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Active host-virus interactions associated with nitrifying populations in soil

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Introduction

While there is substantial knowledge of the diversity and function of prokaryotes contributing to 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).

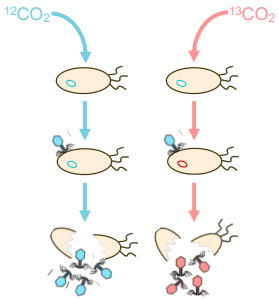

Fig 1. Viruses derived from lysed cells incorporating C from $^{13}CO_2$ will be isotopically enriched in ^{13}C

Approach

DNA-SIP is routinely used to compare the distribution of genomic DNA of AOA after incubation with ^{12}C- or ^{13}C-CO_2 using amplicon sequencing. However, due to the low GC %mol of many AOA populations, ^{13}C-enriched DNA often co-migrates with higher GC %mol DNA (Fig 2).

After incubating acidic and neutral pH soils with urea and CO_2, 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 ^{12}C and ^{13}C incubations to demonstrate activity/recent C incorporation into hosts and viruses.

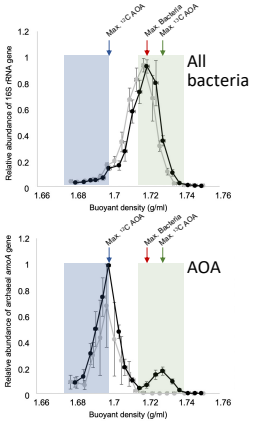

Fig 2. Recovery and sequencing of HBD DNA from ^{12}C and ^{13}C incubations (green area) and LBD DNA from ^{12}C incubation only (blue area).

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

Due to the high proportion of DNA from unlabelled (bacterial) populations with a GC %mol content >60%, only one ^{13}C-enriched AOA MAG was recovered from HBD DNA. However, sequencing ^{12}C-enriched LBD DNA facilitated the recovery of nine medium and high- quality MAGs (Fig 3).

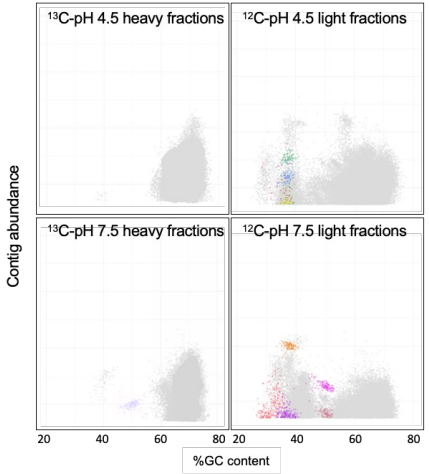

Fig 3. AOA MAGs recovered from ^{13}C-enriched HBD DNA and ^{12}C-enriched LBD DNA.

Result 2: Identifying genomes of active AOA viruses in ^{13}C-enriched metagenomic DNA

Virus contigs assembled from ^{12}C-incubated 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 ^{13}C-DNA and represented active viruses.

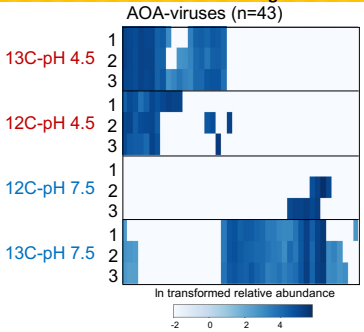

Fig 4. Mapping of reads derived from metagenomic sequencing of HBD DNA of ^{12}C and ^{13}C incubations to viral contigs. Each row represents one of twelve individual microcosms.

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).

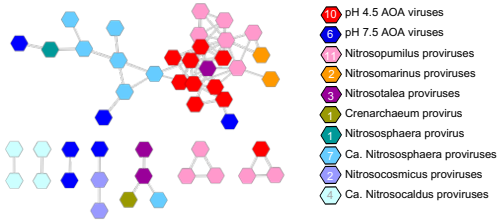

Fig 5. Network of shared homologues between predicted viruses and proviruses using vConTACT (Jang et al., 2019)

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.

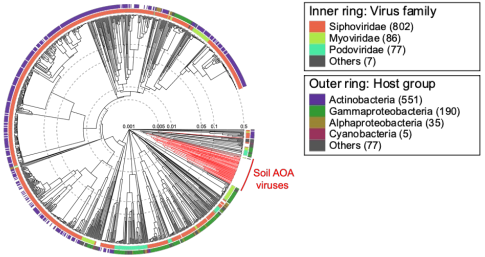

Fig 6. Network of shared homologues between predicted viruses and proviruses using vConTACT (Jang et al., 2019)

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.