

## **Soil Viral Community Composition Differs Spatially and in Response to Wet-up in Mediterranean Grasslands**

Christian Santos-Medellín<sup>1\*</sup> (cmsantosm@ucdavis.edu), Katerina Estera-Molina<sup>2</sup>, Mengting Yuan<sup>2</sup>, Jennifer Pett-Ridge<sup>3</sup>, Mary Firestone<sup>2,4</sup>, and **Joanne B. Emerson<sup>1</sup>**

<sup>1</sup>University of California, Davis, California; <sup>2</sup>University of California, Berkeley, California; <sup>3</sup>Lawrence Livermore National Laboratory, Livermore, California; <sup>4</sup>Lawrence Berkeley National Laboratory, Berkeley, California

**Website:** <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 impacts of drought in a Mediterranean grassland, we harvested rhizosphere-influenced soil samples from 15 experimental plots encompassing two multi-year watering treatments (100% and 50% precipitation since 2017). Collections were performed twice during the 2020 growing season of *Avena barbata*, the annual grass that dominates the ecosystem. To profile the dsDNA viral diversity associated with our 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. By depleting sources of non-viral DNA, this viromic approach facilitates the recovery of a greater richness of viral populations (vOTUs) compared to the recoverable viral diversity from total metagenomes [1, 2].

While precipitation regime and collection time point had significant, albeit minor, effects on overall soil viral community composition, beta diversity trends were largely driven by the location of sampled plots in the field. The observed spatial structuring was defined by a steady turnover of viral populations (vOTUs) along a 16 m transect, with 65% of vOTUs displaying differential abundance patterns impacted by plot position. This distance-decay relationship

highlights potential constraints on the distribution of soil viruses and, possibly, on virus-host interactions at a local scale in these soils. Ongoing characterizations of bacterial and archaeal diversity in these samples should reveal whether the presumed hosts for these viruses exhibit similar spatial patterns, but preliminary data suggest that the observed spatial structuring may be restricted to viral communities.

In a second study, we have focused on rewetting of dry soils, which in Mediterranean ecosystems drives a pulse of CO<sub>2</sub> emissions and a release of inorganic N at magnitudes with global climate implications [3]. Viral activity has been proposed as a contributor to the microbial processes behind this biogeochemical burst, but the specific virus and infected host populations involved have not been identified. To investigate soil viral activity and virus-host dynamics before and after wet-up, while controlling for some of the variation inherent in our field experiments, we have performed laboratory simulations of wet-up. Our preliminary data suggest that viral particle (virion) abundance and diversity are low in dry soils compared to wet soils and, interestingly, that degraded (“relic”) DNA may be more abundant in dry soils. For example, 10 days after rewetting dry grassland soils, we observed a >10-fold increase in the number of viral populations detected in viromes and a >8-fold increase in the number of reads recruited to viral contigs in total metagenomes. This viral bloom seems to be a conserved feature of grassland soils, as evidenced by substantial spikes in viromic DNA yields and decreases in free DNA yields as early as 24 hours after rewetting four compositionally distinct grassland soils. Ongoing temporal surveys will identify the microbial populations and metabolic processes impacted by viral predation during wet-up, along with the relative contributions of dormant viruses and desiccation-resistant virions to these dynamics. Future integration of these results with our ongoing studies of bacterial, fungal, and microfaunal mediation of nutrient cycling will bring us closer to an understanding of viral contributions to terrestrial microbial ecology and biogeochemical cycling.

## References

1. Santos-Medellin C, Zinke LA, ter Horst AM, Gelardi DL, Parikh SJ, Emerson JB. Viromes outperform total metagenomes in revealing the spatiotemporal patterns of agricultural soil viral communities. *bioRxiv*. 2020. , 2020.08.06.237214 *The ISME Journal (in press)*
2. ter Horst AM, Santos-Medellin C, Sorensen JW, Zinke LA, Wilson RM, Johnston ER, Trubl GG, Pett-Ridge J, Blazewicz SJ, Hanson PJ, Chanton JP, Schadt CW, Kostka JE, and Emerson JB. Minnesota peat viromes reveal terrestrial and aquatic niche partitioning for local and global viral populations. *bioRxiv*. 2020. , 2020.12.15.422944 *Microbiome (in review)*
3. Barnard RL, Blazewicz SJ, Firestone MK. Rewetting of soil: Revisiting the origin of soil CO<sub>2</sub> emissions. *Soil Biol Biochem* 2020; **147**: 107819.

**Funding statement:** This research is based upon work supported by the U.S. Department of Energy Office of Science, Office of Biological and Environmental Research Genomic Science program under Award Numbers DE-SC0016247 and DE-SC0020163 to UC Berkeley and UC Davis and award SCW1678 at LLNL. Work at Lawrence Livermore National Laboratory was performed under the auspices of the U.S. Department of Energy Contract DE-AC52-07NA27344.