

**Title:** Litter Microbial Trait-Based Strategies in Response to Drought

**Authors:** Brianna K. Finley<sup>1\*</sup> (bkfinley@uci.edu),<sup>1</sup> Bahareh Sorouri,<sup>1</sup> Kathleen Treseder,<sup>1</sup> Jennifer Martiny,<sup>1</sup> Adam Martiny,<sup>1</sup> Alejandra Rodriguez Verdugo,<sup>1</sup> Michael L. Goulden,<sup>2</sup> Shi Wang,<sup>2,3</sup> Eoin Brodie,<sup>1</sup> and **Steven Allison**

**Institutions:** <sup>1</sup> University of California, Irvine, CA; <sup>2</sup> University of California, Berkeley, CA; <sup>3</sup> Ecology Department, Climate and Ecosystem Sciences, Earth and Environmental Sciences Area, Lawrence Berkeley National Laboratory, Berkeley, CA

**Project Goals:**

The overarching goal of this project was to better understand how microbial trait-based responses under environmental change – such as drought - influence biogeochemical cycling. Specifically, we combine different genomic methods with field-based manipulations of microbiomes to understand microbial functional responses to drought over two years. This project consists of multiple activities. First, we conducted field-based litter microbiome experiments and laboratory assays of whole microbial communities to measure microbiome community shifts and dispersal contributions to drought responses. Secondly, we will assess microbial demographic changes as well as trait tradeoffs of functional genes under different life history strategies (high growth yield, resource acquisition, and stress tolerance) via metagenomic shotgun sequencing. Lastly, we use knockout approaches to validate the genetic basis of drought tolerance phenotypes observed in our experiments.

**Abstract Text:**

Microbial community shifts under environmental change are shown to influence carbon and nutrient cycling. However, the extent to which evolutionary adaptation, demographic changes, and dispersal affect microbial trait-based responses to global change such as drought and subsequent consequences for biogeochemical cycling are poorly understood. Here, we implemented a two-year field plant litter decomposition experiment within the Loma Ridge Global Change Experiment (LRGCE) to assess effects of drought on microbiome functioning with and without the effects of dispersal. The LRGCE has undergone drought manipulations (roughly 50% rainfall reduction) since 2007 on two adjacent vegetation communities: grass and coastal sage shrub. To remove legacy effects of drought on vegetation and microbiomes, we sterilized leaf litter from both vegetation types and inoculated each litter with microbiomes from unsterilized litter from the ambient precipitation plots. We used 0.2  $\mu\text{m}$  pore nylon mesh “microbial cages” to limit dispersal as well as 2 mm pore microbial cages to allow for dispersal within the ambient and drought plots. After two destructive sampling time points 5 and 11 months after microbial cage deployment, we did not observe a difference in litter mass loss between drought and ambient conditions but found about 25% greater litter mass loss from the coastal sage shrub litter compared to the grass across treatments, as well as an interactive effect between vegetation type and dispersal. From the microbiomes isolated from the field experiment,

we will conduct shotgun sequencing to assess changes in microbiome demographics under drought responses with and without the effect of dispersal. Additionally, we will identify and quantify key functional genes associated with different microbial life history strategies under the YAS framework (high growth yield, resource acquisition, and stress tolerance). This will be conducted both at the metagenomic scale of entire microbiomes, as well as quantify different growth rates of genomes using quantitative stable isotope probing within metagenomes. In order to explore the genomic basis for microbial fitness traits, we are using random-barcoded transposon mutagenesis and sequencing (RB-TnSeq). We have developed pools of tagged transposon mutants in a highly-active litter-degrading bacterium (*Erwinia* LR017) originating from our field evolution experiment. Assays were designed to explore the costs of gene disruptions to the fitness of this bacterium in media containing substrates relevant to litter decomposition, including under osmotic stress representative of drought conditions. This information is then used to provide direct genotype-to-phenotype relationships for improved model prediction of how traits and trade-offs interact to control microbial litter decomposition.

**Funding Statement:** This research was supported by the DOE Office of Science, Office of Biological and Environmental Research (BER), grant no. DE-FOA-0002059.