics of the entire consortia. Next, I will simulate population scale traits by running the model 50 times, each initializing with a single

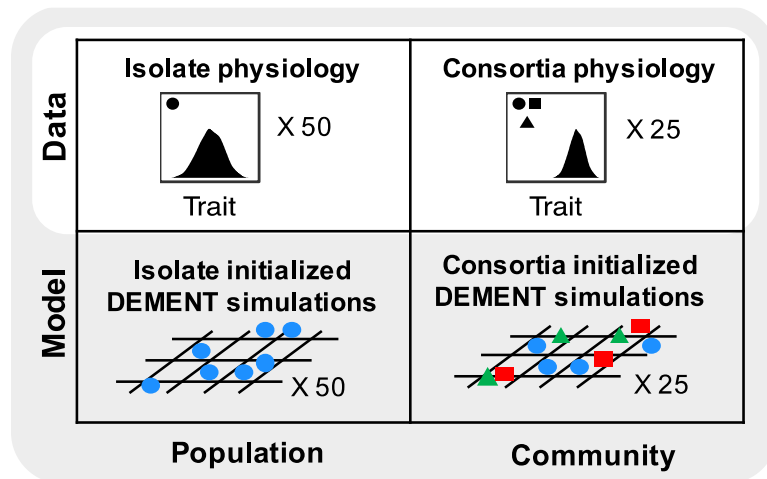


Figure 2. Methodology for testing Hypothesis 1, where Approach 1.1 is denoted in white and Approach 1.2 in grey.

different isolate. I will then calculate community-average traits for the 25 consortia from the average of the 50 isolate simulations based on consortia composition. All model simulations will be conducted in reproducible, executable R and bash scripts with archived parameter values, environmental forcings, and initial conditions. I will compare simulation results to each other as well as to lab-based consortia observations from Approach 1.1 using linear models. I will use the RMSE of the comparison to determine the strength of non-additivity and fit to empirical data.

Hypothesis 2) Positive non-additive effects are more prevalent under moderate drought scenarios.

Moderate drought conditions increase the spatial structuring of leaf litter and decrease opportunities for cheating, allowing for coexistence of cheaters and producers and facilitation²². Conversely, at high moisture conditions, producers are negatively influenced by cheating and, at high drought scenarios, spatial structuring is so extreme that interactions are rare (Fig. 3). I will test the effect of drought using both lab-based and field-based data.

Approach 2.1 (laboratory test): To determine the effect of drought on community interactions and the non-additivity of community-average traits, I will repeat the experimental design of Approach 1.1 with two additional moisture treatments representing moderate (25% water-holding capacity of leaf litter) and low (5%) moisture scenarios. I will generate a linear model of the observed ~ predicted relationship across all three moisture scenarios and determine whether the residuals of the relationship are affected by moisture using an Analysis of Variance test (ANOVA, Fig. 3). Next, I will run additional consortia simulations of the DEMENT model under these scenarios of high and moderate drought. To quantify community interactions in the model, I will test for neutrality and generate interaction potentials of the simulated consortia using the timeseries of microbial abundances^{23,24}. I will determine how the strength and direction of the interaction potentials differ between drought conditions using an ANOVA test and compare interaction potentials to the residuals of the empirical data using linear regression.

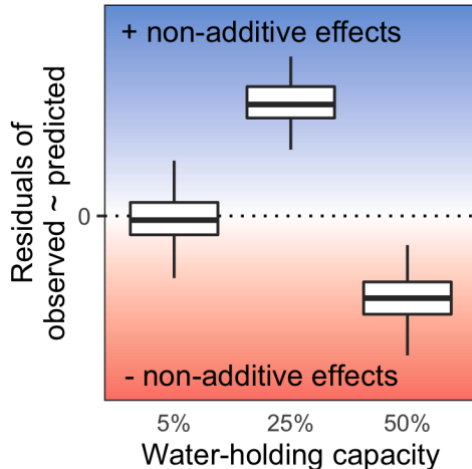


Figure 3. Predicted effect of drought on community interactions and non-additivity. Strength and direction of non-additive effects denoted by divergence of residuals from zero.

Approach 2.2 (field test): To test for the effect of drought at the field scale, I will update the DEMENT model to reflect community interaction potentials from Approach 2.1 and compare simulations to field data collected from the Loma Ridge Global Change Experiment in Southern California. The Loma Ridge Global Change Experiment was established in 2007 to study the effects of precipitation and nitrogen deposition on soil and leaf litter microbial communities and function. To determine the influence of drought, subplots have been treated with two precipitation regimes (ambient and 40% reduction) and microbial community composition has been sampled 3-4 times per year for the last 10 years^{3,25}. I will explicitly parameterize interaction potentials in DEMENT by allowing physiological traits to vary based on community composition at previous time points. Further, interaction potentials from consortia will be translated to complex field communities using statistical models to predict physiological traits of unmeasured genotypes based on

phylogenetically constrained trait distributions⁶. I will use forcing data from Loma Ridge and community composition at the first time point to simulate community-average traits and ecosystem function for each precipitation treatment over the last 10 years. To test for the effect of drought on community interactions and non-additivity, I will generate interaction potentials from both the simulated leaf litter community and the longitudinal timeseries data, compare interaction potentials of microbial communities across treatments, and determine model fit.

Significance, Intellectual Merit, and Fit to Competitive Area: The proposed research will test a RoL for how microbial interactions influence ecosystem function and will also make specific predictions for how Mediterranean grassland ecosystems may respond to drought using data collected across scales. Thus, results will inform both fundamental and predictive understanding of biological processes responding to climate variation. This research will become increasingly important as drought continues to change in both frequency and intensity with climate change^{8,26}, and the process-based modeling approach will allow for prediction even when empirical observations are not available²⁷. Further, my conceptual framework, which incorporates empirical and theoretical approaches across population, community, and ecosystem scales, can be translated to other systems and will further our general understanding of hierarchical biological responses to abiotic environmental change. I am well-qualified to conduct this research on the basis of my previous expertise in dynamic modeling, bioinformatics, and microbial community analyses, and the training I will receive from the sponsoring scientist (Dr. Steven Allison, Institution: UCI), whose lab is at the forefront of mathematical modeling of microbial communities and ecosystem functioning.

TRAINING AND CAREER DEVELOPMENT

Training Objectives and Plan: Basic research in ecology and biogeochemistry is imperative for understanding global change, but often does not address the impacts of global change that disproportionately affect low-income communities of color. My research and training plans are specifically designed to reconcile these two points by combining basic research that can inform policy with mentoring and outreach efforts aimed to increase access to education and research for people from minoritized communities. I will do so with three specific training objectives: 1) Expand my expertise in microbial ecology and carbon biogeochemistry, 2) Advance my computational and modeling skillset, and 3) Gain additional experience in inclusive teaching and mentoring, especially for supporting minoritized students

such as those identifying as Black, Indigenous, and People of Color (BIPOC) and women. To expand my expertise and advance my computational and modeling skillset (objectives 1 and 2), my research plan is designed to provide me with experience in a new microbial system and in incorporating data at all levels of organization, from isolates to natural communities, to predict ecosystem-scale processes. Specifically, my plan will include training in microbial culturing and data generation, parameter selection, and model validation, with an additional focus on partitioning uncertainty between data and process sources. My approach to model development, which equally emphasizes appropriate data generation and uncertainty, will equip me for leading my own research program as I will gain experience translating conceptual models to quantitative, predictive models. Further, it will provide a strong test of the hypothesized Rule of Life and will make specific predictions about drought responses in Southern California. To increase my teaching and mentoring capabilities to serve people from previously excluded communities, I will organize and lead a mentoring program outlined in the **broader impacts** section.

Career Development and Future Research Directions: My proposed research and training plan will provide me the necessary tools for a successful career in academia. I am fascinated by the complexity of microbial communities and I aim to lead a research program which makes inference at the intersection of microbial and ecosystem ecology with both data-driven and theory-driven approaches. Training as a postdoctoral fellow will greatly expand my conceptual understanding of the link between microorganisms and ecosystems and will allow me to develop an interdisciplinary research program that utilizes laboratory generated physiological data to inform ecosystem models. Further, I will be conducting this research in soil ecosystems with a focus on decomposition, which diverges from my dissertation research studying methanogenesis in freshwater lakes and will help me to develop generalizable understanding about how microbes influence function. Finally, as a professor, I plan to lead a research group that values research, teaching, and mentorship as equal tenets for a successful career. Training during a postdoctoral fellowship will equip me to be a more inclusive and effective teacher and mentor throughout my career.

Justification of Sponsoring Scientist and Host Institution: Dr. Steven Allison's group is an ideal place to pursue this research because of his expertise in leaf litter decomposition and process-based modeling, his history of collaborating in the global biogeochemistry community, and his commitment to inclusivity. Additionally, isolates have been previously cultured and genotyped, and methods have been developed within the lab for measuring physiological traits^{3,6}. Further, the faculty at UCI also includes multiple microbial and ecosystem ecologists who study marine and soil ecosystems, allowing for a highly collaborative environment that crosses traditional disciplinary boundaries. This expertise will be key for testing and evaluating a generalizable Rule of Life that is applicable outside of leaf litter ecosystems. UCI also provides the institutional resources to support my research goals, such as high-performance computing through the UCI Research Cyberinfrastructure Center and access to long-term and experimental environmental data at the Loma Ridge Global Change Experiment. Finally, UCI is situated in a diverse collegiate landscape with nearby primarily undergraduate institutions, where efforts to support minoritized students can be most effective.

BROADER IMPACTS

Ecosystem responses to drought have considerable consequences for local communities including lack of access to potable water, increased fire risk, and decreased air quality. My proposed research aims to provide a first principles understanding that can be applied to ecosystem models to better predict future climate change and inform policy decisions. Additionally, as a part of my training plan, efforts to develop my teaching and mentoring capabilities will have substantial impacts towards increasing access to higher education for minoritized communities. Mentorship and outreach have quantifiable impacts on the recruitment of BIPOCs to higher education^{28,29}. I will organize an "Intro to Ecological Research" mentoring program for community college transfer students at UCI. Enrollment of BIPOCs in higher education has been increasing since the 1960s and is primarily driven by community college enrollment³⁰.

However, opportunities for community college students to learn from researchers are lacking, and research careers are often “unknown unknowns”, even for those who later pursue 4-year degrees. At UCI, transfer students represent 31% of the student body and previous informal surveys of UCI undergraduates have indicated a need for additional avenues to learn about undergraduate research. The **goals of this program** will be to increase student sense of belonging, academic confidence, and access to undergraduate research and to provide institutional connections to Biology departments of local community colleges. I will achieve the first goal by conducting bi-weekly group mentoring with transfer students during the Fall and Winter quarters (10 total meetings). I will lead mini-lectures, panel conversations, and career planning activities. Mini-lectures will focus on the impacts of global change and disproportionate impacts on minoritized communities (e.g. coupled pollution and heat wave effects and climate change and fire risk) with reference to current research. Panel conversations with researchers at UCI will allow for skill building and include opportunities to participate in undergraduate research. I will achieve the second goal by conducting outreach events in collaboration with mentees at local community colleges (Irvine Valley College, Orange Coast College, and Saddleback College) in the Spring quarter. I will organize one event per college in which mentees will present on their experiences applying, transitioning, and succeeding at UCI. These events will provide opportunities to showcase mentees’ academic progress and build connections between UCI and surrounding partner schools. Additionally, because financial burdens can be a barrier to participating, I will provide stipends for mentees who participate in outreach events, to be paid from my research fund to cover travel expenses and mentees’ time. To **assess program success**, I will use Likert Scale-based surveys at the beginning and end of each quarter to quantify changes in students’ sense of belonging, academic confidence, and participation in undergraduate research. I will also use these surveys to identify gaps in my mentoring and incorporate changes in the following quarters and in future mentoring and teaching approaches.

TIMELINE

The **anticipated outcomes** of my research include four manuscripts, two associated with each hypothesis. The first will report the effect of community interactions on community-average traits with empirical results from the monoculture and consortia experiments. The second will report results from the theoretical test of non-additivity. The third and fourth will report the effects of drought on non-additivity as tested in the lab and field, respectively. Additionally, my proposed mentoring plan will develop an institutionalized mentoring program to serve UCI Biology transfer students. These outcomes will be **benchmarked** by presenting results of Hypothesis 1 at the Ecological Society of America conference in Aug. 2022, presenting preliminary results of Hypothesis 2 at American Geological Union conference in Dec. 2022, and by submitting an evaluation of mentoring success to the ecology blog Dynamic Ecology.

