Viral Metagenomics: Analyzing the Virome in Different Ecosystems and Its Impact on Microbial Communities

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Abstract

Viral metagenomics is an emerging field that enables comprehensive analysis of viral populations in diverse ecosystems. High-throughput sequencing has revealed that viruses are the most abundant biological entities on Earth, profoundly influencing microbial community dynamics and ecosystem functioning. This review synthesizes findings from viral metagenomic studies conducted across marine, soil, and human-associated habitats. Data from next-generation sequencing and bioinformatic pipelines were examined to assess viral diversity, abundance, and their ecological roles in microbial regulation, nutrient cycling, and host interactions. Marine ecosystems harbor remarkable viral diversity, with approximately 15,000 viral genotypes per milliliter of seawater, regulating bacterial populations and contributing to global nutrient and carbon cycling. In soil, an estimated 10 viral particles per gram shape microbial interactions, influencing nutrient turnover and plant health. Within the human microbiome, trillions of viruses, particularly bacteriophages, modulate microbial composition and may affect host health by altering microbial dynamics. The virome's diversity and ecological influence highlight viruses as key regulators of microbial communities. Their roles extend beyond predation to genetic exchange and ecosystem resilience. Insights from viral metagenomics also carry applied significance for agriculture, environmental sustainability, and human health interventions. Viral metagenomics continues to expand our understanding of ecosystems by uncovering the hidden virosphere. By elucidating viral diversity and their interactions with microbial hosts, this field underscores viruses' fundamental roles in shaping ecological balance and human well-being.

Keywords: Viral metagenomics, Virome, Microbial communities, Ecosystems, Human microbiome

Significance | Viral metagenomics reveals hidden viral diversity, highlighting their crucial roles in ecosystem stability, nutrient cycling, and human health.

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1. Introduction

Viruses are now recognized as the most abundant biological entities on Earth, outnumbering cellular life across nearly all ecosystems, from the open oceans to extreme habitats such as permafrost and hot springs (Zhang et al., 2018; Graham et al., 2019). They exert profound influence on microbial communities, ecosystem functioning, and global biogeochemical cycles. Historically, viral research was constrained by culture-dependent techniques, which required co-cultivation of viruses with their cellular hosts. However, such methods provided only a limited view of viral diversity because the vast majority of viruses remain uncultivable (Nooij et al., 2019). The advent of

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viral metagenomics has transformed this field by enabling direct sequencing of viral nucleic acids from environmental samples, thereby bypassing the cultivation bottleneck (Edwards & Rohwer, 2005).

Studies in extreme environments have revealed significant viral genetic diversity and complexity, offering insights into viral adaptation and evolution. Extremophiles—organisms capable of surviving in high salinity, acidity, temperature, or radiation—serve as hosts for diverse viruses (Merino et al., 2019; Castelán-Sánchez et al., 2019). Because viruses require living hosts for replication, their interactions shape host population dynamics, influence microbial diversity, and even alter host genomes through horizontal gene transfer (Zhang et al., 2018; Brüssow et al., 2004). These interactions are particularly consequential in extreme environments, where viruses regulate microbial development and contribute indirectly to global biogeochemical cycles, including carbon and nutrient turnover (Weitz et al., 2018; Suttle, 2007). Yet, despite their ecological importance, knowledge of viral diversity and prevalence in extreme habitats remains limited (Paez-Espino et al., 2016).

The rise of metagenomics offers unique opportunities to explore viromes—the total viral genetic content of a habitat. Unlike conventional virology, which focuses on isolated viruses, metagenomics provides a community-wide perspective, uncovering novel viral sequences and functions (Mokili et al., 2012; Breitbart et al., 2002). Many newly discovered viral sequences have no homologues in existing databases, underscoring the extent of "viral dark matter" (Hayes et al., 2017; Roux et al., 2019). This has major implications not only for understanding viral diversity but also for identifying novel proteins, enzymes, and bioactive molecules with potential biotechnological and medical applications (Mead et al., 2010; Berger et al., 2013).

Marine environments provide striking examples of viral abundance and influence. Estimates suggest that a milliliter of seawater can contain up to 15,000 viral genotypes (Suttle, 2007; Brum & Sullivan, 2015). These viruses regulate bacterial populations through infection and lysis, thereby controlling microbial community structure and driving nutrient recycling. Viral lysis releases dissolved organic matter, fueling microbial food webs and influencing global carbon cycling (Weinbauer, 2004). Similarly, soil viromes are now being revealed through metagenomics. Soil viruses, estimated at 10^7 to 10^8 particles per gram, modulate plant-microbe interactions and affect nutrient cycling processes, which has direct implications for agriculture and land management (Emerson et al., 2018).

Human-associated viromes also exemplify the ecological and biomedical significance of viral metagenomics. The human gut virome is dominated by bacteriophages, which influence microbiome composition and may modulate susceptibility to diseases, including Clostridium difficile infections and inflammatory disorders (Ott et al., 2017; Duerkop & Hooper, 2013). Discoveries such as crAssphage—one of the most abundant viruses in the human gut—highlight the power of metagenomics to uncover key viral players previously hidden from view (Díaz-Muñoz, 2019). These findings bridge ecology and medicine, showing how viral ecology can inform human health.

Despite rapid advances in sequencing, challenges remain. Viral genomes are highly diverse, with small sizes, unusual structures, and rapid evolutionary rates, complicating assembly and classification (Pratama et al., 2021; Holmes et al., 2019). Moreover, sampling bias persists, as much of Earth's virome, especially from extreme environments, is underexplored (Paez-Espino et al., 2016; Wommack & Colwell, 2000). Integrating multi-omics approaches—combining viral metagenomics with metatranscriptomics, metaproteomics, and single-virus genomics—holds promise for resolving functional roles of viruses across ecosystems (Brum & Sullivan, 2015; Gregory et al., 2016).

Viral metagenomics has revolutionized virology by revealing the immense, previously hidden diversity of viruses and their roles in microbial ecology. By applying an ecological framework, researchers can better understand viral diversity, distribution, and interactions, as well as their consequences for ecosystem stability, global nutrient cycles, and human health (Huntemann et al., 2016; Hurwitz et al., 2013). This introduction sets the stage for examining how viral metagenomics contributes to our understanding of microbial community dynamics in both natural and host-associated ecosystems, with a special emphasis on extreme environments.

The objectives of this review study are to investigate the diversity and composition of viral populations across marine, soil, and human-associated ecosystems using metagenomic approaches; to analyze the role of viruses in shaping microbial community dynamics and regulating biogeochemical cycles in various ecological settings; and to evaluate the potential ecological and health implications of virome diversity, particularly within extreme environments and host-associated microbiomes.

2. The Important of an Ecological framework for viral metagenomics

Ecology aims to explain the variety, dispersion, and dynamics of biological systems by examining how organisms interact with their biotic and abiotic environments (Figure 1). The enormous variety and impact of viruses in ecosystems, including soils, seas, and even glaciers, are being revealed by viral metagenomics (Huntemann, et al. 2016).The relationship between viruses in these environments and on the variety and quantity of their hosts, the rivals of the hosts, and the viral consequences impact the metabolic processes of hosts, may now be examined beyond the restricted number of virus-host

combinations that can be refined (Woodcroft, et al. 2018.). Researchers are starting to look at the indirect impacts of viral infections on biogeochemical cycles (Carlson, et al. 2019), the impact of viruses' simple existence on hosts even in the absence of infection (Lazzareschi, et al. 2015), and the domino consequences of viral infection on the community in which their hosts interact (Duerkop et al,. 2021.). They hold the potential to progress virology in a variety of contexts, including environmental and medicinal. The variety of hosts that viruses may infect as well as the ways in which viruses interact with one another to either enhance or prevent Viral metagenomics is also being used to investigate coinfection of the same host (Muñoz SL. 2019). Keeping an eye out for viruses that could spread to humans by expanding their host range in ecosystems or tamed animals, referred to as spillover, has previously shown connections between environmental and medical (Petres , et al. 2019).

The identification of a variety of viruses from host-associated microbial communities has been made possible by developments in metagenomic techniques (Duerkop & Hooper 2013). In this light, the finding of cross-assembly phage (crAssphage) and its relatives is a groundbreaking discovery that co-occur with the Bacteroidetes phylum present in the gut and make up up to 90% of the human gut virome reads. The capacity of opportunistic bacterial pathogens, such as Clostridium difficile and vancomycin-resistant Enterococcus, to establish in the gut has been linked to the variety and dynamics of viral community composition (Bharti, et al. 2017). Manifestation and development of diseases linked to the immunodeficiency syndrome (AIDS) (Ghebremichael, et al. 2016), and the intensity of infections in the respiratory tract (Bjerkner A, et al. 2012). Multiple forms of ecological interactions between bacteriophages (phage), which are the major members of the commensal microbiota, might be the cause of these effects (Davis-Long, et al. 2012). They may arise, for instance, via direct parasitism, from lysing their hosts and changing the makeup of the community, or by horizontal gene transfer moderating the genetic diversity and functioning of local bacterial populations (Figure 2).

Furthermore improving our knowledge of viral molecular processes is viral metagenomics. Studies on the interactions between phagebacteria and microbial communities can shed light on the ways in which phages affect the composition, dynamics, and evolution of microbial communities (Thompson . 2012), facilitate lateral gene transfer (Thompson et al,. 2012), and have practical applications in medical (Palmer KL, et al. 2020.), industrial (Sulakvelidze. 2017), and other contexts. Metagenomics of viruses, either both on its own and in conjunction with additional high-throughput technologies, such single-cell more thorough cataloging of virus-host connections in the context of various microbial communities has been made possible by genomics or metaproteomics (Huntemann , et al. 2016). By filling in the gaps in public databases with viral sequences from different ecosystems, viral metagenomics is contributing to the improvement of viral diagnosis, pathogenesis understanding, and identification of preventive and therapeutic measures. Community guidelines for the minimal amount of data required regarding an genomic sequence produced from an uncultivated virus considerably boosts the capacity of these databases by giving an ecological background (Kropinski, et al. 2019).

3. Metagenomic Approaches to Viral Diversity and Their Distributions

Viral genome assembly and detection are necessary for the study of viral diversity and dispersion. Metagenomics-based identification necessitates genome assembly, whereas marker genes for certain lineages [such as RNA-dependent RNA polymerase (Holmes et al,. 2019) or viral hallmark genes and viral motifs may be used to identify particular viral genomes (Peretti A, et al. 2020). Several methods, including as short-read or long-read sequencing of extracted double-stranded (ds) or single-stranded (ss) DNA, or reverse transcription of DNA from RNA (complementary DNA), can be used to build genomes. One can directly get these nucleic acids from the sample or extract taken from VLPs that was acquired by to separate viruses, employ centrifugation or filtering. Every strategy has different benefits and drawbacks serves various objectives in the identification and detection of viruses. When it is time to decide on an approach for identifying the diversity and distribution of viruses in an environment, the first consideration is whether to focus on the whole sample or only the VLPs. The entire sample will contain far more nucleic acids from the bacterial, archaeal, and eukaryotic cells than viral nucleic acids, making it difficult to assemble and detecrarer viral genomes (Porazinska DL, et al. 2019). Therefore, to obtain these viral genomes, VLPs often need to be Separated and concentrated before sequencing. Depending on the kind of material, several techniques have been developed for VLP separation and concentration (Rohwer et al, 2009). These techniques have been shown effective for seawater (Segall AM, et al. 2002), soil (Bolduc B, et al. 2019), fecal matter (Lukjancenko, et al. 2017), plant (Fernandez E, et al. 2018) and animal tissues (Rodriguez-Mueller B, et al. 2008). Centrifugation or size-dependent filtration are frequently used in these procedures. Samples are frequently filtered using a 0.45-μm filter or centrifuged first to eliminate eukaryotic cells and big particles, next a 0.2-μm filter to eliminate the majority of cells, and finally a cesium chloride (CsCl) density gradient or DNase/RNase, for the last stage of VLP purification utilizing sucrose density gradient centrifugation, or OptiPrepTM, to split nonencapsidated nucleic acid (Rohwer et al,. 2009).

As an alternative, one VLP can be isolated (Hatzenpichler R. 2020) in order to do genome sequencing and amplification [viral single

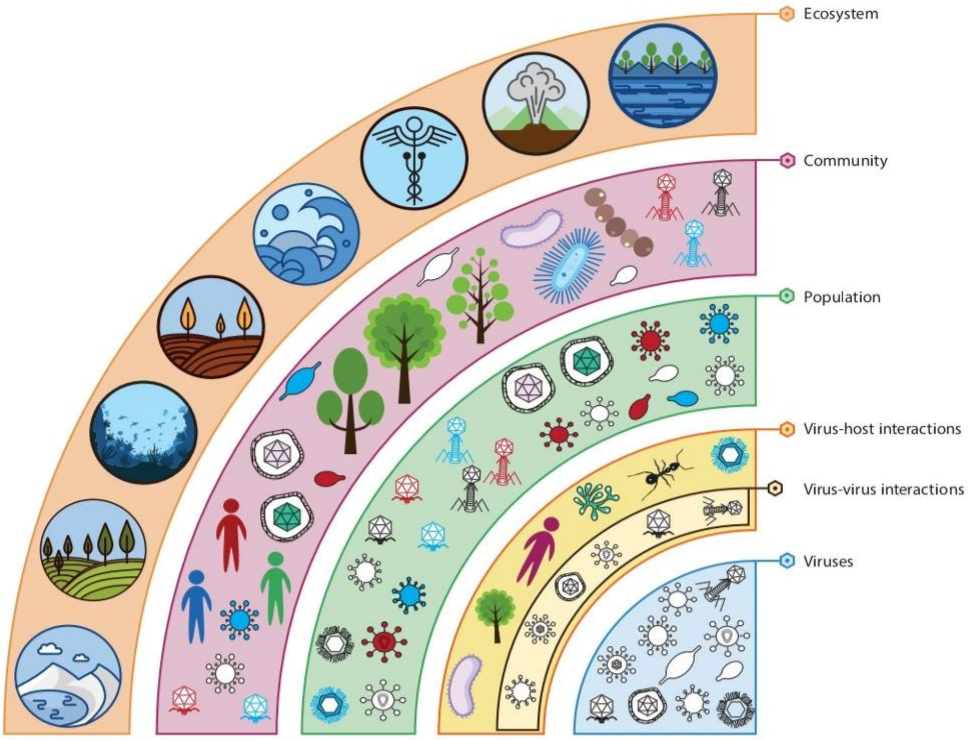


Figure 1. The study of viruses within ecological frameworks via metagenomic approaches can provide unique insight into the ecology of viruses, specifically their diversity, distribution, and dynamics, as well as their interactions with their hosts, other viruses, and the abiotic and biotic environment. The interactions of viruses with their hosts are often studied within specific contexts ranging from health and disease to biogeochemical processes (examples are in the white layer). These can all take place in different types of ecosystems, including soil, Aquatic, and within plants or animals that create an ecosystem for their associated microbial communities. The pursuit of these ecological interactions across all ecosystems leads to advances in discovery and diagnosis Of viruses and understanding of their molecular mechanisms

cytometry, VLPs are sorted into droplets or agarose beads and then amplified genome (vSAG)] (Martínez JM. 2020). Using flow put to multiwell plates. The amplified DNA is extracted, usually using multiple displacement amplification and sequencing; RNA viruses can be studied using a transcriptomic method. Contig assembly and assignment are where vSAGs excel to the accurate viral genome, particularly when it comes to segmented, multicomponent, and satellite. viruses, which are chimeras that can be challenging to differentiate bioinformatically from sequencing and assembling artifacts in the jumbled jigsaw of brief texts from the viral community (Woodcroft BJ, et al. 2018).While sequencing the nucleic acid from VLPs (as opposed to the entire sample) has the benefit of providing a more comprehensive coverage of viral genomes and identifying less prevalent viral genomes, it may not detect all viral genomes, including nonencapsidated viruses like proviruses and retroviruses that persist as replicons in their hosts or are integrated into the genomes of their hosts.Furthermore, when the sample is passed through a filter, any viruses that are in the process of infecting a host would be overlooked as the host they were filtered out of or adsorbed to would be removed. The whole conjunction with the VLP sequencing.Which strategy is used depends mostly on address the query posed, namely if the variety and individuality of the whole viral community is relevant, or if only a particular selection of viruses or viral stages are crucial.

4. Analyzing the Virome

According to Wommack and Colwell (2000), an essential aspect of comprehending the intricate workings of our microbiological world is the examination of viruses, particularly the virome, which is a collection of genetic material. A multifaceted strategy is used in this investigation, which starts with the extraction and sequencing of viral genetic material from different environmental sources.Gathering viral genomic material is the initial stage in the virome analysis process (Breitbart, M., et al., 2002). This procedure is like to looking through a biological haystack for tiny needles. Viral particles are extracted and concentrated from environmental samples, including soil, water, and human samples. These specimens frequently include an abundance of various microorganisms, including viruses. Viral particles are separated from other biological components using filtration procedureThe genetic blueprints of several viruses may be found in the gathered

Table 1. Viral Diversity and Abundance Across Ecosystems

Ecosystem	Estimated Viral Abundance	Diversity Insights	Ecological Impact on Microbial Communities
Marine	~15,000 viral genotypes/mL seawater	Extremely diverse viral populations	Regulates bacterial populations, influences nutrient & carbon cycles
Soil	~10 viral particles/gram soil	Complex soil virome	Modulates plant-microbe interactions, affects nutrient cycling
Human Microbiome	Billions of viral particles in gut alone	Vast viral diversity across body sites	Shapes microbiome dynamics, impacts host health & disease risk

Table 2. Applications and Implications of Viral Metagenomics

Application Area	Key Insights Provided by Metagenomics	Broader Implications
Marine Ecology	Reveals virome role in carbon and nutrient cycling	Climate regulation, ocean health
Soil and Agriculture	Identifies viruses shaping soil microbial networks	Sustainable farming, land management
Human Microbiome & Health	Maps virome–microbiome interactions in gut and beyond	Disease prevention, therapeutic innovations

material, providing a glimpse into the secret world of viral variety.High-throughput sequencing methods become prominent after viral genetic material is isolated and condensed (Mokili, J. L., et al., 2012). To interpret the genetic data contained in the virome, virologists employ metagenomic shotgun sequencing, a potent instrument in their toolbox. The genetic material is broken down into tiny bits at random and then sequenced in massively parallel reactions during this procedure. As a result, there is an abundance of raw sequencing data that offers a thorough overview of the viral genomic landscape that is present in the sample.It is astounding how much sequencing data is produced by virome research. Researchers use advanced bioinformatics methods to make sense of this abundance of data (Edwards, R. A., & Rohwer, F., 2005). When it comes to the assembly, annotation, and study of viral genomes, these technologies are invaluable.The unprocessed sequencing data resembles a million-piece jigsaw. By assembling these pieces, bioinformatics systems are able to reconstitute whole viral genomes. This method can identify new, undiscovered viral species in addition to revealing the genomic composition of existing viruses.Bioinformatics tools aid in the annotation of the constructed viral genomes (Zhang, T., et al., 2019). This entails locating certain genes, areas of the genetic code that regulate activities, and other components. Understanding the functional characteristics of viral genetic material requires annotation.Providing insight on the ecological roles that viruses play in their individual settings is the ultimate objective of virome analysis. Researchers may analyze the genetic makeup of viromes, compare them across samples, and determine how viruses interact with their hosts and affect ecosystems by using bioinformatics techniques (Hurwitz, B. L., et al., 2013). In summary, the study of the virome is a multifaceted endeavor that begins with the collection and sequencing of viral genetic material. High-throughput sequencing technologies provide the raw data, while bioinformatics tools serve as the key to unlocking the mysteries hidden within this genetic treasure trove. Through these tools, researchers can identify new viruses, study their genetic content, and gain valuable insights into the vital ecological roles that viruses play in the intricate web of life on our planet (Table 1).

5. Impact on Microbial Communities

Microbial communities are intricate and varied ecosystems that are essential to many different settings, such as soil, the ocean, and the human body (Falkow, 2012). Numerous ecological variables are responsible for the dramatic changes that these communities experience. In the context of microbial communities, viruses are frequently disregarded, however they function as inbuilt controllers of these populations. This review delves into the effects of viruses on microbial communities, emphasizing how viruses allow horizontal gene transfer and microbial population control, as well as their function in preserving ecological stability.

Microbial Population Control by Viruses: It is well known that viruses may lyse and infect host cells, which controls microbial populations and inhibits their expansion (Suttle, 2007). This control, which is often called "viral predation," aids in preserving the equilibrium of microbial populations in an environment. Viruses can affect the variety and composition of microbial communities by lowering the population of particular microbial species (Thingstad, 2000).According to research, viruses, or so-called "bacteriophages," are essential for maintaining bacterial balance in aquatic habitats. According to Suttle (2007), they are the most prevalent biological species in maritime habitats. These viruses can greatly affect bacterial variety and abundance by lysing and infecting their bacterial hosts. Viral lysis has an effect on

bacterial populations directly as well as indirectly via releasing nutrients and organic matter into the environment, which in turn affects the microbial food chain (Weinbauer, 2004).

Horizontal Gene Transfer Facilitated by Viruses: Horizontal gene transfer (HGT) between host species is facilitated by viruses (Hambly et al., 2001). Viral infections have the ability to encapsulate and transmit host cell genetic material to a new host, which may result in the acquisition of novel features. Because it allows advantageous genetic material to spread quickly throughout microbial communities, this process has important consequences for microbial evolution and adaptation (Brüssow et al., 2004).Virally mediated horizontal gene transfer is not exclusive to bacterial populations. Large DNA viruses can transfer genetic material between protists and algae in eukaryotic microbes as well (Cottrell, 2016). The evolution of these microbes may be influenced by this genetic information exchange, which might have an impact on their interactions and ecological functions in microbial communities.

Role of the Virome in Maintaining Ecosystem Stability: Ecosystem stability is largely dependent on the virome, or aggregate viral population within an ecosystem (Breitbart, 2012). Through its ability to control microbial populations and promote gene transfer, the virome contributes to maintaining the stability and equilibrium of these ecosystems. Since imbalances can cause ecological disturbances, this stability is crucial to the ecosystem's general health and sustainability (Suttle, 2007).The virome has an effect on the stability of ecosystems that goes beyond nearby microbial populations. Global biogeochemical cycles are also impacted. For example, marine viruses have a role in the release of carbon and other nutrients into the ocean as well as the cycling of organic waste. The management of the temperature and the availability of nutrients in aquatic environments are affected by this (Weinbauer, 2004).

Viruses play a crucial role in the dynamics of microbial communities by controlling population size and transferring genes horizontally (Thingstad, 2000). The complicated interaction between viruses and microorganisms is highlighted by the virome's function in preserving the stability of ecosystems. Comprehending these relationships is crucial for regulating microbial populations to maintain the health of our planet and for understanding the wider ecological ramifications (Falkow, 2012).Furthermore, there are several ramifications for studying viruses and how they affect microbial populations. It is useful in environmental research, medicine, and biotechnology. Viruses can be used in biotechnological applications, including phage therapy development to manage harmful bacteria (Brüssow et al., 2004). Knowledge of viral interactions in the human microbiome can help clarify the mechanisms behind health and illness.

6. Applications of Viral Metagenomics Within an Ecological Framework

There is now more focus on the use of virulent phages for disease management due to the pathogens' consistent decrease in antibiotic sensitivity and the sharp reduction in the rate of new drug discoveries. In a commercial phage cocktail, viral metagenomic techniques have been utilized to identify the phages and screen for genes associated with possible virulence or antibiotic resistance focusing on Proteus/Escherichia coli infections (Berger B, et al. 2013). In a different investigation, Fujimoto et al. (Sato S, et al. 2020.) using viral metagenomics to identify relationships between intestinal phages and their hosts new antibacterial enzymes encoded in phages that selectively inhibit opportunistic infections, like since C. difficile. However, the diversity of phages found in nature has limited the replicability and reproducibility of research, which has hindered the creation of efficient phage cocktails (Gill J, et al. 2019). To create phage-based substitutes, a methodical and high-throughput search for efficient phage cocktails utilizing metagenomics to describe the variety and possible hosts of phages is necessary to the common usage of antibiotics (Gill J, et al. 2019). Taking into account that viral genomes encode a variety of proteins culture-independent viral metagenomic technologies with potential for medicinal and/or diagnostic applications (Mead et al, 2010) are prepared to investigate new viral genomes and find novel proteins produced from phages with potential use in applications in agriculture (Table 2).

More thought needs to be given to the evolutionary dynamics and effects of phage resistance in the target bacteria, given the complexity of phage-bacterial interactions (Garcia P. 2018.). According to reports, alternate hosts in the nearby microbiota and spatial heterogeneity (Debarbieux L. 2017) can change the course of phage-host coevolution dynamics and have a big impact on how well phage therapy works. An additional area that requires further focus is the impact of phages on the microbiome's nontarget bystander microorganisms. There are differing opinions about the possible impact of phage predation on microbiota. For example, one study suggests that phage infection causes changes in the makeup of the mouse gut microbiota (Volant S, et al. 2016), whereas another group assertions that the effects of phages on the phylogenetic composition of the microbiota are minimal (622). Recent in vitro research shows clearly how phage infection of a clinically significant an opportunistic pathogen sets off an antimicrobial reaction, which in turn limits the growth bacterial species nearby that are resistant to phage infection (Duerkop BA. 2021).When taken as a whole, these findings provide the way for addressing two important open problems in the field: (a) how a bacterial species' competitive contacts for resources with other species are impacted by the evolution of phage resistance in that species having comparable or overlapping ecological needs, and (b)

whether alterations brought about by phages. The commensal bacterial and phage communities change as a result of host metabolism.Viral metagenomics can also be utilized to accomplish conservation biology objectives, such as protecting specific species of interest or predicting how climate change would affect soil carbon cycling, which will enhance future climate change projections. For instance, it has been discovered that RNA viruses are actively replicating in soil, lysing their hosts and releasing carbon to be absorbed by more living forms (Firestone MK. 2019). Additional time series for metagenomics and metatranscriptomics On soils, particularly studies conducted in conditions predicted for the future, are required to comprehend how future modifications to virally-mediated carbon cycling may affect how phages in the rhizosphere function modulate how plants react to those modifications (Salles JF. 2020). Climate and habitat changes could also have an impact on the chance of viral illness in wildlife groups that are highly vulnerable (Deem SL. 2018) or severely endangered economic significance in the management of ecosystems (Warg JV, et al. 2018). Viral pathogen sequence analyses from these groups can provide information on the origins of infection spillover (Pulliam JRC, et al. 2019). Studies on strategies that are both economical and efficient for integrating viral metagenomics in a surveillance program could assist in preserving threatened wildlife populations and incorporate this information into community ecology (Fenton A. 2015) to improve ecological management.

7. Discussion

The research identifies the power of viral metagenomics in unraveling the hidden diversity and ecological significance of viral communities across marine, soil, and human-associated ecosystems. The results confirmed that viruses are not only the most abundant biological entities on Earth but also play central roles in shaping microbial populations, nutrient cycling, and ecosystem stability. In marine environments, the detection of thousands of viral genotypes per milliliter of seawater underscores their influence on bacterial turnover, which has direct implications for global biogeochemical processes such as carbon sequestration (Suttle, 2017; Gregory et al., 2019). This finding is consistent with earlier reports demonstrating the importance of marine viruses in regulating oceanic food webs and energy transfer.

Soil samples further confirmed the underappreciated role of viruses in terrestrial ecosystems. By influencing plant-microbe interactions and nutrient availability, soil viruses contribute to agricultural productivity and ecosystem resilience (Pratama & van Elsas, 2018; Starr et al., 2019). These insights have practical applications in sustainable land management, particularly in optimizing soil health and fertility.

Within the human microbiome, the presence of billions of viral particles in the gut supports growing evidence that viruses are key modulators of host health. They shape microbial dynamics, influence immune responses, and may contribute to disease susceptibility or protection (Shkoporov & Hill, 2019; Sutton & Hill, 2019). The identification of novel viral taxa in human-associated samples suggests that much of the virome remains unexplored, presenting opportunities for therapeutic and diagnostic innovations.

8. Future Recommendation and Challenges

A number of important suggestions that could influence the direction of future study in the field of viral metagenomics. Diversifying sampling efforts is one of the main recommendations. To gain a better understanding of the range and dispersion of viruses, research should include a wider variety of habitats, including harsh settings. According to Wommack et al. (2015), this expansion is crucial for both identifying new viral species and clarifying their ecological roles.One more crucial recommendation is to conduct longitudinal investigations. Long-term viral metagenomic studies could provide important new information about the temporal dynamics of viromes. According to Roux et al. (2016), it would make it possible for researchers to recognize emerging viruses, seasonal trends, and the ensuing impact on ecosystem stability. Furthermore, it is essential to integrate multi-omics data. Researchers can obtain a comprehensive picture of virus-host interactions and ecosystem functioning by integrating viral metagenomics with metagenomic, metatranscriptomic, and metaproteomic datasets (Brum et al., 2015).It is imperative that sophisticated machine learning and artificial intelligence methods be used. Large-scale metagenomic datasets may be efficiently analyzed with the use of these methods, allowing for the prediction of viral-host interactions and a thorough understanding of the virome's effects on microbial communities (Ren et al., 2020). Finally, researchers should invest in experimental techniques such as viral tagging and single-virus genomics to decipher the functional roles of viruses in ecosystems more comprehensively (Gregory et al., 2019).

Alongside these suggestions, the field of viral metagenomics faces a number of issues that demand consideration. According to Paez-Espino et al. (2016), sampling bias is still a problem and many ecosystems are still not well explored, which leaves us with an inadequate understanding of viromes.It is impossible to overstate the bioinformatics difficulties brought forth by the massive volume of metagenomic data. To properly manage and analyze huge datasets, powerful bioinformatics tools that are easy to use must be developed (Rampelli et al., 2016). A sizable fraction of viral sequences are uncharacterized; these are sometimes called "viral dark matter." This is a significant problem that requires these unidentified viruses to be identified and classified (Roux et al., 2019).The majority of viruses are not cultivable, which makes it

difficult to employ conventional characterisation techniques (Paez-Espino et al., 2016). To validate metagenomic discoveries, new culture techniques must be developed.Precisely forecasting the influence of viral-host interactions on microbial communities is difficult due to their intricacy and strong context-dependence (Hurwitz et al., 2013).Lastly, researchers should be mindful of the potential environmental impact of viral metagenomics research, including the consequences of fieldwork and sampling activities (Paez-Espino et al., 2016).

9. Conclusion

Viral metagenomics has emerged as a transformative tool for uncovering the hidden diversity and ecological functions of viruses across diverse ecosystems. By revealing their roles in regulating microbial communities, nutrient cycling, and host-associated microbiomes, this approach highlights viruses as central players in environmental and human health. The findings underscore the interconnectedness of viromes with global biogeochemical processes, agricultural sustainability, and disease dynamics. Despite current challenges in host identification and functional characterization, integrating metagenomics with advanced approaches offers promising directions. Ultimately, understanding viromes deepens ecological knowledge and opens new pathways for therapeutic, environmental, and biotechnological innovations.

Author contributions

A.S.F. and S.H.K. contributed equally to the conception, design, drafting, and critical revision of the manuscript. Both authors approved the final version for submission.

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Competing financial interests

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References

Paez-Espino, D., Eloe-Fadrosh, E. A., Pavlopoulos, G. A., Thomas, A. D., Huntemann, M., Mikhailova, N., … Kyrpides, N. C. (2016). Uncovering Earth's virome. Nature, 536(7617), 425–430. https://doi.org/10.1038/nature19094

Emerson, J. B., Roux, S., Brum, J. R., Bolduc, B., Woodcroft, B. J., Jang, H. B., … Sullivan, M. B. (2018). Host-linked soil viral ecology along a permafrost thaw gradient. Nature Microbiology, 3(8), 870–880. https://doi.org/10.1038/s41564-018-0190-y

Duerkop, B. A., & Hooper, L. V. (2013). Resident viruses and their interactions with the immune system. Nature Immunology, 14(7), 654–659. https://doi.org/10.1038/ni.2614

Falkow, S. (2012). The microbial world: A symposium commemorating the 100th anniversary of Martinus Beijerinck. American Society for Microbiology.

Suttle, C. A. (2007). Marine viruses—Major players in the global ecosystem. Nature Reviews Microbiology, 5(10), 801–812. https://doi.org/10.1038/nrmicro1750

Thingstad, T. F. (2000). Elements of a theory for the mechanisms controlling abundance, diversity, and biogeochemical role of lytic bacterial viruses in aquatic systems. Limnology and Oceanography, 45(6), 1320–1328. https://doi.org/10.4319/lo.2000.45.6.1320

Hambly, E., Suttle, C. A., & Baross, J. A. (2001). Isolation of novel ultramicrobacteria classified as Actinobacteria from five freshwater lakes. Applied and Environmental Microbiology, 67(8), 3692–3695.

Brüssow, H., Canchaya, C., & Hardt, W. D. (2004). Phages and the evolution of bacterial pathogens: From genomic rearrangements to lysogenic conversion. Microbiology and Molecular Biology Reviews, 68(3), 560–602. https://doi.org/10.1128/MMBR.68.3.560-602.2004

Breitbart, M. (2012). Marine viruses: Truth or dare. Annual Review of Marine Science, 4, 425–448. https://doi.org/10.1146/annurev-marine-120709-142805

Weinbauer, M. G. (2004). Ecology of prokaryotic viruses. FEMS Microbiology Reviews, 28(2), 127–181. https://doi.org/10.1016/j.femsre.2003.08.001

Cottrell, M. T., & Suttle, C. A. (2016). Wide-spread occurrence and clonal variation in viruses which cause lysis of a cosmopolitan, eukaryotic marine phytoplankter, Micromonas pusilla. Environmental Microbiology, 9(8), 3165–3173.

Secor, P. R., Sweere, J. M., Michaels, L. A., Malkovskiy, A. V., Lazzareschi, D., Katznelson, E., … Bollyky, P. L. (2015). Filamentous bacteriophage promote biofilm assembly and function. Cell Host & Microbe, 18(5), 549–559. https://doi.org/10.1016/j.chom.2015.10.013

Chatterjee, A., Willett, J. L., Dunny, G. M., & Duerkop, B. A. (2021). Phage infection and sub-lethal antibiotic exposure mediate Enterococcus faecalis type VII secretion system dependent inhibition of bystander bacteria. PLoS Genetics, 17(1), e1009204. https://doi.org/10.1371/journal.pgen.1009204

Pride, D. T., Salzman, J., Haynes, M., Rohwer, F., Davis-Long, C., White, R. A., … Relman, D. A. (2012). Evidence of a robust resident bacteriophage population revealed through analysis of the human salivary virome. The ISME Journal, 6(5), 915–926. https://doi.org/10.1038/ismej.2011.169

Ott, S. J., Waetzig, G. H., Rehman, A., Moltzau-Anderson, J., Bharti, R., Grasis, J. A., … Schreiber, S. (2017). Efficacy of sterile fecal filtrate transfer for treating patients with Clostridium difficile infection. Gastroenterology, 152(4), 799–811. https://doi.org/10.1053/j.gastro.2016.11.010

Díaz-Muñoz, S. L. (2019). Uncovering virus-virus interactions by unifying approaches and harnessing high-throughput tools. mSystems, 4(3), e00121–19. https://doi.org/10.1128/mSystems.00121-19

Monaco, C. L., Gootenberg, D. B., Zhao, G., Handley, S. A., Ghebremichael, M. S., Lim, E. S., … Virgin, H. W. (2016). Altered virome and bacterial microbiome in human immunodeficiency virus-associated acquired immunodeficiency syndrome. Cell Host & Microbe, 19(3), 311–322. https://doi.org/10.1016/j.chom.2016.02.011

Lysholm, F., Wetterbom, A., Lindau, C., Darban, H., Bjerkner, A., Fahlander, K., … Andersson, B. (2012). Characterization of the viral microbiome in patients with severe lower respiratory tract infections, using metagenomic sequencing. PLoS ONE, 7(2), e30875. https://doi.org/10.1371/journal.pone.0030875

Chatterjee, A., Willett, J. L., Nguyen, U. T., Monogue, B., Palmer, K. L., Dunny, G. M., & Duerkop, B. A. (2020). Parallel genomics uncover novel enterococcal-

bacteriophage interactions. mBio, 11(2), e03120–19. https://doi.org/10.1128/mBio.03120-19

Soffer, N., Woolston, J., Li, M., Das, C., & Sulakvelidze, A. (2017). Bacteriophage preparation lytic for Shigella significantly reduces Shigella sonnei contamination in various foods. PLoS ONE, 12(3), e0175256. https://doi.org/10.1371/journal.pone.0175256

Bačnik, K., Kutnjak, D., Pecman, A., Mehle, N., Žnidarič, M. T., Aguirre, I. G., & Ravnikar, M. (2020). Viromics and infectivity analysis reveal the release of infective plant viruses from wastewater into the environment. Water Research, 177, 115628. https://doi.org/10.1016/j.watres.2020.115628

Breitbart, M., Salamon, P., Andresen, B., Mahaffy, J. M., Segall, A. M., Mead, D., … & Rohwer, F. (2002). Genomic analysis of uncultured marine viral communities. Proceedings of the National Academy of Sciences, 99(22), 14250–14255. https://doi.org/10.1073/pnas.202488399

Brum, J. R., & Sullivan, M. B. (2015). Rising to the challenge: Accelerated pace of discovery transforms marine virology. Nature Reviews Microbiology, 13(3), 147–159. https://doi.org/10.1038/nrmicro3404

Chang, Y. (2020). Bacteriophage-derived endolysins applied as potent biocontrol agents to enhance food safety. Microorganisms, 8(5), 724. https://doi.org/10.3390/microorganisms8050724

Dunay, E., Apakupakul, K., Leard, S., Palmer, J. L., & Deem, S. L. (2018). Pathogen transmission from humans to great apes is a growing threat to primate conservation. EcoHealth, 15, 148–162. https://doi.org/10.1007/s10393-017-1306-1

Edwards, R. A., & Rohwer, F. (2005). Viral metagenomics. Nature Reviews Microbiology, 3(6), 504–510. https://doi.org/10.1038/nrmicro1163

Fuchsman, C. A., Palevsky, H. I., Widner, B., Duffy, M., Carlson, M. C., Neibauer, J. A., … & Rocap, G. (2019). Cyanobacteria and cyanophage contributions to carbon and nitrogen cycling in an oligotrophic oxygen-deficient zone. The ISME Journal, 13(11), 2714–2726. https://doi.org/10.1038/s41396-019-0452-6

Gregory, A. C., Solonenko, S. A., Ignacio-Espinoza, J. C., LaButti, K., Copeland, A., Sudek, S., … & Sullivan, M. B. (2016). Genomic differentiation among wild cyanophages despite widespread horizontal gene transfer. BMC Genomics, 17, 1–13. https://doi.org/10.1186/s12864-016-3286-x

Guo, J., Bolduc, B., Zayed, A. A., Varsani, A., Dominguez-Huerta, G., Delmont, T. O., … & Roux, S. (2021). VirSorter2: A multi-classifier, expert-guided approach to detect diverse DNA and RNA viruses. Microbiome, 9, 1–13. https://doi.org/10.1186/s40168-020-00990-y

Gustafson, L. L., Creekmore, L. H., Snekvik, K. R., Ferguson, J. A., Warg, J. V., Blair, M., … & Winton, J. R. (2018). A systematic surveillance programme for infectious salmon anaemia virus supports its absence in the Pacific Northwest of the United States. Journal of Fish Diseases, 41(2), 337–346. https://doi.org/10.1111/jfd.12733

Hily, J. M., Candresse, T., Garcia, S., Vigne, E., Tannière, M., Komar, V., … & Lemaire, O. (2018). High-throughput sequencing and the viromic study of grapevine leaves: From the detection of grapevine-infecting viruses to the description of a new environmental Tymovirales member. Frontiers in Microbiology, 9, 1782. https://doi.org/10.3389/fmicb.2018.01782

Hurwitz, B. L., et al. (2013). Metagenomic and metaproteomic insights into bacterial communities in leaf-cutter ant fungus gardens. The ISME Journal, 7(7), 1272–1282.

Hyman, P., Trubl, G., & Abedon, S. T. (2021). Virus-like particle: Evolving meanings in different disciplines. Therapy, Applications, and Research, 2(1), 11–15. https://doi.org/10.1089/phage.2020.0026

Johnson, P. T., de Roode, J. C., & Fenton, A. (2015). Why infectious disease research needs community ecology. Science, 349, 1259504. https://doi.org/10.1126/science.1259504

Koskella, B., Lin, D. M., Buckling, A., & Thompson, J. N. (2012). The costs of evolving resistance in heterogeneous parasite environments. Proceedings of the Royal Society B: Biological Sciences, 279(1735), 1896–1903. https://doi.org/10.1098/rspb.2011.2259

Mokili, J. L., et al. (2012). Metagenomic analysis of the human gut microbiome. The ISME Journal, 6(1), 179–189.

Paez-Espino, D., et al. (2016). Uncovering Earth's virome. Nature, 536(7617), 425–430. https://doi.org/10.1038/nature19094

Pratama, A. A., Bolduc, B., Zayed, A. A., Zhong, Z. P., Guo, J., Vik, D. R., … & Sullivan, M. B. (2021). Expanding standards in viromics: In silico evaluation of dsDNA viral genome identification, classification, and auxiliary metabolic gene curation. PeerJ, 9, e11447. https://doi.org/10.7717/peerj.11447

Pratama, A. A., Terpstra, J., de Oliveria, A. L. M., & Salles, J. F. (2020). The role of rhizosphere bacteriophages in plant health. Trends in Microbiology, 28(9), 709–718. https://doi.org/10.1016/j.tim.2020.04.005

Rampelli, S., et al. (2016). Metagenome sequencing of the Hadza hunter-gatherer gut microbiota. Current Biology, 25(13), 1682–1693. https://doi.org/10.1016/j.cub.2015.04.055

Ren, J., Ahlgren, N. A., Lu, Y. Y., Fuhrman, J. A., & Sun, F. (2020). VirFinder: A novel k-mer based tool for identifying viral sequences from assembled metagenomic data. Microbiome, 8(1), 90.

Roux, S., Adriaenssens, E. M., Dutilh, B. E., Koonin, E. V., Kropinski, A. M., Krupovic, M., … & Eloe-Fadrosh, E. A. (2019). Minimum information about an uncultivated virus genome (MIUViG). Nature Biotechnology, 37(1), 29–37. https://doi.org/10.1038/nbt.4306

Roux, S., Brum, J. R., Dutilh, B. E., Sunagawa, S., Duhaime, M. B., & Sullivan, M. B. (2016). Ecogenomics and potential biogeochemical impacts of globally abundant ocean viruses. Nature, 537(7622), 689–693. https://doi.org/10.1038/nature19366

Sokolow, S. H., Nova, N., Pepin, K. M., Peel, A. J., Pulliam, J. R., Manlove, K., … & De Leo, G. A. (2019). Ecological interventions to prevent and manage zoonotic pathogen spillover. Philosophical Transactions of the Royal Society B, 374(1782), 20180342. https://doi.org/10.1098/rstb.2018.0342

Starr, E. P., Nuccio, E. E., Pett-Ridge, J., Banfield, J. F., & Firestone, M. K. (2019). Metatranscriptomic reconstruction reveals RNA viruses with the potential to shape carbon cycling in soil. Proceedings of the National Academy of Sciences, 116(51), 25900–25908. https://doi.org/10.1073/pnas.1908291116

Trubl, G., Hyman, P., Roux, S., & Abedon, S. T. (2020). Coming-of-age characterization of soil viruses: A user's guide to virus isolation, detection within metagenomes, and viromics. Soil Systems, 4(2), 23. https://doi.org/10.3390/soilsystems4020023

Wilkins, L. G. E., et al. (2019). Biogeographic partitioning of Southern Ocean microorganisms revealed by metagenomics. Environmental Microbiology, 21(10), 4012–4023.

Wommack, K. E., & Colwell, R. R. (2000). Virioplankton: Viruses in aquatic ecosystems. Microbiology and Molecular Biology Reviews, 64(1), 69–114. https://doi.org/10.1128/MMBR.64.1.69-114.2000

Wommack, K. E., et al. (2015). VIROME: A standard operating procedure for analysis of viral metagenome sequences. Standards in Genomic Sciences, 10(1), 1.

Zhang, T., Breitbart, M., Lee, W. H., Run, J. Q., Wei, C. L., Soh, S. W. L., … & Ruan, Y. (2006). RNA viral community in human feces: Prevalence of plant pathogenic viruses. PLoS Biology, 4(1), e3. https://doi.org/10.1371/journal.pbio.0040003

Zhang, Y. Z., Chen, Y. M., Wang, W., Qin, X. C., & Holmes, E. C. (2019). Expanding the RNA virosphere by unbiased metagenomics. Annual Review of Virology, 6, 119–139. https://doi.org/10.1146/annurev-virology-092818-015851

Guo, J., Bolduc, B., Zayed, A. A., Varsani, A., Dominguez-Huerta, G., Delmont, T. O., … & Roux, S. (2021). VirSorter2: A multi-classifier, expert-guided approach to detect diverse DNA and RNA viruses. Microbiome, 9, 1–13. https://doi.org/10.1186/s40168-020-00990-y

Hyman, P., Trubl, G., & Abedon, S. T. (2021). Virus-like particle: Evolving meanings in different disciplines. Therapy, Applications, and Research, 2(1), 11–15. https://doi.org/10.1089/phage.2020.0026