Using stable isotopes to track viruses in soils

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Soils are complex ecosystems

Most insights come from isolates & metagenomics

(Figure from Williamson et al. 2017)
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Soil aggregate

Air

-water

Pore-water

Microbes

Plants

Macrofauna

Mesofauna

Microfauna

Viruses

Macrofauna

Mesofauna

Microfauna

Microbes

Viruses

Soil viruses are hard to isolate & metagenomes are swamped by larger genomes = low resolution on viruses

(Figure from Williamson et al. 2017)
Limited Knowledge of Soil Viruses

- $10^7 - 10^9$ viruses/g soil = ~1–1,000 viruses: microbe

(Figure from Williamson et al. 2017)
Limited Knowledge of Soil Viruses

- $10^7$–$10^9$ viruses/g soil = ~1–1,000 viruses: microbe
- Morphologically diverse

Scale bar: 100 nm

(Swanson 2009)
Limited Knowledge of Soil Viruses

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- Recent metagenomics and viromics have revealed:
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  - Direct impacts on microbial biogeochemistry via lysis of dominant microbial lineages or expression of auxiliary metabolic genes (Emerson et al. 2018; Trubl et al. 2018)

### Dominant microbes
- Polysaccharides
- Monomers
- AMGs

### Metabolisms
- Fermentation (e.g. beer)
- Respiration (make CO$_2$)
- Methanogenesis (make CH$_4$)
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Recent advancements made possible by deep sequencing of metagenomes or laborious viromes
Soils are complex ecosystems

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Plants  Macrofauna  Mesofauna  Microfauna  Microbes  Viruses

(Figure from Williamson et al. 2017)
Soils are complex ecosystems

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Microbes exist in different metabolic states and majority of microbes are inactive in soil at a single time-point

(Figure from Williamson et al. 2017)
Soils are complex ecosystems

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Goals:
1. Increase resolution on viruses
2. Target active microbes and their viruses

(Figure from Williamson et al. 2017)
How to track viruses?

Viruses are too small...
Stable isotopes

- Atoms that contain the same number of protons but differ in the number of neutrons

**Hydrogen**

- Proton
- Neutron
- Electron

1. $^1\text{H} = \text{Proton}$ ~99.90%
2. $^2\text{H} = \text{Neutron}$ ~0.012%
Stable isotopes

- Atoms that contain the same number of protons but differ in the number of neutrons

Hydrogen

1 H = Proton
2 H = More mass
Can be tracers of biogeochemical processes

- H$_2^{16}$O
  - 2 Hydrogens and 1 oxygen (8 protons/8 neutrons)

- H$_2^{18}$O
  - 2 Hydrogens and 1 oxygen (8 protons/10 neutrons)
Characterizing viruses via targeted SIP-metagenomics

Blazewicz et al, 2011, SB&B
Blazewicz et al, 2014, Ecology

H₂¹⁶O  H₂¹⁸O
Characterizing viruses via targeted SIP-metagenomics

$H_2^{16}O$  $H_2^{18}O$  $^{12}C$  $^{13}C$

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Characterizing viruses via targeted SIP-metagenomics.

All active organisms & their viruses are labeled.

Specific organisms & their viruses are labeled.

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Characterizing viruses via targeted SIP-metagenomics

- Extract DNA
- Ultracentrifugation

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Characterizing viruses via targeted SIP-metagenomics

- Extract DNA
- Ultracentrifugation

- Dormant or deceased
- New growth

Blazewicz et al, 2011, SB&B
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Characterizing viruses via targeted SIP-metagenomics

- Extract DNA
- Ultracentrifugation
- Sequence
- Comparative Bioinformatics

Dormant/deceased microbial & viral DNA

Active microbial & viral DNA
Characterizing viruses via targeted SIP-metagenomics

- Extract DNA
- Ultracentrifugation
- Sequence
- Comparative Bioinformatics
- Active microbial & viral DNA
- Increased resolution

Dormant/deceased microbial & viral DNA
Characterizing viruses via targeted SIP-metagenomics

Goals:
1. Increase resolution on viruses
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Extract DNA

Dormant/deceased microbial & viral DNA

Comparative Bioinformatics

Increased resolution
Applying SIP to Metagenomics in different biomes

1) Characterize diversity of dsDNA viruses in soil from different biomes

2) Identify active viruses and their microbial hosts

- Two Long-term ecological research (LTER) sites
  - Partially-thaw permafrost bog habitat
  - Highly-dynamic tropical rainforest
Bonanza Creek, Alaska

Source: https://www.lter.uaf.edu/
Bonanza Creek, Alaska

Avg. temperature
-3.1°C

Source: https://www.lter.uaf.edu/
Bonanza Creek, Alaska

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Experimental overview

- Anoxic at 4°C
- 10 cm
- 2 g soil
- Homogenized + pressed to remove porewater
- thermokarst bog
Experimental overview

- **2 g soil thermokarst bog**
- **Anoxic at 4°C**
  - 10 cm
  - Homogenized + pressed to remove porewater
- **Flush w/N₂**
  - 10x at 4°C (10 in Hg/5 psi)
- **N₂H₂**
- **Control**
- **Heavy water (H₂¹⁸O)**
- **4°C → -1.5°C Over 2 days**
- **184 days**
- **370 days**
Experimental overview

- Anoxic at 4°C
- 2 g soil thermokarst bog
- 10 cm

- Homogenized + pressed to remove porewater
- Flush w/N₂ 10x at 4°C (10 in Hg/5 psi)

- N₂H₂
- Control
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- 4°C → -1.5°C Over 2 days

- 184 days
- 370 days

- 16S rRNA gene 184 days
- 23 Metagenomes ¹⁸O & control 184 & 370 days
Bonanza Creek, Alaska

**Overview**

- 23 metagenomes

30 active metagenome-assembled genomes (MAGs)
Bonanza Creek, Alaska

Overview

- 23 metagenomes

30 active metagenome-assembled genomes (MAGs)
Bacterial hosts spanned 3 phyla:

  - Proteobacteria
  - Bacteroidetes
  - Firmicutes
Bonanza Creek, Alaska

Overview
- 23 metagenomes

Bacteria active below freezing!
Bonanza Creek, Alaska

Overview

- 23 metagenomes
- ~4,000 viruses detected by VirSorter (categories 1 & 2) & DeepVirFinder (score $\geq 0.9$ and P value $< 0.05$)
Bonanza Creek, Alaska

Overview
- 23 metagenomes
- ~4,000 viruses detected by VirSorter (categories 1 & 2) & DeepVirFinder (score $\geq 0.9$ and P value $< 0.05$)
- 332 vOTUs (>10kb)

Identifying viral populations (>10kb) Identified viruses were clustered by 95% nucleotide identity over 85% of the shorter contig
Bonanza Creek, Alaska

Overview
- 23 metagenomes
- ~4,000 viruses detected by VirSorter (categories 1 & 2) & DeepVirFinder (score \( \geq 0.9 \) and P value < 0.05)
- 332 vOTUs (\( \geq 10kb \))
- 9 virus-host linkages via nucleotide identity (threshold 95% ANI & \( \geq 1500bp \))...more to come
A lot of soil viruses detected!

332 vOTUs

Normalized relative abundance
(90% ANI across 75% of contig)
Increased resolution by removing non-active viruses

332 vOTUs

H$_2^{16}$O$_{184d}$
H$_2^{16}$O$_{370d}$

H$_2^{18}$O$_{184d}$
H$_2^{18}$O$_{370d}$

Not active

Normalized relative abundance
(90% ANI across 75% of contig)
Many viruses are active

- 256 vOTUs considered active

Normalized relative abundance
(90% ANI across 75% of contig)
Viral community is dynamic

Active vOTUs (H$_2^{18}$O samples)

1. Many viruses persist

256 vOTUs

Normalized relative abundance
(90% ANI across 75% of contig)
What does persistence mean, ecologically?

- 68% (173 vOTUs) increased in abundance over time
What does persistence mean, ecologically?

- 68% (173 vOTUs) increased in abundance over time
- 1) Temperate viruses propagating in microbial hosts
- 2) Virions persist
- 3) Viral DNA accumulates in environment as ‘relic DNA’
Viral community is dynamic

Active vOTUs ($H_2^{18}O$ samples)

1. Many viruses persist
2. Some viruses are gone

256 vOTUs

Normalized relative abundance
(90% ANI across 75% of contig)
Viral community is dynamic

- Many viruses persist
- Some viruses are gone
- New viruses become active

Active vOTUs (H$_2^{18}$O samples)

256 vOTUs

Normalized relative abundance (90% ANI across 75% of contig)
Bonanza Creek, Alaska

Overview

- 23 metagenomes
- ~4,000 viruses detected by VirSorter (categories 1 & 2) & DeepVirFinder (score ≥ 0.9 and P value < 0.05)
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Viruses active below freezing!
Two Long-term ecological research (LTER) sites
- Partially-thaw permafrost bog habitat
- Highly-dynamic tropical rainforest
Luquillo Experimental Forest, Puerto Rico

- Soils naturally oscillate between oxic and anoxic conditions
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$^{13}$C enriched plant biomass added to 20 g of soil

Figure credit: Alex Paya
13C enriched plant biomass added to 20 g of soil

**Treatments:**
1. static oxic (Oxic)
2. 4-day-oxic/4-day-anoxic (High frequency)
3. 8-day-oxic/4-day-anoxic (Low frequency)
4. static anoxic (Anoxic)

Oxic/anoxic conditions controlled by headspace
Oxic = air
Anoxic = N₂

**Figure credit:** Alex Paya
Luquillo Experimental Forest, Puerto Rico

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2. 4-day-oxic/4-day-anoxic (**High frequency**) 
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4. static anoxic (**Anoxic**) 

Incubated for 44 days

85 SIP-fractionated metagenomes

10 bulk soil metagenomes

Figure credit: Alex Paya
Luquillo Experimental Forest, Puerto Rico

Overview
- 95 metagenomes
Luquillo Experimental Forest, Puerto Rico

Overview

- 95 metagenomes
- 214 MAGs
  - 4 phyla of bacterial hosts:
    - Acidobacteria
    - Actinobacteria
    - Bacteroidetes
    - Proteobacteria
Luquillo Experimental Forest, Puerto Rico

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- ~45,000 viruses detected by VirSorter (categories 1 & 2) & DeepVirFinder (score ≥ 0.9 and P value < 0.05)
- 640 vOTUs (>10kb)

Identifying viral populations (>10kb)

Identified viruses were clustered by 95% nucleotide identity over 85% of the shorter contig
Luquillo Experimental Forest, Puerto Rico

Overview
- 95 metagenomes
- 214 MAGs
  - 4 phyla of bacterial hosts:
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- ~45,000 viruses detected by VirSorter (categories 1 & 2) & DeepVirFinder (score ≥ 0.9 and P value < 0.05)
- 640 vOTUs (≥10kb)
- 11% of viruses are linked to a host via nucleotide identity (threshold 95% ANI & ≥1500bp)
Luquillo Experimental Forest, Puerto Rico

Sample treatments

- Bulk metagenome ($^{12}$C)
- $^{12}$C-SIP fractions
- $^{13}$C-SIP fractions (host ate added plant biomass)

640 vOTUs (>10kb)

Normalized relative abundance
(90% ANI across 75% of contig)
SIP-fractions recovered more vOTUs

640 vOTUs (>10kb)

Field_Bulk (476)
Oxic_Bulk (519)
Low Frequency_Bulk (513)
High Frequency_Bulk (496)
Anoxic_Bulk (522)
Oxic\textsuperscript{12}C (463)
Low Frequency\textsuperscript{12}C (503)
High Frequency\textsuperscript{12}C (500)
Anoxic\textsuperscript{12}C (414)
Oxic\textsuperscript{13}C (539)
Low Frequency\textsuperscript{13}C (528)
High Frequency\textsuperscript{13}C (493)
Anoxic\textsuperscript{13}C (497)

Normalized relative abundance
(90% ANI across 75% of contig)

8% more vOTUs
Focus on specific active viruses

Sample treatments

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Oxic_{12}C (463)
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640 vOTUs (>10kb)

Normalized relative abundance (90% ANI across 75% of contig)
Redox conditions impact viral community

More oxygen = more vOTUs?

64 vOTUs (>10kb)

Sample treatments

Low Frequency\textsuperscript{13}C (12)

High Frequency\textsuperscript{13}C (9)

Anoxic\textsuperscript{13}C (9)

Oxic\textsuperscript{13}C (30)

Normalized relative abundance
(90% ANI across 75% of contig)
Redox conditions impact viral community

More oxygen = more vOTUs?

7 viruses active in anoxic conditions only?

WHY?
Summary

Applied SIP-metagenomics on two dramatically different LTER soils

1. Bonanza Creek, Alaska
   - Identified microbes and viruses active in subzero temperatures
   - Temporal succession of vOTUs
   - Evidence that viruses may persist in the environment > 1 year
Summary

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   - Linked viruses to key microbes involved in the degradation and fate of organic carbon compounds
   - Redox strongly influences virus activity
   - SIP fractions recovered more vOTUs
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Synthesis
   - A lot of novel viruses
   - Tropical soils had 8x more sequencing, but only ~2x more vOTUs
     - Less organisms, reducing metagenome complexity?
   - The metabolic repertoire was different between dormant/deceased & active microbes
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Thank You!
Ongoing work

Are viruses important...beyond being cool...for reducing uncertainty?

Using SIP to identify active viral populations

Active vOTUs?

How to do qSIP?