Virus Activity in Soil Revealed Through SIP-Metagenomics

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Project Goals: Microorganisms play key roles in soil carbon turnover and stabilization of persistent organic matter via their metabolic activities, cellular biochemistry, and extracellular products. Microbial residues are the primary ingredients in soil organic matter (SOM), a pool critical to Earth's soil health and climate. We hypothesize that microbial cellular-chemistry, functional potential, and ecophysiology fundamentally shape soil carbon persistence, and we are characterizing this via stable isotope probing (SIP) of genome-resolved metagenomes and viromes. We focus on soil moisture as a 'master controller' of microbial activity and mortality, since altered precipitation regimes are predicted across the temperate U.S. *Our SFA's ultimate goal is to determine how microbial soil ecophysiology, population dynamics, and microbe-mineral-organic matter interactions regulate the persistence of microbial residues under changing moisture regimes.*

Abstract: Viruses are known to have critical ecosystem functions, via mechanisms such as nutrient cycling through host lysis, host metabolism reprogramming and shuffling of host genes via horizontal gene transfer [1,2]. In soils these mechanisms are under-explored, however recent work suggests that viruses may impact their surroundings by expressing a variety of auxiliary metabolic genes (AMGs) involved in carbon cycling [3] and even sporulation [4]. Here, we investigated the ecology of the viral component of bulk metagenomes sampled from three grassland Californian soils that occur along a precipitation gradient, and evaluated the subset of viruses that were 'active' via a stable isotope probing (SIP) experiment with ¹⁸O-water.

Across the three sites, 8,617 viral operational taxonomic units ('vOTUs') were identified (a 5fold gain over previous similar surveys), 37% of which were distributed into 495 novel viral clusters (approximate genus-level taxonomy). Virus diversity varied significantly (Shannon's H, p-value < 0.005) between the three soil sites, and was most diverse in the driest soil. Virus communities were strongly structured by soil site origin (PERMANOVA, p-value=0.001), and vOTU composition was strongly influenced by soil composition, pH and inorganic elements present. Host-linkage trends revealed that Actinobacteriota – specifically Mycobacteria - were the dominant host taxa (range: 68 – 83%) across the three soils. These were followed by Proteobacteria or Acidobacteria hosts, depending on the soil origin. The level of virus activity (determined via SIP incubation) varied significantly (range: 29 – 70% active viruses from total viruses) between soil sites, and followed an inverse relationship with soil moisture. Actinobacteriophages (mainly mycobacteriophages) were the most active viruses in two soils, except for Angelo. At the wettest site, viruses of Bacteriovoracia (order: Bdellovibrio; common soil-dwelling obligatory bacterial parasites) were among the most active. Uniquely in the driest soil site, we found active Archaeal viruses, predicted to infect members of the Nitrososphaeraceae. This family plays important roles in nitrogen cycling, implying that viral infection could impact soil nutrient cycling. Lastly, 3% of the active pool of viruses encoded ecologically-relevant AMGs, including those for carbon utilization, nitrogen and energy metabolism. In summary, our data suggest that soils harbor substantial viral diversity, most of which is unknown; these viruses are actively replicating and can manipulate keystone microbial taxa responsible for nutrient transformation in soils.

References

- Roux S, Brum JR, Dutilh BE, Sunagawa S, Duhaime MB, Loy A, et al. Ecogenomics and potential biogeochemical impacts of globally abundant ocean viruses. Nature. 2016;537:689– 93.
- 2. Gregory AC, Zayed AA, Conceição-Neto N, Temperton B, Bolduc B, Alberti A, et al. Marine DNA Viral Macro- and Microdiversity from Pole to Pole. Cell. 2019;177:1109-1123.e14.
- 3. Emerson JB, Roux S, Brum JR, Bolduc B, Woodcroft BJ, Jang HB, et al. Host-linked soil viral ecology along a permafrost thaw gradient. Nat Microbiol [Internet]. 2018;3:870–80. Available from: https://www.ncbi.nlm.nih.gov/pubmed/30013236
- 4. Trubl G, Jang H Bin, Roux S, Emerson JB, Solonenko N, Vik DR, et al. Soil Viruses Are Underexplored Players in Ecosystem Carbon Processing. mSystems. 2018;3:1–21.

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