

## Spatial Turnover of Soil Viral Communities and Genotypes Overlain by Cohesive Responses to Moisture in Grasslands

Christian Santos-Medellin<sup>1\*</sup> (cmsantosm@ucdavis.edu), Katerina Estera-Molina,<sup>2</sup> Mengting Yuan,<sup>2</sup> Jennifer Pett-Ridge,<sup>3</sup> Mary K. Firestone,<sup>2</sup> and Joanne B. Emerson<sup>1</sup>

<sup>1</sup>University of California, Davis; <sup>2</sup>University of California, Berkeley; <sup>3</sup>Lawrence Livermore National Laboratory, Livermore, CA

**Website URL:** <https://nature.berkeley.edu/crosskingdominteractions>

**Project Goals:** The overarching goal of our project is to understand how cross-kingdom and within-kingdom interactions (involving viruses, bacteria, archaea, fungi, protists, microfauna, and plant roots) provide a functional foundation for nutrient cycling in grassland soils. Within this framework, we seek to identify the biotic and abiotic factors that govern the structure, variation, and assembly of viral communities inhabiting these environments. By revealing the conditions under which viral community compositional patterns are and/or are not tightly coupled to their microbial host communities and biogeochemistry, we can begin to unravel the extent to which virus-host interactions impact soil carbon and nutrient cycling.

**Abstract Text:** Soil and rhizosphere microorganisms play key roles in biogeochemical cycling and plant productivity, and by infecting soil microbiota, viruses likely have substantial direct and indirect impacts on these processes. In the oceans, viruses lyse (burst and kill) an estimated 20-40% of microbial cells daily, impacting global ocean food webs, carbon and nutrient cycling, and climate. At  $\sim 10^7$  to  $10^{10}$  viruses per gram, soil viruses may play similarly important roles in terrestrial ecosystems and have been recognized as abundant but virtually unknown members of the soil microbiome.

As part of our large-scale field manipulation study on the impact of reduced precipitation on soil biotic interactions in a Mediterranean grassland, we collected surface (0-15cm) soils from 22 densely-rooted locations distributed across 15 experimental plots that have received treatments of 100% or 50% 50-year averaged precipitation since 2017. Soils were collected twice during the 2020 growing season of *Avena barbata*, the naturalized annual grass that dominates the ecosystem, for a total of 44 samples. To profile dsDNA viral diversity in these samples, we generated 44 viral size-fraction metagenomes (viromes) by separating smaller virions from larger microbes with 0.2  $\mu\text{m}$  filtration prior to DNA extraction and sequencing.

Viral community beta-diversity was most significantly explained by spatial distance, a pattern driven by the steady turnover of viral populations (vOTUs) along a 16 m horizontal transect. This spatial structuring also impacted the genetic microdiversity of the more ubiquitous vOTUs, such that genome sequences from predominant allelic variants tended to diverge with increasing spatial distance. Both viral community composition and the genetic makeup of viral populations exhibited significant distance-decay relationships across the field, highlighting potential

constraints on the spatiotemporal scales of soil viral dispersal and virus-host interactions in these soils.

In addition to spatial structuring, reduced precipitation was significantly correlated with viral community composition, with evidence for more substantial viral responses to current or very recent moisture regimes than to legacy precipitation treatment. Interestingly, many of the vOTUs that were enriched in lower moisture samples were also grouped according to shared predicted protein content in a network analysis, suggesting that viral populations with higher-level genome conservation displayed a cohesive response to reduced moisture content. Furthermore, many of these vOTUs shared genomic features with known actinophages, suggesting that their responses to desiccation could result from interactions with host Actinobacteria, which have been established as drought responsive in prior studies.

In a companion study, we are exploring virus-host dynamics in response to laboratory rewetting of dry soils from four distinct grasslands, including those adjacent to the Hopland field experiment described above. Tens of thousands of vOTUs were recovered from these 10-day laboratory experiments, in which viral communities separated most significantly by soil source and then by dry versus wet soils. For all wet soils analyzed so far, highly diverse viral communities exhibited rapid temporal succession that was reproducible across replicate microcosms, with viromes from 24 hours and 10 days post-rewetting sharing on average fewer than 25% of their vOTUs.

Together, results suggest active and highly dynamic grassland viral communities that seem to respond rapidly to both increases and decreases in soil moisture. The high degree of spatial turnover within one field during one growing season suggests dispersal limitation for most viral populations on scales of meters and months. During the final year of this project, we will focus on further incorporation of host community composition and activity in our analyses to better understand how virus-host interactions contribute to carbon and nitrogen cycling in grassland soils.

**Funding Statement:** This research was supported by the DOE Office of Science, Office of Biological and Environmental Research (BER), grant no. DE-SC0020163. Lawrence Livermore National Laboratory is managed by UT-Battelle, LLC for the U.S. Department of Energy under contract no. xx. This program is supported by the U.S. Department of Energy, Office of Science, through the Genomic Science Program, Office of Biological and Environmental Research, under FWP xx.