Infective Viruses and Inert Virions: Illuminating Abundant Unknowns in Terrestrial Biogeochemical Cycles

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Project Goals: The overarching goal of this project is to assess and compare the contributions of active, infectious viruses and degraded, inert viral particles to biogeochemistry across diverse terrestrial ecosystems. Using a multi-omics approach, we seek to establish spatiotemporal patterns in soil viral community composition and activity linked to host carbon and nitrogen metabolism in forests, chaparrals, grasslands, and wetlands. Leveraging a prescribed forest fire and a temperature and atmospheric CO_2 manipulation experiment, we will also explore feedbacks between soil viruses and carbon dynamics in response to environmental change. Finally, through laboratory experiments, we will investigate the composition, fate, and transport of viral particles in soil. By integrating field and laboratory experiments across a variety of soil chemical compositions and spatiotemporal scales, this project will expand our understanding of the soil virosphere and its influence on carbon and nutrient cycling.

Abstract: Viruses have been recognized as highly abundant but poorly characterized members of the soil microbiome. By infecting soil microbes, viruses likely have substantial impacts on terrestrial biogeochemical processes under their hosts' control. Viral particles (virions) may also play more direct roles in soil biogeochemical cycling as packets of carbon, nitrogen, and phosphorous, but the time scales and environmental conditions that determine virion infectivity, transport, and/or sorption to soil particles are unknown. This project uses a combination of field, laboratory, and computational approaches to distinguish between infective and degraded virions and to assess their respective contributions to soil biogeochemical cycling.

With a focus on bacterial and archaeal viruses with dsDNA genomes, we use viral size-fraction metagenomics (viromics) as our primary approach, separating smaller virions from larger microbes via 0.2 μm filtration prior to DNA extraction and sequencing. By depleting sources of non-viral DNA, we have shown that a far greater diversity of soil viral populations can be recovered from viromes, compared to total metagenomes. However, we do not have a firm understanding of what it means, biologically and ecologically, to recover a viral genome in a virome. Was the virion containing that genome produced yesterday or last year, and is it still capable of infecting a new host, or has it decayed past the point of infectivity? Our comparisons of viromes from the same soils prepared with and without a DNase treatment (to remove free DNA prior to virion lysis) are yielding preliminary insights into the conditions and temporal scales over which virions are produced, remain infective, and decay in soil. Interestingly, when a DNase treatment is not applied, this post-0.2 μm metagenomics approach also seems to enrich for free DNA, allowing for the potential characterization of "relic" DNA in the environment.

Coincident with known decreases in microbial activity during the dry season, we find low virion abundance and diversity in dry compared to wet Mediterranean soils, and free DNA seems to be more abundant in dry soils. Though preliminary, these results are reproducible under field and laboratory conditions in a variety of soils, suggesting new virion production during wet-up and

virion decay during the dry season. Ongoing multi-omics investigations are testing the hypothesis that Mediterranean dry seasons are marked by low viral activity and the accumulation of free DNA, while viral blooms occur throughout the rainy season, with the onset of microbial and viral activity in wet soils fueled in part by free DNA and other necromass as substrates.

We hypothesize that high-temperature fires inactivate virions in near-surface soils. Preliminary results from chaparral and woodland habitats at Quail Ridge Natural Reserve, which burned in the California LNU Complex Fires in August 2020, include DNase-treated viromic DNA yields below detection limits throughout the dry season post-fire. Substantial increases in viromic DNA yields after rain suggest that a bloom of new viral particles appeared in these burned soils at the onset of the rainy season, similar to our results from unburned soils. We infer that, at least for viruses, the timing of fire (during the wet or dry season) and the associated soil moisture content are likely to be important for distinguishing the effects of fire from those of desiccation alone. We are continuing to follow soil viral community recovery in these burned chaparrals and woodlands, which experienced wildfire during the dry season. To assess the impacts of fire during the rainy season, common for management, we will analyze soils before and after a prescribed burn in Blodgett Forest (a mixed conifer forest), scheduled to occur in Spring 2021. Laboratory experiments are also being performed to tease apart the relative effects of fire, temperature, and soil moisture content on viral community composition and virion integrity.

The DOE Spruce and Peatland Responses Under Changing Environments (SPRUCE) experiment provides a platform for testing the vulnerability of boreal peat viruses and processes under their control, including host biogeochemical cycling, to elevated temperature and atmospheric CO_2 concentrations. Over the first two years of whole ecosystem warming and deep peat heating (2015-2016), peat viral community composition was significantly correlated with peat depth, water content, and porewater CH_4 and CO_2 concentrations, but not with temperature. In order to more thoroughly assess feedbacks between peat viruses and carbon cycling, we are tracking peat viral community composition and virus-host dynamics over longer time scales in the SPRUCE experiment (through 2022), with a focus on viral predation of methanogens and methanotrophs responsible for CH_4 cycling and release to the atmosphere.

To compare the chemical composition of soil virions, bacteria, and lysed host necromass, we are separating and/or enriching each of these fractions from four different soils (forest, chaparral, grassland, and wetland habitats) for a variety of laboratory analyses. Metabolomics and incubations with ^{32}P radiolabeled nucleotides and orthophosphate will be used to compare the chemical and phosphorous contents of these soil constituents. Virion isoelectric points will be measured with a zetasizer, revealing whether viral particles tend to have isoelectric points above, below, or near the pH of their native soils, indicating the relative degrees of potential sorption to minerals and/or transport within soil hydrological conduits. Soil viral community composition will also be compared across a range of buffer pHs, aggregate size fractions, and soil moisture contents to assign traits to viral populations, which will be tracked across our field experiments.

Results from this project will facilitate a better understanding of viral contributions to terrestrial biogeochemical cycling, both through their infections of hosts responsible for carbon and nutrient cycling and as components of soil organic matter.

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