

**Bringing Soil Viruses into the Limelight.** G. Trubl<sup>1</sup>, S. Roux<sup>1,2</sup>, J. B. Emerson<sup>1</sup>, B. Bolduc<sup>1</sup>, HB. Jang<sup>1</sup>, N. Solonenko<sup>1</sup>, V. I. Rich<sup>1</sup>, & M. B. Sullivan<sup>1,3</sup>, <sup>1</sup>The Ohio State University, Department of Microbiology, 484 West 12<sup>th</sup> Ave. Columbus, OH, 43210, USA, <sup>2</sup>Department of Energy Joint Genome Institute, 2800 Mitchell Dr., Walnut Creek, CA, 94598, USA, <sup>3</sup>The Ohio State University, Department of Civil, Environmental and Geodetic Engineering, 2070 Neil Avenue Columbus, OH, 43210, USA

We are discovering more places in which life could exist in our solar system, from the icy worlds of Europa and Enceladus to the permafrost-covered polar caps and subsurface of Mars. To date, the search for extra-terrestrial life has mostly focused on understanding the metabolic potential of microorganisms in extreme environments. But, on Earth, there are ~10 viruses for every microorganism, and viruses play central roles in modulating microbial metabolism through cellular lysis (e.g. viruses drive the largest ocean C flux, of ~150 Gt yr<sup>-1</sup>), as well as gene transport and expression during infection (10<sup>29</sup> transduction events day<sup>-1</sup>). Because of this, there has been a resurgence of viral ecology, and the tremendous importance of viruses in regulating microbial ecosystems is becoming more apparent. Viral research in soils has so far been outpaced by viral research in aquatic environments, largely due to the technical challenges of accessing these soil viruses as well as the vast physical diversity among different soil types. Here, we highlight several major advances in soil virology, in the context of our research on permafrost-associated Arctic soils. First, we optimized viral resuspension and purification methods for a range of active-layer soil types. Second, after characterizing the recovered viruses via metagenomic sequencing, we applied cutting-edge viral-specific informatics methods to recover genome fragments, define their gene content and identity, connect to potential hosts, and analyze their relationships to environmental parameters. The results revealed over 400 novel viral populations, the majority (75%) of which are viral “dark matter”, with no significant similarity to publicly available viral genome sequences. Viral abundance and diversity increased with permafrost thaw stage, while individual viruses were endemic to their soil type, and soil moisture content, pH, and host community composition most strongly predicted viral community composition. Of the viruses that could be annotated, a third of them were linked to highly abundant microbial hosts, and several viruses (25%) contained genes with the potential to alter host carbon metabolism and survivability in this cold ecosystem (e.g. heat-shock proteins). By developing laboratory protocols to resuspend viruses from challenging soils and to extract their genetic material, and by leveraging recently developed bioinformatic tools (via iVirus pipeline, part of the CyVerse infrastructure), it is our goal to make soil viral research more accessible to all, while

also characterizing viral roles in an astrobiologically-relevant earth habitat.