

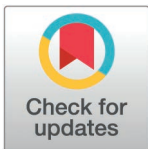
RESEARCH ARTICLE

Virus-host interactions in iron-oxidizing mats of the East Pacific Rise

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Abstract

Iron-oxidizing microbial mats are commonly encountered features at low-temperature (<100°C) hydrothermal vents found along the seafloor at tectonically active settings. The microbial communities in these mats are uniquely adapted to exploit the specific ecological niches within the physical and chemical gradients of their environment. A vital component for these adaptations is viral infections, although their impacts are poorly constrained. While the microbial communities of iron-oxidizing mats at seafloor hydrothermal vents have been well studied, the role of viruses in affecting these communities with respect to biogeochemical cycling, diversity, and/or population control remains unclear. The goal of this study was to assess the role and impact of viruses within iron-oxidizing mats of the well-studied 9°N East Pacific Rise (EPR) segment. We found unique viral assemblages at each site, with 41% of the viral operational taxonomic units (vOTUs) shared across all sites, but with differing relative abundances. The virally-encoded auxiliary metabolic genes (AMGs) were also differentially abundant among the sites and included processes related to carbon and sulfur cycling. Virus–host linkages showed that the viral assemblages infect different members of the microbial communities within each mat, as well as the potential to affect ecological shifts in the metabolisms of various hosts due to viral infection, which could affect the rates of microbial processes. In these iron-oxidizing mats, viruses are therefore likely playing a role in the diversity and evolution of the microbial taxa through lysis and the potential to alter host metabolisms. Exploration of virus–host dynamics within iron-oxidizing mats in hydrothermal vent systems like the EPR aid in constraining a vital component of the deep ocean food webs and give critical insight to the ecology of these productive microbial communities.

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Introduction

Hydrothermal vent habitats support abundant and diverse chemosynthetic microbial communities and their associated food webs. While the microbial assemblages have been studied since the discovery of hydrothermal venting [1–4], the role viruses play within these environments is largely unknown. Viruses are pivotal components of microbial communities as they are catalysts for microbial adaptation to changing environmental conditions [5–7], a significant source of microbial mortality through lysis [5,8], and viruses affect biogeochemical cycles [9,10]. Understanding the role viruses play within hydrothermal vent microbial communities is critical to understanding community dynamics that could affect primary productivity [1] and consequently secondary production [2,11].

One common feature of hydrothermal vent habitats is diffuse flow vents, which form when warm subsurface fluids mix in the shallow seafloor with oxygenated bottom water, resulting in warm (<100°C), diffusely flowing fluids exiting the seafloor. This creates habitats for chemolithoautotrophic organisms that are hot spots of microbial activity and are often associated with iron-oxidizing microbial mats [3]. Zetaproteobacteria are key microbes that use the diffuse Fe(II)-rich fluids to engineer the mat habitat via neutrophilic iron oxidation, which results in deposition of iron-oxide stalks [12–16]. Zetaproteobacteria serve as the most abundant primary producers in these iron-oxide microbial mats and are supported by a variety of microbial members capable of iron reduction, carbon fixation, fermentation, nitrate assimilation, and denitrification, among others [17–19]. Heterotrophs and fermenters are provided a reservoir of organic carbon from the polysaccharides and adsorbed organic carbon contained in the iron-oxyhydroxide stalk structures produced through Zetaproteobacteria metabolism [19–21]. It is unclear how the viruses of these prokaryotic communities, while present [19], affect biogeochemical cycles, diversity, and/or exert population control within the food web of these iron mats. Diversity, high biomass, and metabolic cooperation that are common in iron-oxidizing mats [19] can result in unique virus–host interactions.

Phages, viruses that infect bacteria, can have a range of interactions with their host that encompass both antagonistic and/or mutualistic relationships that alter and affect the diversity of microbial populations and contribute to biogeochemical processes [22]. Lytic viruses (lytic cycle: commandeer host, replicate, and lyse host) can redirect the metabolic activity of their hosts as well as release labile nutrients through lysis, providing food for the surrounding microbial community and altering biogeochemical cycles; an overall process known as the viral shunt [23,24]. In contrast, temperate phages (lysogenic cycle: integrates into host genome and passively replicates with host cell or enters lytic cycle) have a relationship with their host that is mutualistic prior to entering the lytic phase; it can lead to lysogenic conversion, where phage integration into the host genome results in a new stable phenotype or phenotypic plasticity [6]. Lysogenic conversion can increase fitness by allowing hosts to occupy novel niches within the environment, increasing the survivability of the host and phage simultaneously [6]. High incidences of temperate phages have been found in diffuse flow fluids [25,26], which may result from the diverse and unpredictable

conditions associated with diffuse flow vents making it challenging for free-living organisms and therefore selecting for a survival strategy that is more mutualistic in nature. A shared component between these two types of infection cycles are phage encoded auxiliary metabolic genes (AMGs), which allow phages to metabolically reprogram their host during infection, affecting diversity and function of marine microbial populations [5]. Phages encode genes that participate in novel pathways in hydrothermal sediments [10] and plumes [9], allowing them to directly participate in important biogeochemical cycles.

Given the integral role that viruses play within microbial communities in general, it is likely that there are important host–virus interactions within iron-oxidizing mats that help shape and sustain these isolated environments as well as influence important ecological processes in these mats, such as iron oxidation, carbon cycling, and nitrate reduction. To date, viral ecology of iron-oxidizing mats in hydrothermal vent habitats is unexplored. Therefore, the goal of this work was to provide a first assessment of the role and impact of viruses in iron-oxidizing microbial mats. We chose the well-studied 9°50' N segment of the East Pacific Rise as a field site and we hypothesized that the viral community would reflect key iron-oxidizing microbial mat community members as well as encode AMGs that are ecologically important for the various microbial metabolic processes (e.g., iron, sulfur, carbon, and nitrogen cycling). We sampled three iron-oxidizing mats, induced and collected the viral communities, and present the analysis of their metagenomes here.

Materials and methods

Sampling

In collaboration with the hot2cold Vents project [1,27], samples were taken during cruise AT42–09 (March/April of 2019) aboard the *R/V Atlantis* at 9°50'N segment of the East Pacific Rise, which is a fast-spreading mid-ocean ridge, with a spreading rate of ~5.5 cm yr⁻¹ [28]. Three flocculent iron-oxidizing mats found in diffuse flow areas, characteristically brown/orange in color, were collected using a hydraulic slurp sampler aboard HOV Alvin on dives 5020 (Marker 28), 5022 (Io) and 5024 (Riftia field -1, 2) (Fig 1, Table 1). The hydraulic slurp sampler was used here in single chamber mode – a water pump applies vacuum via a hose connected to one liter chamber (bottle) and collects material sucked up as the hose is applied to a surface (microbial mats in this case). A mesh screen at the top of the chamber filters out rocks and macrofauna. The collected sample remains in the chamber on the basket of HOV Alvin until recovery after the HOV is on deck. Temperatures of hydrothermal fluids exiting the seafloor closest to the collected mats were measured using the resistance temperature device (RTD) aboard HOV Alvin. Additional background seawater was also collected using a 1.2 L Niskin bottle connected to HOV Alvin. Once onboard the ship, the mat samples were subsampled from the slurp chambers into two sets of subsamples: (1) microbial mat collected into 50 ml centrifuge tubes and stored at 4°C until processing for viral induction experiments 14 months later, and (2) microbial mat mixed with an equal volume of RNALater and stored for 24 hours at 4°C before being frozen at -80°C until further processing to determine initial bacterial community composition. The Riftia Field samples are subsets of the same sampled mat.

Mitomycin C prophage induction of microbial mats

The permeate, or flow-through, of tangential flow filtration (TFF) [30 kDa TFF PrepScale (MilliPore)] of background seawater was used to generate virus-free seawater shipboard and kept in the dark until use. After a 14-month storage period, enough virus-free seawater was added to bring the volume up to 50 ml for each sample. The samples were gently mixed using sterile serological pipettes, then split into equal volumes in two sterile 50 ml centrifuge tubes, one for the control (i.e., uninduced) and one for the induction experiment. For each of the induction samples, mitomycin C was added to a final concentration of 1 µg/ml and inverted to mix [29]. All samples were incubated at 13°C for 24 hours. After the incubation period the samples were spun at 4,000 x g for 45 minutes and the supernatants were filtered through a 0.2 µm syringe filter (Supor® membrane, Pall Laboratory) into sterile 50 ml centrifuge tubes. The filtrate was stored at 4°C until DNA extraction, which was less than a week after the incubations were completed.

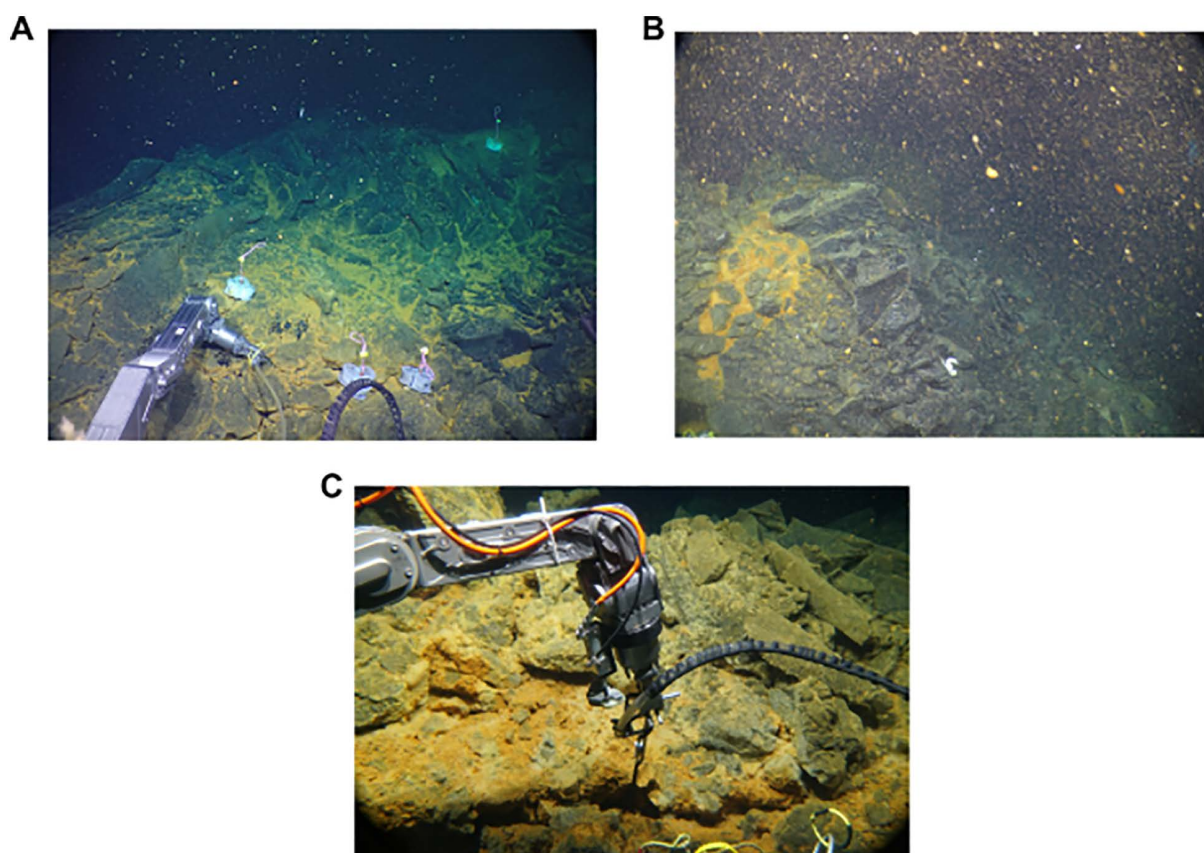


Fig 1. Images from sampling sites taken aboard HOV Alvin. A) Marker 28 location (dive 5020). B) Io location (dive 5022). C) Riftia Field location (dive 5024). Reproduced under a CC BY license, with permission from Woods Hole Oceanographic Institution (WHOI), original copyright 2020.

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Table 1. Sample recovery of iron-oxidizing mats.

Sample IDs	Sample Dive	Sample Site	Sample Coordinates	Depth (m)	Volume (mL)	Temperature (°C)
M28	5020	Marker 28	9.83614°N, 104.29089W	2509	20	12.5
IO	5022	Io	9.83512°N, 104.29100°W	2507	27	10
RF1	5024-1	Riftia Field	9.84548°N, 104.29300°W	2510	27.5	16
RF2	5024-2	Riftia Field	9.84548°N, 104.29300°W	2510	30	16

<https://doi.org/10.1371/journal.pone.0357099.t001>

DNA extraction and sequencing

Samples were concentrated using Sartorius VivaSpin Turbo 15 columns with a 30,000 MWCO filter to a final volume of <1 ml. Each sample was washed 3x in the columns with 15 ml virus-free seawater and eluted to a final volume between 1.5–1.75 ml. DNA was extracted using Qiagen DNeasy PowerSoil kit (Qiagen, MD) for DNA using the manufacturer's protocol. Due to low DNA recovery, samples were subjected to whole genome amplification (WGA) with Qiagen Repli-g Mini kit (Qiagen, MD). Each sample (controls and induced for all four samples) was amplified in triplicate using 5 µl of template per reaction tube and following the manufacturer's protocol. The samples were then cleaned using AMPure XL beads (Beckman, CA) according to the manufacturer's recommendations. All samples (0.018–3 µg) were sent for library

preparation (PerkinElmer NEXTFLEX Rapid XP DNA-Seq Kit HT) and sequencing (32M reads per sample) to TAMU AgriLife Genomics and Bioinformatics Service using Illumina NovaSeq with 150 bp paired-ends.

To determine initial bacterial community composition, DNA was extracted as previously described [30]. Briefly, DNA was extracted from 0.5g of microbial mat material using the manufacturer's protocol with minor modifications (FastDNA SPIN Kit for Soil, MP Biomedicals, Santa Ana, CA). In place of the sodium phosphate buffer, 250µl of 0.5M sodium citrate, pH 5.8 was used, and lysis was performed using two rounds of bead beating at a setting of 5.5 using the FastPrep instrument, with samples being placed on ice between rounds (MP Biomedicals). Then quantified by a Qubit 4.0 fluorometer with high-sensitivity reagents (ThermoFisher Scientific, Waltham, MA). The bacterial V3-V4 SSU rRNA gene was amplified by PCR using 340F and 784R primers with Illumina adaptors [30,31] by NC State Genomic Sciences Laboratory. The resulting amplicons were sequenced using a MiSeq (Illumina, San Diego, CA) following the manufacturer's protocol to generate 2 X 300 bp reads. After sequencing, reads were trimmed of primers using CutAdapt (v 3.5) [32]. Amplicon sequence data processing and analysis were performed using a pipeline involving the DADA2 (v 1.34.0) [33] and Phyloseq [34] packages in R (version 4.4.0). ASVs were created using default commands in DADA2, followed by removing Chimeric sequences using the removeBimeraDenovo function and the consensus method. The SILVA SSU rRNA database (v 138.1) was used for the taxonomic classification of ASVs. DNA was extracted only from Marker 28 and Riftia Fields due to constraints imposed by field sampling.

Viral metagenome analysis

Sequences were quality controlled using BBtools to trim adapter sequences (bbduk) using 'ktrim=r k=23 mink=11 hdist=1 qtrim=r trimq=20 tpe tbo', merge pair-end reads (bbmerge) with parameters 'rem k=62 extend2=50 ecct vstrict', remove contamination (bbduk) with parameters 'minid=0.95 maxindel=3 bwr=0.16 bw=12 qtrim=r trimq=10 untrim' and mask low-complexity regions (bbmask) with 'entropy=0.50' [35]. Sequences were assembled using MEGAHIT v1.2.8 [36] with default parameters. Viral identification was performed on the individual assemblies with VIBRANT (v1.2.1) [37] using the '-virome' flag. The resulting viral sequences were then filtered by length to keep viral contigs ≥ 5 kbp before clustering into viral operational taxonomic units (vOTUs) using CD-HIT-EST v4.8.1 [38] at 95% ANI and 85% alignment coverage. A cut off of 5 kb (instead of 10 kb) was used to account for the genomes of single stranded DNA (ssDNA) viruses, which can be between 5.3–6.3 kb, given the likelihood of ssDNA virus overamplification during WGA [39–41]. The quality-controlled reads (from BBTools) were mapped back to the vOTUs using bwa-mem2 v2.2.1 [42] and the number of mapped reads to each vOTU was calculated using samtools [43]. Taxonomy of each of the vOTUs was performed using geNomad v1.5.2 [44] and any unclassified vOTUs were categorized as "Unknown viruses". AMG identifications were compiled from the VIBRANT output for each vOTU. Lifestyles of vOTUs were ignored due to the inconsistencies and variability associated with mitomycin C inductions in terms of producing reliable chemical induction of lysogens; additionally, the use of WGA would potentially skew any lifestyles predicted and enumerated via relative abundance and therefore would be unreliable distinctions [45,46]. Hosts of the vOTUs were predicted utilizing iPHoP v1.3.3 [47], which uses both host-based (tRNAs, CRISPRs, etc) and phage-based tools (machine-learning approach based on association of viral genes to hosts) [48–51]. The resulting host predictions were additionally filtered to only keep the ones that were either called by more than one tool or had $\geq 95\%$ confidence score. The resulting hosts were compared to amplicon sequence variants of the 16S rRNA gene from the bulk mat for Marker 28 and Riftia Field sites. Tabular results for each of the various software used can be found in S2-5 Tables in [S1 File](#).

Statistical analysis

To assess the differences between the viral populations, a Bray-Curtis dissimilarity matrix was calculated with the vegan package [52] in R [53] using the number of reads mapped per vOTU per viral metagenome
$$\frac{\text{Mapped reads}}{\text{Size of vOTU genome (kbp)}} \div \frac{\text{Size of whole metagenome (Mbp)}}{\text{Size of whole metagenome (Mbp)}}$$

as count data for each sample (n=8 samples). From this matrix, a principal coordinate analysis was performed using the ape package [54] in R. To assess the statistical significance of the differences associated with the PCoA, a PERMANOVA was performed using the vegan package [52]. To directly compare the abundances of vOTUs across sites, the z-score was calculated by vOTU to convert values to standard deviations $[Z = \frac{\text{abundance} - \text{mean abundance}}{\text{standard deviation of abundance}}]$. A heatmap of the z-score values of all vOTUs was generated using ggplot2 in R [55]. To compare the AMGs between sites, a dissimilarity matrix was calculated between the AMGs encoded by the vOTUs using the count data associated with each vOTU. The dissimilarity matrix was used to generate a nonmetric multidimensional scaling (NMDS) plot using ggplot2 [55] in R. A PERMANOVA was performed to assess the statistical significance of any differences in the distribution of AMGs by site using the vegan package [52]. AMGs were also compared using z-scores calculated across AMGs, to compare abundances across the sites and visualized using a heatmap using ggplot2 in R [55].

Results

Viral assemblage composition

A total of 27,493 viral contigs ≥ 5 kb were assembled. Clustering at 95% ANI resulted in a total of 3,639 viral operational taxonomic units (vOTUs) (Table 2), which were used to assess taxonomy, identify auxiliary metabolic genes (AMGs), and predict potential hosts.

Comparison of the viral assemblages between the three sites, Marker 28, Io, and Riftia Field, showed that both the single-stranded DNA (ssDNA) (Fig 2A) and double-stranded DNA (dsDNA) (Fig 2B) viral populations were distinct, which were statistically significant ($p\text{-value} < 0.05$).

Most of the viruses across all samples occupied either the designation of an unknown virus (2.6–24.1%), unknown class Caudoviricetes (10.2–75.5%), or family *Microviridae* (0.08–70.9%) (Fig 3A). The remainder of the composition varied and consisted of no more than ~5% of the relative abundance. The majority were classified within ssDNA viruses and dsDNA. ssDNA viruses that were shared across all sites included members of the family *Inoviridae*, and viruses from orders Geplafuvirales, and class Arfiviricetes. Marker 28 and Io sites shared viruses from class Cressdnaviricota and order Cirli-virales. Marker 28 and both Riftia Field samples shared viruses from order Petitvirales. dsDNA viruses that were shared across all sample sites from class Caudoviricetes included *Zobellviridae*, *Herelleviridae*, and viruses from order Crassvira-les. *Drexelvira* was only found at Io sites, while *Demericviridae* was shared between Marker 28 and both Riftia Field samples. Giant viruses of the order Pimascovirales were observed at Marker 28 and one of the Riftia Field samples. Site Io also was observed to include the presence of giant viruses, but from class Megaviricetes and virophages from order Priklauovirales that were unique to the Io site.

The viral community compositions were distinct between each of the sites (Fig 2), although the vOTUs unique to each site accounted for less than 7% of all vOTUs. Marker 28, Io, and the two Riftia Field samples harbored 124 unique vOTUs (3% of all vOTUs), 237 (7%), 29 (1%) and 11 (<1%), respectively (Fig 3B). Despite 41% of the vOTUs being shared between the sites (Fig 3B), the vOTUs exhibit variable abundances where each site has a unique assemblage of vOTUs (Fig 4).

Table 2. Summary of the reads and vOTUs for each sampling location.

Sample	Marker 28	Io		Riftia Field			
	Induced	Control	Induced	Control	Induced	Control	Induced
Total # reads (x10 ⁶)	2.9	32.9	50.0	55.7	44.7	47.5	49.0
#vOTUs	2143	2518	2388	3085	3098	2796	2821

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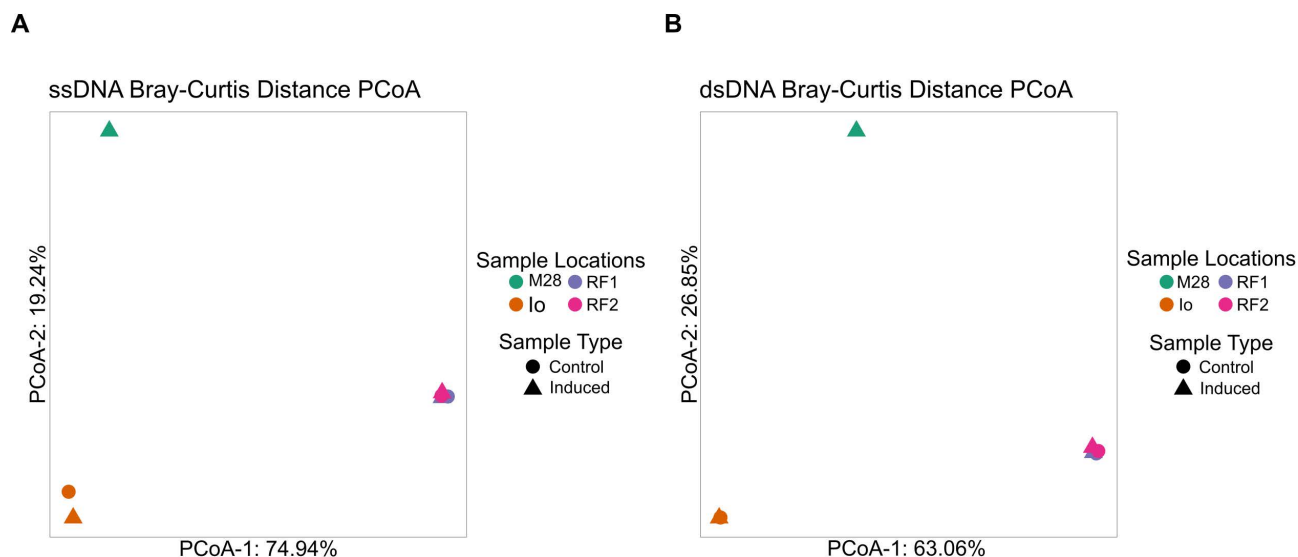


Fig 2. Principal coordinate analysis of vOTUs. Principal coordinate analysis of the Bray-Curtis dissimilarity matrix based on normalized read counts per vOTU (mapped reads per vOTU genome size in kbp per total metagenome size in Mbp) between each site for ssDNA [A] and dsDNA [B] viral populations, which accounts for 94.2% and 92.9% of the variation, respectively. The sites are clustering by location.

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Viral AMG metabolisms and pathways

There was a total of 72,783 proteins found in the vOTUs, of which only 4,904 (6.7%) were annotated. AMGs were identified in 10.7% of the vOTUs, representing a total of 557 proteins (0.77%). The function of these proteins consisted of carbohydrate metabolisms, amino acid metabolism, metabolism of cofactors and vitamins, metabolism of terpenoids and polyketides, energy metabolisms, biosynthesis of other secondary metabolisms, and glycan biosynthesis and metabolism (Fig 5A). Despite these similarities, the AMGs were distinct among each site (Fig 5B), which may be a result of the differences in relative abundances of the vOTUs and the AMG composition of each site. However, the differences were determined not to be statistically significant ($p\text{-value} > 0.05$).

In order to understand the potential ecological impacts of viruses in iron-oxidizing mats, we focused on the specific processes that affect the physiology of the hosts. These processes include carbohydrate metabolisms, energy metabolisms, biosynthesis of other secondary metabolites, metabolism of terpenoids and polyketides, glycan biosynthesis and metabolism, as well as folding, sorting and degradation within the sulfur relay system, and xenobiotics biodegradation and metabolism. Each sampling site showed different relative abundances of the various pathways (Fig 6), although the differences were not statistically significant ($p\text{-value} > 0.05$).

For carbohydrate metabolisms, Marker 28 site had relative abundances above the mean compared to the other sites for AMGs classified for propanoate metabolism and pyruvate metabolism; Io site had relative abundances above the mean in AMGs for fructose and mannose metabolism and galactose metabolism as well as for butanoate metabolism. The Riftia Field site showed the most carbohydrate pathways including amino sugar and nucleotide sugar metabolism, pentose and glucuronate interconversions, ascorbate and aldarate metabolism, and glyoxylate and dicarboxylate metabolism present above the mean for both samples and butanoate metabolism, pentose phosphate pathway, and starch and sucrose metabolism present at Riftia Field sample 1 but not sample 2 (Fig 6).

Relative abundances of AMGs related to energy metabolisms and carbon fixation were also different among the sites. Genes for methane metabolism were present at the greatest abundance compared to the mean in both Riftia Field samples, while AMGs for sulfur metabolism were only elevated above the mean in Riftia Field sample 1. Marker 28 showed

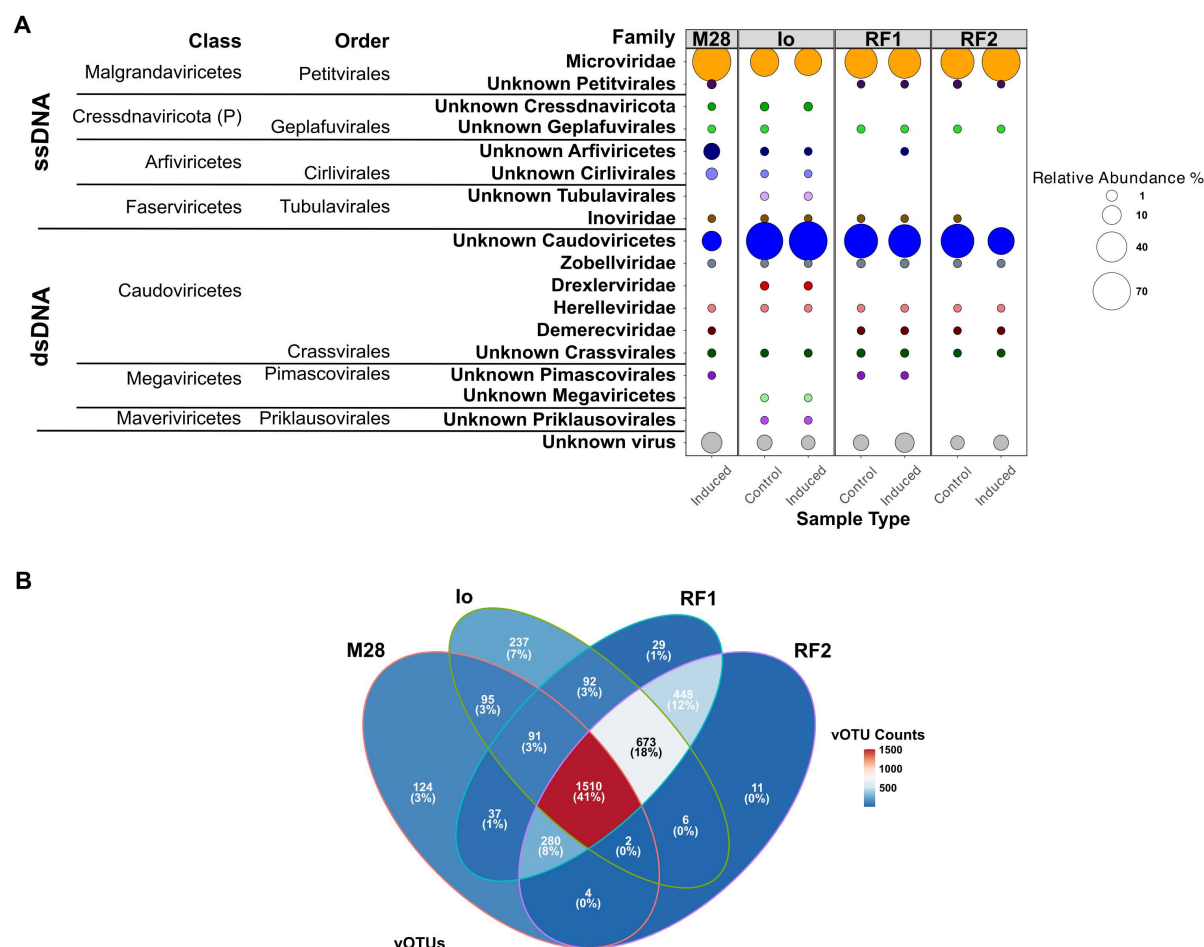


Fig 3. Taxonomy and relative abundance of the viral assemblages from each site (A) and Venn diagrams of the distribution of the viruses across the different sites using counts of vOTUs (B). The assemblages were dominated by *Microviridae*, unknown viruses of class Caudoviricetes, and Unknown viruses (i.e., unclassified viruses). 41% of the vOTUs were shared between the three sites.

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higher than the mean relative abundance for carbon fixation pathways and nitrogen metabolism for all sites. vOTUs from Io had the highest relative abundance of oxidative phosphorylation AMGs and some connected to sulfur metabolism.

AMGs for biosynthesis of other secondary metabolites and metabolism of terpenoids and polyketides were higher than the mean for Io and Riftia Field sites. Microbial mats from Io harbored AMGs above the mean relative abundance for polyketide sugar unit biosynthesis, flavonoid biosynthesis, phenylpropanoid biosynthesis, stilbenoid diarylhelthanoid, and gingerol biosynthesis and other unclassified secondary metabolites. The Riftia Field sample 1 was above the mean for AMGs related to biosynthesis of vancomycin group of antibiotics, polyketide sugar unit biosynthesis, streptomycin biosynthesis, and acarbose and validamycin biosynthesis. Marker 28 had elevated abundance for tropane, piperidine and pyridine alkaloid biosynthesis.

The relative abundance of AMGs responsible for glycan biosynthesis and metabolism was different for each site as well. Marker 28, Io, and Riftia Field sample 1 showed higher than the mean abundance of AMGs for lipopolysaccharide biosynthesis. Io also showed elevated abundance of lipoarabinomannan biosynthesis. The Riftia Field sample 1 had higher than the mean abundance in AMGs for glycosphingolipid biosynthesis, N-glycan biosynthesis, arabinogalactan biosynthesis, and peptidoglycan biosynthesis. The sample from Io was the one that showed high abundance of AMGs that participate in benzoate degradation and sulfur relay system.

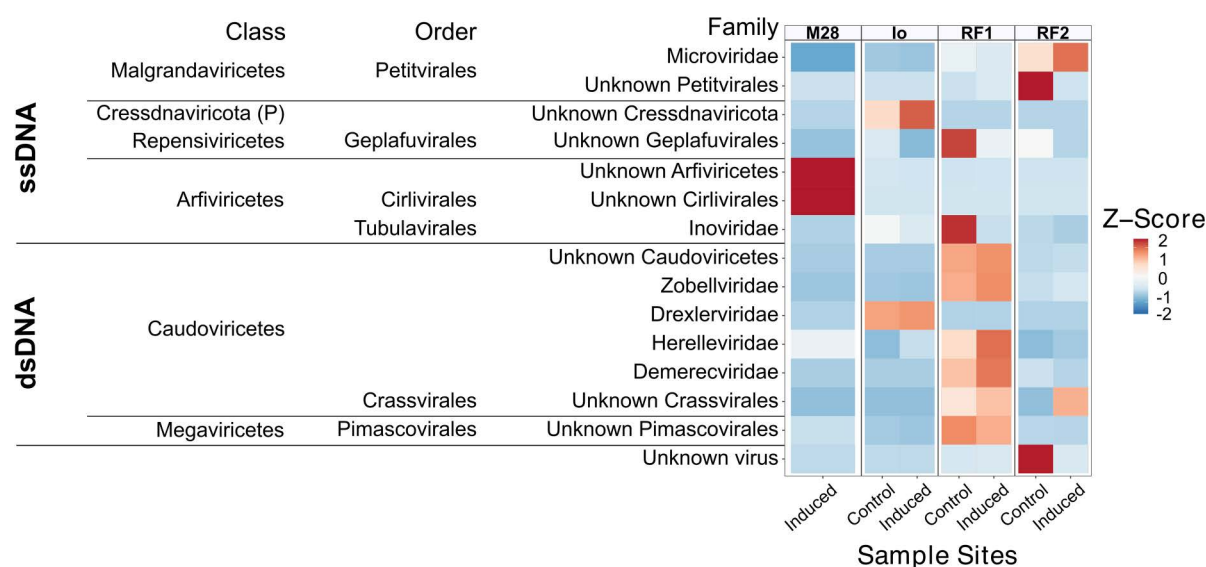


Fig 4. Heatmap of the z-score of the vOTU population abundance across each sampling site. The viral assemblages at each site, while sharing 41% of all viruses, differ in their population abundance. Red indicates population abundances above the mean, while blue indicates population abundances below the mean in units of standard deviations.

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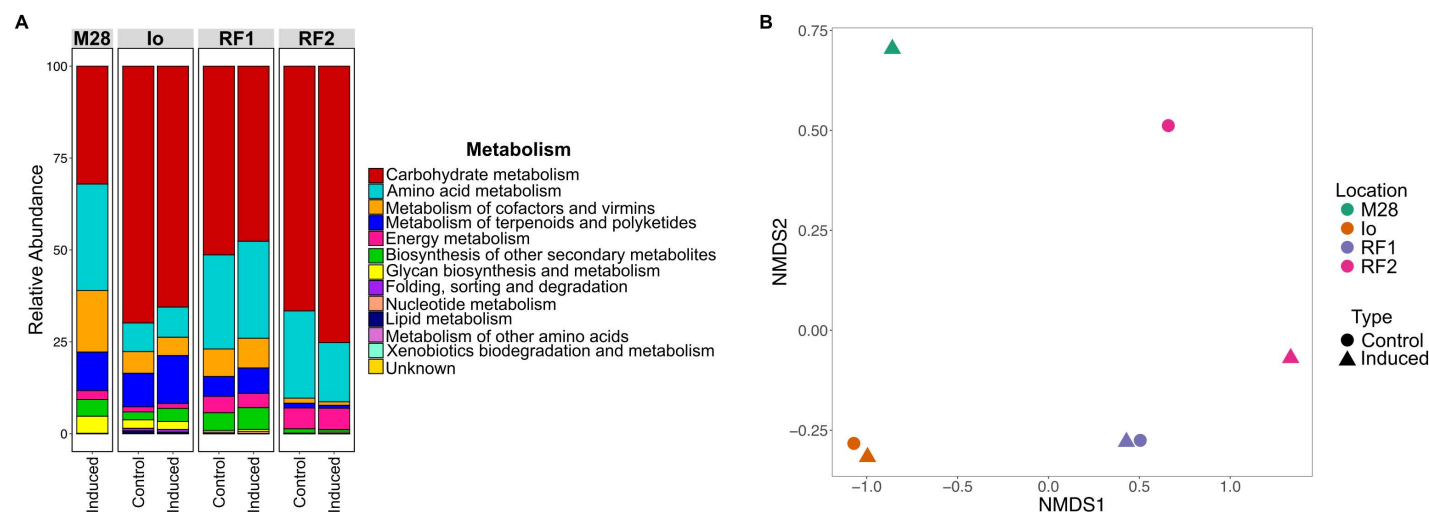


Fig 5. Comparison of virally-encoded genes. A) Relative abundances of virally-encoded metabolisms. Stacked bar histogram of the various metabolisms present within the vOTUs. The relative abundance of each AMG is commensurate with the relative abundance of the associated vOTU. B) Bray-Curtis NMDS of AMGs. The distribution of AMGs at each of the sample sites were compared using a Bray-Curtis dissimilarity matrix. As observed with the viral composition comparison, each site groups separately.

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Predicted hosts

A total of 68 high confidence hosts, encompassing 10 classes of Bacteria and two classes of Archaea, were predicted for the vOTUs, representing ~7.3% of the viral community (Fig 7A, B). Hosts were predicted for *Microviridae* (ssDNA), viruses from class Caudoviricetes (dsDNA), and viruses that were unclassified and reflected prevalent members of the

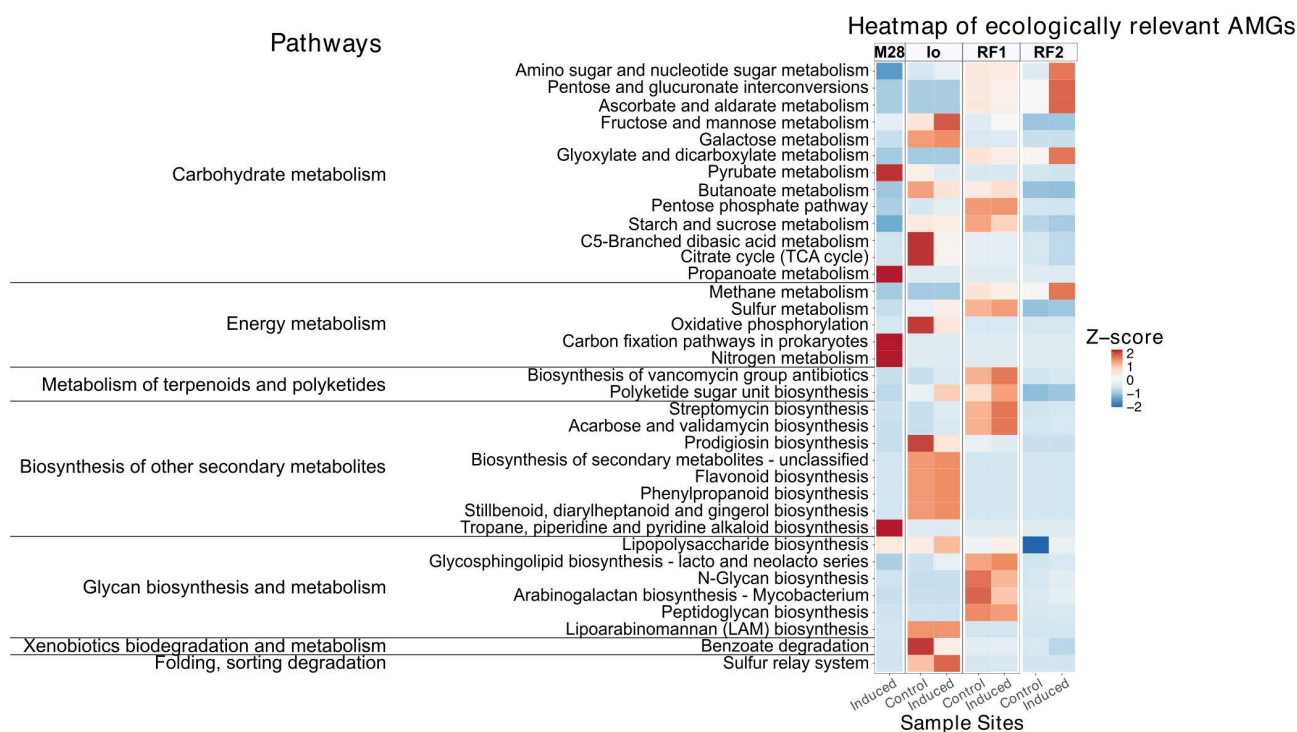


Fig 6. Heatmap of the different ecologically relevant metabolisms. The z-score was calculated using relative abundance across each of the AMGs to compare between the sites. Red indicates relative abundances above the mean, while blue indicates relative abundances below the mean in units of standard deviations.

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16S rRNA gene community (S1 Fig) with classes Gammaproteobacteria (27.1-34.6%, including order Methylococcales at 1.4-20.5%), Zetaproteobacteria (9.2-30.8%), Bacteroidia (11.7-19.5%), and Alphaproteobacteria (3.7-8.6%). The hosts predicted for *Microviridae* matched the bacterial classes Negativicutes and Bacteroidia. Viral class Caudoviricetes had predicted hosts from all classes present except for Negativicutes and Nitrososphaeria. Unclassified viruses had hosts predicted within Bacteroidia, Alphaproteobacteria, Gammaproteobacteria, and Nitrososphaeria.

There were several classes that were predicted to be hosts but not observed within the 16S rRNA gene community. These were Negativicutes, Thermoproteia (Archaea), Paceibacteria, and Actinomycetia that were present in both Marker 28 and Riftia Field sites. Note that the 16S rRNA primers used do not target Archaea, which explains the absence of Thermoproteia in the amplicon data. lo did not have a 16S rRNA gene community analysis for comparison, however, it displayed similar predicted hosts to the other samples. While the classes Negativicutes, Thermoproteia, Paceibacteria, and Actinomycetia were not explicitly determined as part of the 16S rRNA gene community, the associated phyla Firmicutes, Thermoproteota, Patescibacteria, and Actinobacteriota were all present at or below 1% relative abundance ("Others", S1 Fig and S1 Table).

Discussion

The viral community in microbial mats has the potential for influencing biogeochemical cycles

In this study, we sought to determine what viruses are present in iron-oxidizing microbial mat communities. These habitats are commonly found in hydrothermal vent fields at the base of sulfide chimneys and near/over diffuse hydrothermal vent flow [56]. Despite their high prevalence, their viral ecology has not previously been explored. We examined the natural

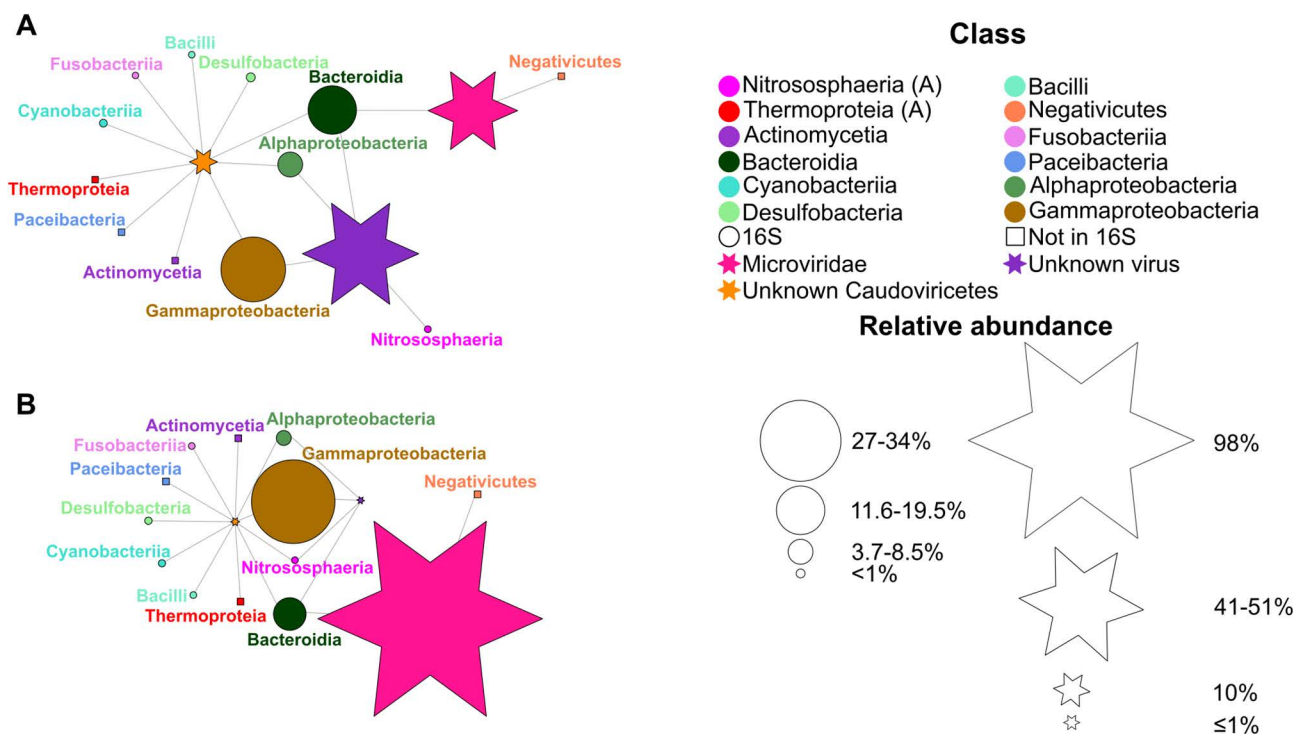


Fig 7. Network of predicted hosts. The predicted hosts and their associated viral families of Marker 28 (A) and an average across both control and induced samples of Riftia Field (B). Size and shape of host class (circles) was determined by its presence within the 16S rRNA gene community. If present, the class was represented by a circle and sized by the relative abundance associated with the 16S rRNA gene community. If the class was not present, the class was represented by a square using the smallest size (i.e., not associated with a relative abundance). The viruses, indicated by the star shape, are also expressed in relative abundance. Prokaryote host classes that contain an (A) are Archaea, ssDNA: Microviridae, dsDNA: Unknown Caudoviricetes, Unknown: unknown virus.

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viral community as well as the viral community after induction with mitomycin C. We found that, while 41% the viruses were shared between the three sites sampled here, there were differences in the relative abundances of vOTUs and AMG composition between the sites, and the AMGs detected were ecologically relevant for the iron-oxidizing mat community, which is exposed to reduced fluids and likely has organic carbon sticking to the iron-oxide matrix [20].

The AMGs abundant at each site highlight a variety of different metabolic pathways that indicate the potential to respond to different nutrient availability and environmental stresses on the microbial community. The viruses at the lo site displayed several mechanisms that capitalize on the use of sulfur in different ways, including the presence of the main metabolic pathway for assimilatory sulfate reduction, a pathway potentially providing electron donors for sulfate reducing bacteria [57], and the synthesis of cofactors that may enhance sulfur metabolism as well as other carbon and/or nitrogen metabolisms [58]. There was also the potential for viral manipulation of the cell envelope [59], which may increase cell membrane fluidity [60], consequently allowing easy passage of particular nutrients into the cell and fueling viral production. Further manipulation of host metabolism was possible with AMGs related to aldose scavenging [61] which could increase or supplement the carbon degradation repertoire of particular taxa, consequently manipulating carbon pools within the community. Additionally, AMGs related to a high-affinity complex for oxidative respiration [62–66] and heavy metal stress [67] were present in vOTUs at lo, indicating aerobic respiration could be maintained during infection, which could be advantageous during times when oxygen is limited. The host-virus interactions at the lo site suggests sulfur, low oxygen, heavy metals, and aldoses are ecologically important factors influencing to the microbial communities.

The AMG unique to Marker 28 site demonstrated the potential for manipulation of a variety of nutrient cycles including carbon, sulfur, and nitrogen. Viruses at Marker 28 site encoded AMGs that participate in the tricarboxylate acid (TCA) cycle [68], autotrophic carbon fixation [69,70], and central carbon utilization [71], all of which could be involved in energy production for the virus [72]. Assimilatory sulfate reduction to incorporate sulfide into cysteine was targeted by two different AMGs, *cysH* and *cysC*, that could be targeting rate-limiting steps [73]. Additionally, there was an AMG for benzoate degradation that has been found as an electron donor for sulfate reducing bacteria (SRB), providing an additional carbon pathway [74]. Viruses targeted nitrogen and/or carbon cycling through an AMG unique to Marker 28 (*gdhA*), which could be used in a dissimilatory pathway to generate α -ketoglutarate to fuel the TCA cycle or assimilatory pathway that generates nitrogen [75,76], which could be used to generate more proteins, via more amino acid production, by phages for creation of progeny [77]. Tolerance to oxidative stress and maintenance of redox homeostasis may be attributed to the AMGs *gloB* and *gloC*, which participate in a methylglyoxal detoxification pathway [78,79]. These AMGs may be involved in niche creation for an otherwise anaerobic taxon. Marker 28 viruses may be targeting abundant taxa in a kill-the-winner strategy, where the most locally abundant taxa are infected and lysed more frequently [8,80]. This could have an impact on central carbon pathways and carbon fixation, as well as SRB by directly affecting the metabolic functioning of the microbial community. Viruses may also be providing novel functions to different taxa creating niches to reduce competition and streamline viral production. Overall, Marker 28 site viral assemblages highlight carbon fixation and utilization pathways, expanding or providing oxidative stress tolerance, as well as sulfur and nitrogen cycling as ecologically important processes within the microbial community.

Riftia Field viruses harbored strategies involving carbon cycling, potentially methane and sulfur as well as manipulating community dynamics. There were several AMGs related to the degradation of carbohydrates (*UGDH*), participation in the pentose phosphate pathway (*pgl*) [81], and cellulolytic capabilities (*cbhA*) [82,83], all of which could be ways viruses are acquiring energy for viral production. In a similar strategy, there was an AMG (*hprA*) suggested to be integral in formaldehyde oxidation or as part of the serine cycle for methylotrophic bacteria [84]. Supporting data was provided by the detection of methylotrophic order Methylococcales in the 16S rRNA analysis from the same samples. Additionally, there was an AMG (*glyA*), which may have a similar function to *hprA* of shunting carbon into the pentose phosphate pathway for either nucleotide synthesis and generating reducing power [85] or it may participate in methane metabolism by catalyzing an intermediate for methanogenesis. Assimilatory sulfate reduction AMGs (*cysH*) were present at Riftia Field similarly to the other two sites, but viruses at Riftia Field had a unique set of glycan biosynthesis AMGs. There was one set of AMGs (*rffB/rffG*) involved in the production of vancomycin, streptomycin, and validomycin [86] and may either be imbuing resistance or a way to eliminate competition for resources [87]. Other glycan biosynthesis AMGs (*MGAT3*, *vanY*) may aid in biofilm formation through the creation of polysaccharides or increasing cell membrane fluidity [60] or can provide vancomycin resistance [88]. The combination of several AMGs related to remineralization of different carbohydrates as well as the presence of antimicrobials and resistance mechanisms may suggest that carbon is more limited at the Riftia Field site increasing the competition between the taxa [89]. Riftia Field is the only site where viruses have the potential ability to alter methane related metabolisms, which highlights this particular process as ecologically important at Riftia Field and but less so at the other sites.

Overall, the viral assemblages encoded genes involved in ecologically important functions within the microbial communities primarily related to carbon and sulfur rather than iron. While not statistically different, the virally-encoded AMGs were differentially distributed across sites, which is consistent with previous studies [25,90]. The temperatures between the sites were not drastically different, however, they do suggest different seawater and hydrothermal fluid interactions that could affect the geochemical gradients [91] producing a distinct microbial mat community and consequently viral assemblages in both composition and ecological functions within each site [90,92]. It is important to note that a large proportion of the community remained unclassified, with 29.1% – 99.6% vOTUs falling into “unknown” or “unclassified” consistent with other studies [90], which highlights the large and unexplored diversity within hydrothermal vents, but also makes it

challenging to know the various hosts and biogeochemical cycles they impact. Additionally, the long storage time may have biased the surviving microbial community towards hardier taxa, which could decouple the induction results from the 16S rRNA amplicons for the host composition, i.e., the potential reason we did not see any Zetaproteobacteria viruses or even iron-related AMGs. Moreover, mitomycin C does not exist naturally in marine ecosystems [5], which may result in underestimating inducible lysogens by limiting burst size, i.e., number of phage progeny released, and limiting the growth of the phage in general [93]. Finally, due to the long storage time and short incubation period after mitomycin C treatment the number of inducible lysogens may have been limited.

Regardless, each site contains a distinct viral assemblage that performs different ecological roles and contains unique AMGs that highlight the different environmental factors that could be affecting the host community. The large proportion of shared vOTUs is likely the reflection of how similar these iron-oxidizing mats are as compared to other hydrothermal vent communities found in sediments, cold seeps, or plumes that share very little of their viral communities [90]. There is the possibility that the viruses may have different host ranges depending on which environment, i.e., host population, is present [94], adding nuance even to very similar communities. While the majority of the vOTUs were classified as unknown/unclassified, there are still important virus–host interactions that give insight into the role viruses play within iron-oxidizing mats. The viral assemblages have the potential to expand host carbohydrate usage, alter nutrient cycling through energy metabolism, and affect the surrounding community with antibiotic resistance or production and biofilm formation. The role of viruses within these communities is likely underestimated as only a small fraction of the proteins present within the vOTUs were annotated. Further exploration of virus–host dynamics within iron-oxidizing mats in hydrothermal vent systems like the EPR aid in constraining a vital component of the deep ocean food web.

Host predictions highlight a broad range of affected taxa within iron oxidizing mats

Identifying the hosts of the vOTUs helps to contextualize the viral assemblages both in terms of which microbial members are present, and which potential metabolic functions are potentially being altered. If viruses are targeting active members of the community, determining which members and what potential functions they would be performing under infection would help to assess the impact these viruses have on the ecology of these iron-oxidizing mats. It may further reveal the unique structure of the different sampling sites and identify important virus–host interactions within the mat.

The identified host taxa represented a broad range of relative abundances from 50–55% (e.g., Classes Gammaproteobacteria, Bacteroidia, and Alphaproteobacteria), as well as less than 1% (e.g., phyla Firmicutes, Thermoproteota, Patescibacteria, and Actinobacteriota) suggesting the viruses can target both abundant and more rare taxa. The identification of an archaeal host may suggest that Archaea play an important role within iron-oxidizing mats albeit at low relative abundance as seen in other iron-oxidizing mats [19]. The low representation of archaeal ASVs within the 16S rRNA gene community reflects the use of bacterial-specific primers, which was done because they show less bias for these iron-oxidizing mats [95]. Gammaproteobacteria and Bacteroidia have been found to perform a variety of functions within iron-oxidizing microbial mats, including sulfur cycling, methanotrophy, aromatic carbon degradation, metal, and nitrogen cycling [96–100]. Viruses infecting these classes could have significant impacts to metabolic rates within the mat. The conspicuous absence of any Zetaproteobacteria hosts likely reflects both the novelty associated with the viral community with the majority only classified to the class or order level as well as a bias within the databases mined for host identification. Bias of the databases could also be the reason the predicted hosts were so similar across sites, and/or it could reflect how similar the microbial communities are themselves. Interestingly, lysogenic viruses were detected in Zetaproteobacteria-dominated iron-oxidizing mats at Kama’ehuakanaloa (formerly Loihi) Seamount by searching in MAGs [19]. In our work, only ~7.3% of the viral community had predicted hosts, which is consistent with other studies [90]. Combined, this indicates that much work is needed to improve host predictions for vOTU hosts in hydrothermal vent environments. Lack of knowledge about host specificity for vOTUs hampers our understanding of the potential impact of viruses within iron-oxidizing mats.

Conclusion

Hydrothermal vents are found in every ocean basin and are important ecosystems to the overlying water column in terms of biogeochemical cycles, and serve as the base of the food web in the dark ocean. Viruses reflect the environmental conditions and physiological state of their hosts. Viruses are top-down controls that affect the diversity and abundance of the microbial community. Assessing rates of mortality, as well as improving host association with whole community metagenomics, is crucial to constrain the role and impact of viruses and rates of primary productivity. Here, we described the genetic novelty found in the viral communities sampled from iron-oxidizing microbial mats at 9°50'N EPR. We showed that viral communities shared features but were specific to their site. Our work highlights important dynamics and host predictions to iron-oxidizing mats within the EPR and potentially other hydrothermal vent systems. Understanding the mechanisms that constrain and influence the viral communities has important implications to understanding and constraining microbial community processes and dynamics within hydrothermal vents that could affect primary productivity and consequently secondary production.

Supporting information

S1 Fig. Relative abundance of the 16S rRNA gene from Marker 28 and Riftia field sites.

(TIFF)

S1 Table. Amplicon sequence variant table of the 16S rRNA gene for Marker 28 and Riftia Field sites.

(CSV)

S1 File. S2-S5 Tables. Results from VIBRANT, checkV, genNomad, and iPhoP. Various tabular results from each of the softwares used to identify and classify vOTUs as well as predict hosts.

(XLSX)

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References

- Achberger AM, Jones R, Jamieson J, Holmes CP 2nd, Schubotz F, Meyer NR, et al. Inactive hydrothermal vent microbial communities are important contributors to deep ocean primary productivity. *Nat Microbiol*. 2024;9(3):657–68. <https://doi.org/10.1038/s41564-024-01599-9> PMID: [38287146](https://pubmed.ncbi.nlm.nih.gov/38287146/)
- Hu SK, Herrera EL, Smith AR, Pachiadaki MG, Edgcomb VP, Sylva SP, et al. Protistan grazing impacts microbial communities and carbon cycling at deep-sea hydrothermal vents. *Proc Natl Acad Sci U S A*. 2021;118(29):e2102674118. <https://doi.org/10.1073/pnas.2102674118> PMID: [34266956](https://pubmed.ncbi.nlm.nih.gov/34266956/)
- Dick GJ. The microbiomes of deep-sea hydrothermal vents: distributed globally, shaped locally. *Nat Rev Microbiol*. 2019;17(5):271–83. <https://doi.org/10.1038/s41579-019-0160-2> PMID: [30867583](https://pubmed.ncbi.nlm.nih.gov/30867583/)
- McNichol J, Stryhanyuk H, Sylva SP, Thomas F, Musat N, Seewald JS, et al. Primary productivity below the seafloor at deep-sea hot springs. *Proc Natl Acad Sci U S A*. 2018;115(26):6756–61. <https://doi.org/10.1073/pnas.1804351115> PMID: [29891698](https://pubmed.ncbi.nlm.nih.gov/29891698/)
- Breitbart M, Bonnain C, Malki K, Sawaya NA. Phage puppet masters of the marine microbial realm. *Nat Microbiol*. 2018;3(7):754–66. <https://doi.org/10.1038/s41564-018-0166-y> PMID: [29867096](https://pubmed.ncbi.nlm.nih.gov/29867096/)
- Obeng N, Pratama AA, Elsas JD van. The Significance of Mutualistic Phages for Bacterial Ecology and Evolution. *Trends Microbiol*. 2016;24(6):440–9. <https://doi.org/10.1016/j.tim.2015.12.009> PMID: [26826796](https://pubmed.ncbi.nlm.nih.gov/26826796/)
- Sime-Ngando T. Environmental bacteriophages: viruses of microbes in aquatic ecosystems. *Front Microbiol*. 2014;5:355. <https://doi.org/10.3389/fmicb.2014.00355> PMID: [25104950](https://pubmed.ncbi.nlm.nih.gov/25104950/)
- Suttle CA. Marine viruses—major players in the global ecosystem. *Nat Rev Microbiol*. 2007;5(10):801–12. <https://doi.org/10.1038/nrmicro1750> PMID: [17853907](https://pubmed.ncbi.nlm.nih.gov/17853907/)
- Anantharaman K, Duhaime MB, Breier JA, Wendt KA, Toner BM, Dick GJ. Sulfur oxidation genes in diverse deep-sea viruses. *Science*. 2014;344(6185):757–60. <https://doi.org/10.1126/science.1252229> PMID: [24789974](https://pubmed.ncbi.nlm.nih.gov/24789974/)
- He T, Li H, Zhang X. Deep-Sea Hydrothermal Vent Viruses Compensate for Microbial Metabolism in Virus-Host Interactions. *mBio*. 2017;8(4):e00893-17. <https://doi.org/10.1128/mBio.00893-17> PMID: [28698277](https://pubmed.ncbi.nlm.nih.gov/28698277/)
- Hu SK, Anderson RE, Pachiadaki MG, Edgcomb VP, Serres MH, Sylva SP, et al. Microbial eukaryotic predation pressure and biomass at deep-sea hydrothermal vents. *ISME J*. 2024;18(1):wrae004. <https://doi.org/10.1093/ismej/wrae004> PMID: [38366040](https://pubmed.ncbi.nlm.nih.gov/38366040/)
- Astorch-Cardona A, Odin GP, Chavagnac V, Dolla A, Gaussier H, Rommevaux C. Linking Zetaproteobacterial diversity and substratum type in iron-rich microbial mats from the Lucky Strike hydrothermal field (EMSO-Azores observatory). *Appl Environ Microbiol*. 2024;90(2):e0204123. <https://doi.org/10.1128/aem.02041-23> PMID: [38193671](https://pubmed.ncbi.nlm.nih.gov/38193671/)
- Chan CS, Emerson D, Luther GW 3rd. The role of microaerophilic Fe-oxidizing micro-organisms in producing banded iron formations. *Geobiology*. 2016;14(5):509–28. <https://doi.org/10.1111/gbi.12192> PMID: [27392195](https://pubmed.ncbi.nlm.nih.gov/27392195/)
- Emerson D. Biogeochemistry and microbiology of microaerobic Fe(II) oxidation. *Biochem Soc Trans*. 2012;40(6):1211–6. <https://doi.org/10.1042/BST20120154> PMID: [23176456](https://pubmed.ncbi.nlm.nih.gov/23176456/)
- Hribovšek P, Olesin Denny E, Dahle H, Mall A, Øfstegaard Viflot T, Boonnawa C, et al. Putative novel hydrogen- and iron-oxidizing sheath-producing Zetaproteobacteria thrive at the Fåvne deep-sea hydrothermal vent field. *mSystems*. 2023;8(6):e00543-23. <https://doi.org/10.1128/msystems.00543-23> PMID: [37921472](https://pubmed.ncbi.nlm.nih.gov/37921472/)
- Edwards KJ, Glazer BT, Rouxel OJ, Bach W, Emerson D, Davis RE, et al. Ultra-diffuse hydrothermal venting supports Fe-oxidizing bacteria and massive amber deposition at 5000 m off Hawaii. *ISME J*. 2011;5(11):1748–58. <https://doi.org/10.1038/ismej.2011.48> PMID: [21544100](https://pubmed.ncbi.nlm.nih.gov/21544100/)
- Fullerton H, Hager KW, McAllister SM, Moyer CL. Hidden diversity revealed by genome-resolved metagenomics of iron-oxidizing microbial mats from Lō'ihi Seamount, Hawai'i. *ISME J*. 2017;11(8):1900–14. <https://doi.org/10.1038/ismej.2017.40> PMID: [28362721](https://pubmed.ncbi.nlm.nih.gov/28362721/)
- McAllister SM, Moore RM, Gartman A, Luther GW, Emerson D, Chan CS. The Fe(II)-oxidizing Zetaproteobacteria: historical, ecological and genomic perspectives. *FEMS Microbiol Ecol*. 2019;95(4):fiz015. <https://doi.org/10.1093/femsec/fiz015> PMID: [30715272](https://pubmed.ncbi.nlm.nih.gov/30715272/)
- McAllister SM, Vandzura R, Keffer JL, Polson SW, Chan CS. Aerobic and anaerobic iron oxidizers together drive denitrification and carbon cycling at marine iron-rich hydrothermal vents. *ISME J*. 2021;15(5):1271–86. <https://doi.org/10.1038/s41396-020-00849-y> PMID: [33328652](https://pubmed.ncbi.nlm.nih.gov/33328652/)
- Chan CS, Fakra SC, Emerson D, Fleming EJ, Edwards KJ. Lithotrophic iron-oxidizing bacteria produce organic stalks to control mineral growth: implications for biosignature formation. *ISME J*. 2011;5(4):717–27. <https://doi.org/10.1038/ismej.2010.173> PMID: [21107443](https://pubmed.ncbi.nlm.nih.gov/21107443/)
- Bennett SA, Toner BM, Barco R, Edwards KJ. Carbon adsorption onto Fe oxyhydroxide stalks produced by a lithotrophic iron-oxidizing bacteria. *Geobiology*. 2014;12(2):146–56. <https://doi.org/10.1111/gbi.12074> PMID: [24428517](https://pubmed.ncbi.nlm.nih.gov/24428517/)
- Zimmerman AE, Howard-Varona C, Needham DM, John SG, Worden AZ, Sullivan MB, et al. Metabolic and biogeochemical consequences of viral infection in aquatic ecosystems. *Nat Rev Microbiol*. 2020;18(1):21–34. <https://doi.org/10.1038/s41579-019-0270-x> PMID: [31690825](https://pubmed.ncbi.nlm.nih.gov/31690825/)

23. Fuhrman JA. Marine viruses and their biogeochemical and ecological effects. *Nature*. 1999;399(6736):541–8. <https://doi.org/10.1038/21119> PMID: [10376593](#)
24. Wilhelm SW, Suttle CA. Viruses and nutrient cycles in the sea: Viruses play critical roles in the structure and function of aquatic food webs. *BioScience*. 1999;49:781–8. <https://doi.org/10.2307/1313569>
25. Anderson RE, Sogin ML, Baross JA. Evolutionary strategies of viruses, bacteria and archaea in hydrothermal vent ecosystems revealed through metagenomics. *PLoS One*. 2014;9(10):e109696. <https://doi.org/10.1371/journal.pone.0109696> PMID: [25279954](#)
26. Williamson SJ, Cary SC, Williamson KE, Helton RR, Bench SR, Winget D, et al. Lysogenic virus-host interactions predominate at deep-sea diffuse-flow hydrothermal vents. *ISME J*. 2008;2(11):1112–21. <https://doi.org/10.1038/ismej.2008.73> PMID: [18719614](#)
27. Fullerton H, Patel N, Syverson DD, Moyer CL, Sylvan JB. Establishing community structure and diversity within hydrothermal vent bacterial communities of the East Pacific Rise at 9°50'N. *Microbiol Spectr*. 2026;14:e00067-26. <https://doi.org/10.1128/spectrum.00067-26>
28. Fornari D, Von Damm K, Bryce J, Cowen J, Ferrini V, Fundis A, et al. The East Pacific Rise Between 9°N and 10°N: Twenty-Five Years of Integrated, Multidisciplinary Oceanic Spreading Center Studies. *Oceanog*. 2012;25(1):18–43. <https://doi.org/10.5670/oceanog.2012.02>
29. Williamson SJ, Houchin LA, McDaniel L, Paul JH. Seasonal variation in lysogeny as depicted by prophage induction in Tampa Bay, Florida. *Appl Environ Microbiol*. 2002. <https://doi.org/10.1128/AEM.68.9.4307-4314.2002>
30. Hager KW, Fullerton H, Butterfield DA, Moyer CL. Community structure of lithotrophically-driven hydrothermal microbial mats from the Mariana Arc and back-arc. *Front Microbiol*. 2017;8.
31. Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, et al. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res*. 2013;41(1):e1. <https://doi.org/10.1093/nar/gks808> PMID: [22933715](#)
32. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet J*. 2011;17:10–2.
33. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods*. 2016;13(7):581–3. <https://doi.org/10.1038/nmeth.3869> PMID: [27214047](#)
34. McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*. 2013;8(4):e61217. <https://doi.org/10.1371/journal.pone.0061217> PMID: [23630581](#)
35. Bushnell B, Rood J, Singer E. BBTools Software Package. *PLOS ONE*. 2017.
36. Li D, Luo R, Liu C-M, Leung C-M, Ting H-F, Sadakane K, et al. MEGAHIT v1.0: A fast and scalable metagenome assembler driven by advanced methodologies and community practices. *Methods*. 2016;102:3–11. <https://doi.org/10.1016/j.ymeth.2016.02.020> PMID: [27012178](#)
37. Kieft K, Zhou Z, Anantharaman K. VIBRANT: automated recovery, annotation and curation of microbial viruses, and evaluation of viral community function from genomic sequences. *Microbiome*. 2020;8(1):90. <https://doi.org/10.1186/s40168-020-00867-0> PMID: [32522236](#)
38. Fu L, Niu B, Zhu Z, Wu S, Li W. CD-HIT: accelerated for clustering the next-generation sequencing data. *Bioinformatics*. 2012;28(23):3150–2. <https://doi.org/10.1093/bioinformatics/bts565> PMID: [23060610](#)
39. Duhaime MB, Deng L, Poulos BT, Sullivan MB. Towards quantitative metagenomics of wild viruses and other ultra-low concentration DNA samples: a rigorous assessment and optimization of the linker amplification method. *Environ Microbiol*. 2012;14(9):2526–37. <https://doi.org/10.1111/j.1462-2920.2012.02791.x> PMID: [22713159](#)
40. Kim K-H, Bae J-W. Amplification methods bias metagenomic libraries of uncultured single-stranded and double-stranded DNA viruses. *Appl Environ Microbiol*. 2011;77(21):7663–8. <https://doi.org/10.1128/AEM.00289-11> PMID: [21926223](#)
41. Székely AJ, Breitbart M. Single-stranded DNA phages: from early molecular biology tools to recent revolutions in environmental microbiology. *FEMS Microbiol Lett*. 2016;363(6):fnw027. <https://doi.org/10.1093/femsle/fnw027> PMID: [26850442](#)
42. Vasimuddin Md, Misra S, Li H, Aluru S. Efficient Architecture-Aware Acceleration of BWA-MEM for Multicore Systems. In: 2019 IEEE International Parallel and Distributed Processing Symposium (IPDPS). 2019. p. 314–24.
43. Danecek P, Bonfield JK, Liddle J, Marshall J, Ohan V, Pollard MO, et al. Twelve years of SAMtools and BCFtools. *GigaScience*. 2021;10(2):giab008. <https://doi.org/10.1093/gigascience/giab008> PMID: [33590861](#)
44. Camargo AP, Roux S, Schulz F, Babinski M, Xu Y, Hu B, et al. Identification of mobile genetic elements with geNomad. *Nat Biotechnol*. 2023;42(8):1303–12. <https://doi.org/10.1038/s41587-023-01953-y> PMID: [37735266](#)
45. Knowles B, Bailey B, Boling L, Breitbart M, Cobián-Güemes A, Del Campo J, et al. Variability and host density independence in inductions-based estimates of environmental lysogeny. *Nat Microbiol*. 2017;2:17064. <https://doi.org/10.1038/nmicrobiol.2017.64> PMID: [28452987](#)
46. Köstner N, Jürgens K, Labrenz M, Herndl GJ, Winter C. Uneven host cell growth causes lysogenic virus induction in the Baltic Sea. *PLoS One*. 2019;14(8):e0220716. <https://doi.org/10.1371/journal.pone.0220716> PMID: [31386696](#)
47. Roux S, Camargo AP, Coutinho FH, Dabdoub SM, Dutilh BE, Nayfach S, et al. iPHoP: An integrated machine learning framework to maximize host prediction for metagenome-derived viruses of archaea and bacteria. *PLoS Biol*. 2023;21(4):e3002083. <https://doi.org/10.1371/journal.pbio.3002083> PMID: [37083735](#)
48. Ahlgren NA, Ren J, Lu YY, Fuhrman JA, Sun F. Alignment-free \$d_2^{*}\$ oligonucleotide frequency dissimilarity measure improves prediction of hosts from metagenomically-derived viral sequences. *Nucleic Acids Res*. 2017;45(1):39–53. <https://doi.org/10.1093/nar/gkw1002> PMID: [27899557](#)
49. Coutinho FH, Zaragoza-Solas A, López-Pérez M, Barylski J, Zieleszinski A, Dutilh BE, et al. RaFAH: Host prediction for viruses of Bacteria and Archaea based on protein content. *Patterns (N Y)*. 2021;2(7):100274. <https://doi.org/10.1016/j.patter.2021.100274> PMID: [34286299](#)

50. Galiez C, Siebert M, Enault F, Vincent J, Söding J. WisH: who is the host? Predicting prokaryotic hosts from metagenomic phage contigs. *Bioinformatics*. 2017;33(19):3113–4. <https://doi.org/10.1093/bioinformatics/btx383> PMID: [28957499](https://pubmed.ncbi.nlm.nih.gov/28957499/)
51. Lu C, Zhang Z, Cai Z, Zhu Z, Qiu Y, Wu A. Prokaryotic virus Host Predictor: a Gaussian model for host prediction of prokaryotic viruses in metagenomics. *bioRxiv*. 2020;2020.12.02.408310. <https://doi.org/10.1101/2020.12.02.408310>
52. Oksanen J, Simpson GL, Blanchet FG, Kindt R, Legendre P, Minchin PR, et al. *vegan: Community Ecology Package*. 2022. Available: <https://cran.r-project.org/package=vegan>
53. RStudio Team. *RStudio: Integrated Development for R*. Boston (MA): RStudio. PBC; 2020. Available: <http://www.rstudio.com/>
54. Paradis E, Schliep K. *ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R*. *Bioinformatics*. 2019;35(3):526–8. <https://doi.org/10.1093/bioinformatics/bty633> PMID: [30016406](https://pubmed.ncbi.nlm.nih.gov/30016406/)
55. Wickham H. *ggplot2: Elegant Graphics for Data Analysis*. New York: Springer-Verlag; 2016. Available: <https://ggplot2.tidyverse.org>
56. McAllister SM, Davis RE, McBeth JM, Tebo BM, Emerson D, Moyer CL. Biodiversity and emerging biogeography of the neutrophilic iron-oxidizing Zetaproteobacteria. *Appl Environ Microbiol*. 2011;77(15):5445–57. <https://doi.org/10.1128/AEM.00533-11> PMID: [21666021](https://pubmed.ncbi.nlm.nih.gov/21666021/)
57. Tsiola A, Michoud G, Daffonchio D, Fodelianakis S, Giannakourou A, Malliarakis D, et al. Depth-driven patterns in lytic viral diversity, auxiliary metabolic gene content, and productivity in offshore oligotrophic waters. *Front Microbiol*. 2023;14:1271535. <https://doi.org/10.3389/fmicb.2023.1271535> PMID: [38029212](https://pubmed.ncbi.nlm.nih.gov/38029212/)
58. Hasnat MA, Leimkühler S. Shared functions of Fe-S cluster assembly and Moco biosynthesis. *Biochim Biophys Acta Mol Cell Res*. 2024;1871(5):119731. <https://doi.org/10.1016/j.bbamcr.2024.119731> PMID: [38631442](https://pubmed.ncbi.nlm.nih.gov/38631442/)
59. Guerin ME, Kaur D, Somashekar BS, Gibbs S, Gest P, Chatterjee D, et al. New insights into the early steps of phosphatidylinositol mannoside biosynthesis in mycobacteria: PimB' is an essential enzyme of *Mycobacterium smegmatis*. *J Biol Chem*. 2009;284(38):25687–96. <https://doi.org/10.1074/jbc.M109.030593> PMID: [19638342](https://pubmed.ncbi.nlm.nih.gov/19638342/)
60. Huang D, Xia R, Chen C, Liao J, Chen L, Wang D, et al. Adaptive strategies and ecological roles of phages in habitats under physicochemical stress. *Trends Microbiol*. 2024;32(9):902–16. <https://doi.org/10.1016/j.tim.2024.02.002> PMID: [38433027](https://pubmed.ncbi.nlm.nih.gov/38433027/)
61. Skoog A, Vlahos P, Rogers KL, Amend JP. Concentrations, distributions, and energy yields of dissolved neutral aldoses in a shallow hydrothermal vent system of Vulcano, Italy. *Organic Geochem*. 2007;38(8):1416–30. <https://doi.org/10.1016/j.orggeochem.2007.03.005>
62. Thöny-Meyer L, Beck C, Preisig O, Hennecke H. The ccoNOQP gene cluster codes for a cb-type cytochrome oxidase that functions in aerobic respiration of *Rhodobacter capsulatus*. *Mol Microbiol*. 1994;14(4):705–16. <https://doi.org/10.1111/j.1365-2958.1994.tb01308.x> PMID: [7891558](https://pubmed.ncbi.nlm.nih.gov/7891558/)
63. Dong Y, Sanford RA, Inskeep WP, Srivastava V, Bulone V, Fields CJ. Physiology, metabolism, and fossilization of hot-spring filamentous microbial mats. *Astrobiology*. 2019;19:1442–58. <https://doi.org/10.1089/ast.2018.1965>
64. Forte E, Giuffrè A. How bacteria breathe in hydrogen sulfide-rich environments. *Biochemist*. 2016;38(5):8–11. <https://doi.org/10.1042/bio03805008>
65. Stolper DA, Revsbech NP, Canfield DE. Aerobic growth at nanomolar oxygen concentrations. *Proc Natl Acad Sci U S A*. 2010;107(44):18755–60. <https://doi.org/10.1073/pnas.1013435107> PMID: [20974919](https://pubmed.ncbi.nlm.nih.gov/20974919/)
66. Gazitúa MC, Vik DR, Roux S, Gregory AC, Bolduc B, Widner B, et al. Potential virus-mediated nitrogen cycling in oxygen-depleted oceanic waters. *ISME J*. 2021;15(4):981–98. <https://doi.org/10.1038/s41396-020-00825-6> PMID: [33199808](https://pubmed.ncbi.nlm.nih.gov/33199808/)
67. Cheng Z, He Y, Wang Y, Yang X, Chen J, Wu L, et al. Interactive dynamics between rhizosphere bacterial and viral communities facilitate soybean fitness to cadmium stress revealed by time-series metagenomics. *Soil Biol Biochem*. 2024;190:109313. <https://doi.org/10.1016/j.soilbio.2024.109313>
68. Kather B, Stingl K, van der Rest ME, Altendorf K, Molenaar D. Another unusual type of citric acid cycle enzyme in *Helicobacter pylori*: the malate:quinone oxidoreductase. *J Bacteriol*. 2000;182(11):3204–9. <https://doi.org/10.1128/JB.182.11.3204-3209.2000> PMID: [10809701](https://pubmed.ncbi.nlm.nih.gov/10809701/)
69. Ramos-Vera WH, Weiss M, Strittmatter E, Kockelkorn D, Fuchs G. Identification of missing genes and enzymes for autotrophic carbon fixation in crenarchaeota. *J Bacteriol*. 2011;193(5):1201–11. <https://doi.org/10.1128/JB.01156-10> PMID: [21169482](https://pubmed.ncbi.nlm.nih.gov/21169482/)
70. Wang Y, Ferrinho S, Connaris H, Goss RJM. The Impact of Viral Infection on the Chemistries of the Earth's Most Abundant Photosynthesizers: Metabolically Talented Aquatic Cyanobacteria. *Biomolecules*. 2023;13(8):1218. <https://doi.org/10.3390/biom13081218> PMID: [37627283](https://pubmed.ncbi.nlm.nih.gov/37627283/)
71. Slobodkina G, Allieux M, Merkel A, Cambon-Bonavita MA, Alain K, Jebbar M. Physiological and genomic characterization of a hyperthermophilic archaeon *Archaeoglobus neptunius* sp. nov. isolated from a deep-sea hydrothermal vent warrants the reclassification of the genus *Archaeoglobus*. *Front Microbiol*. 2021;12.
72. Howard-Varona C, Lindback MM, Bastien GE, Solonenko N, Zayed AA, Jang H, et al. Phage-specific metabolic reprogramming of virocells. *ISME J*. 2020;14(4):881–95. <https://doi.org/10.1038/s41396-019-0580-z> PMID: [31896786](https://pubmed.ncbi.nlm.nih.gov/31896786/)
73. Kieft K, Breister AM, Huss P, Linz AM, Zanetakos E, Zhou Z, et al. Virus-associated organosulfur metabolism in human and environmental systems. *Cell Rep*. 2021;36(5):109471. <https://doi.org/10.1016/j.celrep.2021.109471> PMID: [34348151](https://pubmed.ncbi.nlm.nih.gov/34348151/)
74. Wang H, Sun L. Comparative metagenomic analysis of the microbial communities in the surroundings of Iheya north and Iheya ridge hydrothermal fields reveals insights into the survival strategy of microorganisms in deep-sea environments. *J Mar Syst*. 2018;180:102–11. <https://doi.org/10.1016/j.jmarsys.2016.10.006>
75. Oliveira T, Sharkey MA, Engel PC, Khan AR. Crystal structure of a chimaeric bacterial glutamate dehydrogenase. *Acta Crystallogr F Struct Biol Commun*. 2016;72(Pt 6):462–6. <https://doi.org/10.1107/S2053230X16007305> PMID: [27303899](https://pubmed.ncbi.nlm.nih.gov/27303899/)

76. Riba L, Becerril B, Servín-González L, Valle F, Bolívar F. Identification of a functional promoter for the *Escherichia coli* *gdhA* gene and its regulation. *Gene*. 1988;71(2):233–46. [https://doi.org/10.1016/0378-1119\(88\)90040-6](https://doi.org/10.1016/0378-1119(88)90040-6) PMID: 2465204
77. Waldbauer JR, Coleman ML, Rizzo AI, Campbell KL, Lotus J, Zhang L. Nitrogen sourcing during viral infection of marine cyanobacteria. *Proc Natl Acad Sci U S A*. 2019;116(31):15590–5. <https://doi.org/10.1073/pnas.1901856116> PMID: 31308237
78. Reiger M, Lassak J, Jung K. Deciphering the role of the type II glyoxalase isoenzyme YcbL (GlxII-2) in *Escherichia coli*. *FEMS Microbiol Lett*. 2015;362(2):1–7. <https://doi.org/10.1093/femsle/fnu014> PMID: 25670698
79. Reva O, Messina E, La Cono V, Crisafi F, Smedile F, La Spada G, et al. Functional diversity of nanohaloarchaea within xylan-degrading consortia. *Front Microbiol*. 2023;14:1182464. <https://doi.org/10.3389/fmicb.2023.1182464> PMID: 37323909
80. Thingstad TF. Elements of a theory for the mechanisms controlling abundance, diversity, and biogeochemical role of lytic bacterial viruses in aquatic systems. *Limnol Oceanogr*. 2000;45(6):1320–8. <https://doi.org/10.4319/lo.2000.45.6.1320>
81. Thomason LC, Court DL, Datta AR, Khanna R, Rosner JL. Identification of the *Escherichia coli* K-12 *ybhE* gene as *pgl*, encoding 6-phosphogluconolactonase. *J Bacteriol*. 2004;186(24):8248–53. <https://doi.org/10.1128/JB.186.24.8248-8253.2004> PMID: 15576773
82. Pérez Castro S, Borton MA, Regan K, Hrabe de Angelis I, Wrighton KC, Teske AP, et al. Degradation of biological macromolecules supports uncultured microbial populations in Guaymas Basin hydrothermal sediments. *ISME J*. 2021;15(12):3480–97. <https://doi.org/10.1038/s41396-021-01026-5> PMID: 34112968
83. Meinke A, Gilkes NR, Kwan E, Kilburn DG, Warren RAJ, Miller JRC. Cellobiohydrolase A (CbhA) from the cellulolytic bacterium *Celulomonas fimi* is a β -1,4-exocellobiohydrolase analogous to *Trichoderma reesei* CBH II. *Mol Microbiol*. 1994;12:413–22. <https://doi.org/10.1111/j.1365-2958.1994.tb01030.x>
84. Chistoserdova LV, Lidstrom ME. Genetics of the serine cycle in *Methylobacterium extorquens* AM1: identification of *sgaA* and *mtaA* and sequences of *sgaA*, *hprA*, and *mtaA*. *J Bacteriol*. 1994;176(7):1957–68. <https://doi.org/10.1128/jb.176.7.1957-1968.1994> PMID: 8144463
85. Anderson CL, Sullivan MB, Fernando SC. Dietary energy drives the dynamic response of bovine rumen viral communities. *Microbiome*. 2017;5(1):155. <https://doi.org/10.1186/s40168-017-0374-3> PMID: 29179741
86. Brunel C, Beifen Y, Pouteau R, Li J, van Kleunen M. Responses of Rhizospheric Microbial Communities of Native and Alien Plant Species to *Cuscuta* Parasitism. *Microb Ecol*. 2020;79(3):617–30. <https://doi.org/10.1007/s00248-019-01438-z> PMID: 31598761
87. Farias P, Espírito Santo C, Branco R, Francisco R, Santos S, Hansen L, et al. Natural hot spots for gain of multiple resistances: arsenic and antibiotic resistances in heterotrophic, aerobic bacteria from marine hydrothermal vent fields. *Appl Environ Microbiol*. 2015;81(7):2534–43. <https://doi.org/10.1128/AEM.03240-14> PMID: 25636836
88. Meziane-Cherif D, Stogios PJ, Evdokimova E, Savchenko A, Courvalin P. Structural basis for the evolution of vancomycin resistance D, D-peptidases. *Proc Natl Acad Sci U S A*. 2014;111(16):5872–7. <https://doi.org/10.1073/pnas.1402259111> PMID: 24711382
89. Xu Q, Zhang H, Vandenkoornhuyse P, Guo S, Kuzyakov Y, Shen Q, et al. Carbon starvation raises capacities in bacterial antibiotic resistance and viral auxiliary carbon metabolism in soils. *Proc Natl Acad Sci U S A*. 2024;121(16):e2318160121. <https://doi.org/10.1073/pnas.2318160121> PMID: 38598339
90. Cheng R, Li X, Jiang L, Gong L, Geslin C, Shao Z. Virus diversity and interactions with hosts in deep-sea hydrothermal vents. *Microbiome*. 2022;10(1):235. <https://doi.org/10.1186/s40168-022-01441-6> PMID: 36566239
91. Wankel SD, Germanovich LN, Lilley MD, Genc G, DiPerna CJ, Bradley AS, et al. Influence of subsurface biosphere on geochemical fluxes from diffuse hydrothermal fluids. *Nature Geosci*. 2011;4(7):461–8. <https://doi.org/10.1038/ngeo1183>
92. Gao S, Paez-Espino D, Li J, Ai H, Liang J, Luo Z, et al. Patterns and ecological drivers of viral communities in acid mine drainage sediments across Southern China. *Nat Commun*. 2022;13(1):2389. <https://doi.org/10.1038/s41467-022-30049-5> PMID: 35501347
93. Santoro F, Pastore G, Fox V, Petit M-A, Iannelli F, Pozzi G. *Streptococcus pyogenes* Φ 1207.3 Is a Temperate Bacteriophage Carrying the Macrolide Resistance Gene Pair *mef(A)*-*msr(D)* and Capable of Lysogenizing Different *Streptococci*. *Microbiol Spectr*. 2023;11(1):e0421122. <https://doi.org/10.1128/spectrum.04211-22> PMID: 36625667
94. Hwang Y, Roux S, Coclet C, Krause SJE, Girguis PR. Viruses interact with hosts that span distantly related microbial domains in dense hydrothermal mats. *Nat Microbiol*. 2023;8(5):946–57. <https://doi.org/10.1038/s41564-023-01347-5> PMID: 37024618
95. Smith L, Fullerton H, Moyer CL. Complex hydrothermal vent microbial mat communities used to assess primer selection for targeted amplicon surveys from Kama'euhuanalao Seamount. *PeerJ*. 2024;12:e18099. <https://doi.org/10.7717/peerj.18099> PMID: 39301056
96. Emerson D, Fleming EJ, McBeth JM. Iron-Oxidizing Bacteria: An Environmental and Genomic Perspective. *Ann Rev Microbiol*. 2010;561–83. <https://doi.org/10.1146/annurev.micro.112408.134208>
97. Florentino AP, Weijma J, Stams AJM, Sánchez-Andrea I. Ecophysiology and Application of Acidophilic Sulfur-Reducing Microorganisms. *Grand Challenges in Biology and Biotechnology*. Springer International Publishing; 2016. p. 141–75.
98. Khomyakova MA, Merkel AY, Slobodkin AI. *Perlabentimonas gracilis* gen. nov., sp. nov., a gliding aerotolerant anaerobe of the order Bacteroidales, isolated from a terrestrial mud volcano. *Syst Appl Microbiol*. 2021;44:126245. <https://doi.org/10.1016/j.syapm.2021.126245>
99. Meier DV, Bach W, Girguis PR, Gruber-Vodicka HR, Reeves EP, Richter M, et al. Heterotrophic Proteobacteria in the vicinity of diffuse hydrothermal venting. *Environ Microbiol*. 2016;18(12):4348–68. <https://doi.org/10.1111/1462-2920.13304> PMID: 27001712
100. Wasmund K, Mußmann M, Loy A. The life sulfuric: microbial ecology of sulfur cycling in marine sediments. *Environ Microbiol Rep*. 2017;9(4):323–44. <https://doi.org/10.1111/1758-2229.12538> PMID: 28419734