Title: Evidence for Active, Dynamic Viral Communities in Wet Soils across Habitats

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Project Goals: The overarching goal of this project is to assess and compare the contributions of active, infectious viruses and 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 grasslands, shrublands, woodlands, and wetlands. Leveraging a prescribed forest fire and a peatland temperature and atmospheric CO_2 manipulation experiment, we are also exploring feedbacks between soil viruses and carbon dynamics in response to environmental change. Through laboratory experiments, we are investigating the chemical composition, fate, and transport of viral particles in soil. By integrating field and laboratory experiments across a variety of soil edaphic properties and spatiotemporal scales, this project is expanding our understanding of the soil virosphere and its influence on carbon and nutrient cycling.

Abstract Text: 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 phosphorus, 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 inert virions and to assess their respective contributions to soil biogeochemical cycling.

Using a 'viral size-fraction' metagenomics (viromics) approach, we are exploring the conditions and temporal scales over which virions are produced, remain infective, and decay in soil. We have shown that viromes can recover 1-2 orders of magnitude more viral sequence than total metagenomes, but the temporal scales over which a soil virome might be integrated were unknown at the start of this project. In Mediterranean habitats that experience a dry season, we find that the majority of the viral community seems to decay during seasonal dry-down, such that DNA contained in viral particles is largely undetectable throughout the dry season. In moist soils, results suggest that viral communities are active and dynamic and thus likely represent recently produced virions. However, dry periods early in the rainy season can render virion DNA undetectable within three weeks. Taken together, results suggest that soil viromes tend to capture very recently active viral communities, likely integrated over days to weeks.

Our interpretation that most soil viromes capture a short window of recent activity is consistent with our repeated findings of highly divergent soil viral communities over spatial distances as short as 1 m in nearly all habitats explored thus far. In other words, if viral communities turn over rapidly in time, communities far enough apart to experience dispersal limitation would likely be decoupled in both space and time, thus appearing very dissimilar at a single time point. Centered on a highly spatiotemporally resolved viromic study of the Jepson Prairie grassland (8 locations, 28 time points since November 2020), our ongoing work seeks to unravel the relative contributions of space, time, and dispersal on patterns of soil viral community composition.

We are also performing a suite of laboratory analyses on purified viral fractions to assess the chemical composition, sorption, and transport of soil virions. In soils from three habitats, viral community composition did not differ significantly across a range of buffer pHs for removing viral particles from the soil matrix, suggesting that our viromics protocol is relatively robust to different virion chemistries. We are using a ZetaSizer to determine whether viral particles tend to have isoelectric points above, below, or near the pH of their native soils to assess their potential sorption to minerals and/or transport within soil hydrological conduits. Exploratory imaging analyses in collaboration with EMSL and SSRL, planned for early 2022, are expected to reveal the phosphorus content and chemistry in virions from low-phosphorus wetlands.

To further investigate physicochemical constraints on virion integrity and viral community composition, we are analyzing data from three burned habitats. In shrublands and woodlands that burned during the dry season in the LNU Complex Fires in August 2020, viromic DNA yields remained below detection limits throughout the dry season, increased substantially after rain, and remained high until the following dry season. As these results mirrored patterns observed in unburned Mediterranean soils, we sought to decouple the effects of fire from desiccation alone by leveraging a prescribed burn in a mixed conifer forest that occurred during the rainy season (common for management) in Spring 2021. One week post-fire, viromic DNA yields were lower in burned compared to unburned soils, but this pattern was short-lived, suggesting that the impact of fire on virion abundance may be ephemeral. These preliminary results suggest that the degree of virion inactivation and the timing of viral community recovery post-fire seem to depend on soil moisture and depth. Viromes from these datasets are currently being sequenced, and laboratory experiments are being developed to tease apart the relative effects of temperature and soil moisture 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.

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

Publications

1. ter Horst, A.M., Santos-Medellín, C., Sorensen, J.W., Zinke, L.A., Wilson, R.M., Johnston, E.R., Trubl, G.G., Pett-Ridge, J., Blazewicz, S.J., Hanson, P.J., Chanton, J.P., Schadt, C.W., Kostka, J.E., Emerson, J.B. (2021) Minnesota peat viromes reveal terrestrial and aquatic niche partitioning for local and global viral populations. *Microbiome*. 9:233.

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