Stable isotope-enabled metagenomics reveal phages active in rhizosphere soil

Evan Starr[1]* (starr.evan@berkeley.edu), Alexa Nicolas[1], Shengjing Shi[2], Sneha Couvillion[3], Alexander Probst[4], Donald Herman[1,6], Steve Blazewicz[5], **Mary Lipton[3]**, Jillian Banfield[1,6], and **Mary Firestone[1,6]**

[1]University of California, Berkeley, California; [2] Lincoln Science Centre, AgResearch Ltd, Christchurch, New Zealand; [3]Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA; [4] Biofilm Center, University of Duisburg-Essen, Essen 45141, Germany; [5] Lawrence Livermore National Laboratory, Livermore, California; and [6]Lawrence Berkeley National Laboratory, Berkeley, California

Project goals: The flow and fate of carbon from roots into soil is controlled by a myriad of interactions. Our project explores the impact of phages on the rhizosphere community and ultimately on the fate of carbon in soil. We are tracing carbon movement, through stable isotope labelling, from plant fixation to exudation in the root zone, uptake into the microbial community, and finally through replication and lysis into active phages. The combined approach of stable isotope probing with genome resolved metagenomics gives us insight into the flow of carbon, novel phage diversity, and phage activity. Additionally, we are employing metaproteomics, Nanoscale Secondary Ion Mass Spectrometry (NanoSIMS), and fluorescence imaging of viral like particles to quantify and follow phage activity and dynamics. This combination of approaches will allow us to better understand the impact of phages on carbon flow from roots into soil, microbial community dynamics and the soil food web, all with implications for soil health and agricultural productivity.

The zone of influence around plant roots, the rhizosphere, is of great importance to plant health and carbon cycling. There have been numerous experiments exploring plant-bacteria interactions, but phages have been largely overlooked in rhizosphere studies. We hypothesize that phages have an effect on the rhizosphere community and ultimately on the fate of soil carbon. The impact of phages in soil may be analogous to the “phage shunt” or “biological pump” from marine systems in which phage lysis either keeps carbon in the dissolved organic carbon pool which is quickly metabolized by microbes and respired back to the atmosphere or phage lysis causes organic carbon to be sorbed onto mineral surfaces, creating persistent soil organic carbon. To begin to address our larger hypotheses we need a fundamental understanding of our system. Phages have been understudied in soil, not because of their perceived lack of impact, but because of the difficulty of phage research and the abiotic and biotic diversity of soil more generally. Therefore, we needed to determine how best to study phage activity, diversity, and dynamics in soil. We combined stable isotope probing (SIP) and genome resolved metagenomics (DNA sequencing) to find and analyze recently active phage populations in the rhizosphere. In this study we grew *Avena fatua*, common wild oat, in $^{13}CO_2$ and collected rhizosphere soil, and bulk soil (soil not associated with roots) at weeks 0, 6, and 9. The DNA extracted from these samples underwent 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.

We are thus able to identify active phages in the rhizosphere. The populations of phages that incorporated the label into their genomes were different from the non-labelled phages, indicating that the development of rhizosphere-competent bacterial consortia enabled the production of a new (different) phage population. We were also able to link several phages with their hosts and show that there were differences in phage-host abundances between samples, indicating a dynamic system. Our discovery of active phages is supported by metaproteomic analyses which identified expressed phage proteins in the rhizosphere soil. In preparation for future, more targeted studies, we have improved our quantitative methods including enumeration of phage-like particles using fluorescent microscopy and assessment of phage C isotope content using NanoSIMS.

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-SC0010570 and DE-SC0016247 to MF at UC Berkeley and DE-SC10010566 to JB at UC Berkeley. AJP was supported by the German Science Foundation under DFG1603-1/1. Work at LLNL was performed under the auspices of the U.S. Department of Energy under Contract DE-AC52-07NA27344 and work at the Environmental Molecular Sciences Laboratory (EMSL located at PNNL) under Contract DE-AC05-76RLO 1830.