

Active host-virus interactions associated with nitrifying populations in soil

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Active host-virus interactions associated with nitrifying populations in soil

Result 1: Recovery of AOA MAGs from LBD 12C- and HBD 13C-enriched DNA

¹³C-pH 4.5 heavy fractions | ¹²C-pH 4.5 light fractions

%GC content

In transformed relative abundance

¹²C-pH 7.5 light fractions

Sungeun Lee, Christina Hazard and Graeme W. Nicol

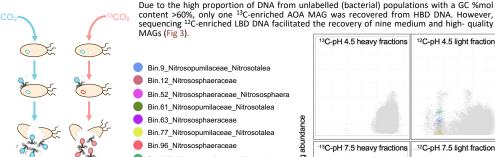
Contig abundance

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While there is substantial knowledge of the diversity and function of prokarvotes contributing nitrification in soil, nothing is known of the scale or frequency of interactions with viruses in situ. Recent advances in bioinformatic tools has enabled identification of linkages between viruses and hosts, typically at broad taxonomic levels. However, this does not determine if these represent current or historical interactions nor whether a virus or host are active.

The aim of this study was to identify interactions between viruses and nitrifying prokaryotic hosts. Specifically, we aimed to identify active interactions in situ between AOA cells and lytic viruses by following the transfer of recently assimilated inorganic carbon (Fig 1).



lysed cells incorporating C from 13CO2 will be isotopically enriched in 13C

Approach DNA-SIP is routinely used to compare the distribution of genomic DNA of AOA after incubation with 12C- or 13C-CO2 using amplicon sequencing. However, due to the low GC %mol of many AOA populations. 13C-enriched DNA often co-migrates with higher GC %mol DNA (Fig 2).

After incubating acidic and neutral pH soils with urea and CO2, metagenomic DNA was sequenced from low buoyant density (LBD) which is naturally enriched in AOA, and mapped reads from high buoyant density (HBD) DNA from both 12C and ¹³C incubations to demonstrate activity/recent C incorporation into hosts and viruses.

Fig 2. Recovery and sequencing of HBD DNA from 12C and 13C incubations (green area) and LBD DNA from 12C incubation only (blue area).

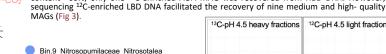


Fig 1. Viruses derived from

Bin.12 Nitrososphaeraceae Bin.52 Nitrososphaeraceae Nitrososphaera Bin.61 Nitrosopumilaceae Nitrosotalea Bin.63 Nitrososphaeraceae

Bin.77 Nitrosopumilaceae Nitrosotalea

Bin.96 Nitrososphaeraceae Bin.107 Nitrosopumilaceae Nitrosotalea

Bin.112 Nitrososphaeraceae

Bin.11 Nitrososphaeraceae

Unbinned

bacteria

1.72 1.74 1.76

1.72

Fig 3. AOA MAGs recovered from 13Cenriched HBD DNA and 12C-enriched LBD DNA.



Virus contigs assembled from 12Cincubated LBD DNA were predicted using established bioinformatic tools (Roux et al., 2015; Ren et al., 2020). To identify active viruses recently derived from lysed AOA, individual reads from sequenced HBD DNA were mapped to LBD DNA-derived viral contigs (Fig 4) and demonstrated that a large proportion were enriched in 13C-DNA

and represented active viruses.

Fig 4. Mapping of reads derived from metagenomic sequencing of HBD DNA of 12C and 13C incubations to viral contigs. Each row represents one of twelve individual microcosms

Result 3: Gene sharing network of AOA viruses and proviruses

53% of predicted free virus contigs from both soils shared genome content with predicted proviruses identified in sequenced AOA genomes (Fig 5).

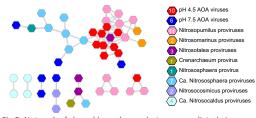
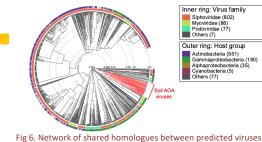


Fig 5. Network of shared homologues between predicted viruses and proviruses using vConTACT (Jang et al., 2019)

Result 4: Proteomic analysis of active soil AOA viruses

Proteomic tree of predicted soil virus and integrated provirus sequences indicates that most recovered viral sequences associated with AOA belong share genetic content that is distinct from other soil viruses (Fig 6). Soil AOA viruses showed no relationships with viruses of other archaea.



and proviruses using vConTACT (Jang et al., 2019)

Conclusion

Combining unlabelled DNA buoyant density-fractionation and DNA-SIP sequencing identified active viruses infecting low GC %mol AOA within a complex ecosystem by following carbon flow.

Result 2: Identifying genomes of active AOA viruses in ¹³C-enriched metagenomic DNA AOA-viruses (n=43)

13C-pH 4.5

12C-pH 4.5

12C-pH 7.5

13C-pH 7.5

13C-pH 7.5 heavy fractions