Diversity of Viruses in Soil: Do Interactions with Soil Organisms Impact Carbon Flow?

Alexa Nicolas[1]* (alexa_nicolas@berkeley.edu), Evan Starr[1], Erin Nuccio[2], Donald Herman[1, 3], Steve Blazewicz[2], Peter Weber[2], **Jennifer Pett-Ridge[2]**, Jillian Banfield[1,3],and **Mary Firestone[1, 3]**

[1]University of California, Berkeley, California; [2]Lawrence Livermore National Laboratory, Livermore, California; and [3]Lawrence Berkeley National Laboratory, Berkeley, California

Project goals: Understanding the flow and fate of carbon from roots into soil is critical to unravelling soil carbon sequestration. Our project explores the impact of phages on soil microbial communities and ultimately on the fate of carbon in soil. To realize the full extent of the impact of viruses on soil microbial communities and soil carbon flow we have synthesized meta-'omic techniques. These approaches enable capturing a broad diversity of viruses: active viruses revealed through stable isotope genome-resolved metagenomes, RNA viruses mined from soil metatranscriptomes, and filtered double and single-stranded DNA viruses from soil viromes. Additionally, we are optimizing imaging of viral-like particles to pair dynamics quantified from fluorescence imaging with genomic abundances, and to quantify viral contribution to carbon turnover using nanoscale secondary ion mass spectrometry (NanoSIMS). Taken together, analysis of soil viral diversity and dynamics with stable isotope probing will allow us to connect viral populations to carbon flow from roots into soil, microbial community dynamics and the soil food web, with important implications for soil health and agricultural productivity.

Disentangling the complex network of inter-organismal interactions is required to fully understand and manage carbon flow through soil. While viruses outnumber microbial cells in soil, recent analysis of CRISPR spacers suggests upwards of 90% of viruses infecting bacteria (phage) have not been identified. We identified novel viral populations in soil which may impact soil carbon flow, through interactions with and lysis of hosts. Little is known about environmental RNA viruses, especially in soils. By assembling 48 metatranscriptomes from different soil zones – root-influenced, litter-influenced, and bulk soil (no addition) – we resolved highly diverse RNA viral communities from multiple soil habitats. Viruses changed over time and by environmental condition; Narnaviridae and Leviviridae were the most diverse and dominant viral families. Much of the observed viral diversity seemed to parasitize either fungi or Proteobacteria. A mechanistic understanding of how RNA viruses affect host metabolism and impact carbon flow remains to be studied.

Simultaneously, we identified DNA viruses produced through host infections, and recently active DNA viruses. We hypothesize that one role of phage in soil may be to lyse host cells allowing for host material to become part of the dissolved organic carbon pool, which can be respired back to the atmosphere or stabilized on mineral surfaces – similar to the marine "phage shunt." Toward understanding a soil phage shunt we have isolated viral-like particles from soil, which represent viruses resulting from host infections and potential lysis events and thus may serve as a proxy for microbial death. We have also resolved active soil phage genomes through stable isotope probing. From the resulting genomes of these efforts we have begun to understand which viruses are present in soil, what are their genomic contents and how this may impact soil carbon. From viral metagenomes (viromes) derived from isolated viral-like particles we have tapped a vast

unexplored, unannotated domain of soil microbial communities. Using recently optimized methods, we were able to isolate a "viral fraction," smaller than 0.22μm and larger than 100kDa, from field soil growing *Avena barbata* (wild oat plant), and extract nucleotides from this fraction for Illumina HiSeq sequencing. Based on analysis of terminase genes, these soil virome sequences are more related to each other than to previously identified viral genomes from NCBI. We are in the process of ground-truthing these viromes with relevant soil metagenomes.

To trace atmospheric carbon into soil microbes and phages, we grew *Avena spp.* plants under $^{13}CO_2$ and collected rhizosphere soil, and bulk soil (soil not associated with roots) at weeks 0, 6, and 9. Following extraction of DNA from the samples, we performed density-gradient centrifugation, yielding DNA samples with a range of ^{13}C label: unlabeled, partially labeled, and heavily labeled DNA. DNA from the separated fractions were then sequenced, and the sequence data were assembled, binned for host genomes, and phage contigs were identified. This enabled us to identify active phages in the rhizosphere—i.e. phage incorporating newly added ^{13}C label. Of the viral genomes resolved from this stable isotope probing dataset, we identified 13 complete phage genomes. For two of the phages we resolved near complete genomes of their hosts, which allowed us to examine specific anti-phage defense systems in context, including CRISPR and restriction modification systems. We observed different populations of phages that incorporated the label into their genomes as compared to non-labelled phages, indicating that the development of rhizosphere-competent bacterial consortia enabled the production of new (different) phage populations. These combined approaches to discovering and understanding soil viruses allow us to track phage population dynamics through time in relation to identified hosts and uncover a previously unseen wealth of viral genomes in soil.

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