4-5-2023

# Astrovirology: How Viruses Enhance our Understanding of Life in the Universe

Gareth Trubl
*Lawrence Livermore National Laboratory*

Ken Stedman
*Portland State University*, kstedman@pdx.edu

Kathryn F. Bywaters
*Honeybee Robotics, Altadena, CA*

Emily E. Matula
*NASA Johnson Space Center, Houston, TX*

multiple additional authors



## Citation Details

Trubl, G., Stedman, K. M., Bywaters, K. F., Matula, E. E., Sommers, P., Roux, S., ... & Boston, P. J. (2023). Astrovirology: how viruses enhance our understanding of life in the Universe. International Journal of Astrobiology, 1-25.

*International Journal of Astrobiology* (2023), 1–25
doi:10.1017/S1473550423000058

RESEARCH ARTICLE

# Astrovirology: how viruses enhance our understanding of life in the Universe

Gareth Trubl[1], Kenneth M. Stedman[2], Kathryn F. Bywaters[3], Emily E. Matula[4], Pacifica Sommers[5], Simon Roux[6], Nancy Merino[1], John Yin[7], Jason T. Kaelber[8], Aram Avila-Herrera[9], Peter Anto Johnson[10], John Christy Johnson[10], Schuyler Borges[11], Peter K. Weber[1], Jennifer Pett-Ridge[1,12] and Penelope J. Boston[13]

[1]Physical and Life Sciences Directorate, Lawrence Livermore National Laboratory, Livermore, CA, USA
[2]Center for Life in Extreme Environments, Department of Biology, Portland State University, Portland, OR, USA
[3]Honeybee Robotics, Altadena, CA, USA
[4]NASA Johnson Space Center, Houston, TX, USA
[5]University of Colorado Boulder, Boulder, CO, USA
[6]DOE Joint Genome Institute, Berkeley, CA, USA
[7]Chemical and Biological Engineering, Wisconsin Institute for Discovery, University of Wisconsin-Madison, Madison, WI, USA
[8]Institute for Quantitative Biomedicine, Rutgers, the State University of New Jersey, Piscataway, NJ, USA
[9]Computing Directorate, Lawrence Livermore National Laboratory, Livermore, CA, USA
[10]Faculty of Medicine & Dentistry, University of Alberta, Edmonton, AB, Canada
[11]Northern Arizona University, Flagstaff, AZ, USA
[12]Life & Environmental Sciences Department, University of California Merced, Merced, CA, USA
[13]NASA Ames Research Center, Moffett Field, CA, USA
**Author for correspondence:** Gareth Trubl, E-mail: Trubl1@llnl.gov

**Received:** 28 September 2022; **Revised:** 5 February 2023; **Accepted:** 6 February 2023

**Keywords:** ecology, evolution, exobiology, life, origin, planetary protection, space

## Abstract

Viruses are the most numerically abundant biological entities on Earth. As ubiquitous replicators of molecular information and agents of community change, viruses have potent effects on the life on Earth, and may play a critical role in human spaceflight, for life-detection missions to other planetary bodies and planetary protection. However, major knowledge gaps constrain our understanding of the Earth's virosphere: (1) the role viruses play in biogeochemical cycles, (2) the origin(s) of viruses and (3) the involvement of viruses in the evolution, distribution and persistence of life. As viruses are the only replicators that span all known types of nucleic acids, an expanded experimental and theoretical toolbox built for Earth's viruses will be pivotal for detecting and understanding life on Earth and beyond. Only by filling in these knowledge and technical gaps we will obtain an inclusive assessment of how to distinguish and detect life on other planetary surfaces. Meanwhile, space exploration requires life-support systems for the needs of humans, plants and their microbial inhabitants. Viral effects on microbes and plants are essential for Earth's biosphere and human health, but virus–host interactions in spaceflight are poorly understood. Viral relationships with their hosts respond to environmental changes in complex ways which are difficult to predict by extrapolating from Earth-based proxies. These relationships should be studied in space to fully understand how spaceflight will modulate viral impacts on human health and life-support systems, including microbiomes. In this review, we address key questions that must be examined to incorporate viruses into Earth system models, life-support systems and life detection. Tackling these questions will benefit our efforts to develop planetary protection protocols and further our understanding of viruses in astrobiology.

## Contents

**Introduction** **2**
**Potential role(s) of viruses in the early stages of life** **5**
Future directions . . . 6
**Habitability, persistence and process limits of viruses and cellular life** **7**
Future directions . . . 10
**Biogeochemical cycling and biosignature detection** **10**
Future directions . . . 11
**Impact of viruses on human space exploration** **12**
Viral response to spaceflight environments . . . 12
Environmental control and life support systems . . . 13
Bioregenerative ECLSS . . . 13
Human physiology and the viral ecology of crewed spacecraft . . . 14
Spaceflight operational impacts . . . 15
Future directions . . . 15
**Detection of viruses on Earth and elsewhere** **16**
Future directions . . . 17
**Conclusion** **17**

## Introduction

Viruses are numerically abundant and an integral part of life on Earth. However, there is very little known about viruses across many of Earth's environments, including the many extreme habitats that serve as astrobiology analogues, when compared with the understanding of viruses relevant to human health. Even with respect to human health, relatively little is known about viral responses to the space environment beyond Earth's atmosphere. Additionally, almost no research has been conducted to identify the impact of these viruses on the spacecraft systems used to sustain humans. Understanding viruses and virus–host interactions in space-relevant contexts will inform our grasp of their role(s) in both human spaceflight missions, including environmental control and life support systems (ECLSS), and in the search for life elsewhere. Here, we review viruses in diverse Earth environments, the very limited studies conducted on viruses in space and lay out a roadmap for future research. For a more extensive overview of environmental viruses and introduction to viruses, see Berliner *et al*. (2018); for a specific review on the roles of viruses in spaceflight affecting human health, see Pavletic *et al*. (2022); for a complementary review on the need to incorporate viruses more in astrobiology, see De La Higuera and Lázaro (2022).

Viruses are sometimes defined as 'very small obligate intracellular parasites' (Acheson, 2011), and more broadly as 'entities whose genomes are elements of nucleic acids that replicate inside living cells using the cellular synthetic machinery and causing the synthesis of specialized elements that can transfer the viral genome to other cells' (Luria *et al*., 1978). These 'specialized elements' or virions are crucial to the definition of viruses. Viruses contain genetic information that can be transferred on massive scales due to the very large numbers of virions on Earth.

The sheer ubiquity of virions on Earth, with up to $10^{31}$ in the Earth's oceans alone (Suttle, 2005; Mushegian, 2020), makes them an attractive target for the search for life on other worlds if one only knew what to search for! Viruses that infect bacteria and archaea typically range in size from tens to a few hundred nanometres, while viruses that infect eukaryotic organisms (e.g. amoebae or humans) can even range from tens to thousands of nanometres (Fig. 1). These so-called giant viruses can be larger than some bacteria or archaea and can even be infected by viruses themselves (Sommers *et al*., 2021). For example, virophages have been observed to infect giant viruses that are themselves infecting cellular organisms (e.g. amoebae). In these cases, the virophage infection co-opts the giant virus and may improve the condition of the host organism (Roux *et al*., 2017; Backstrom *et al*.,

https://doi.org/10.1017/S1473550423000058 Published online by Cambridge University Press

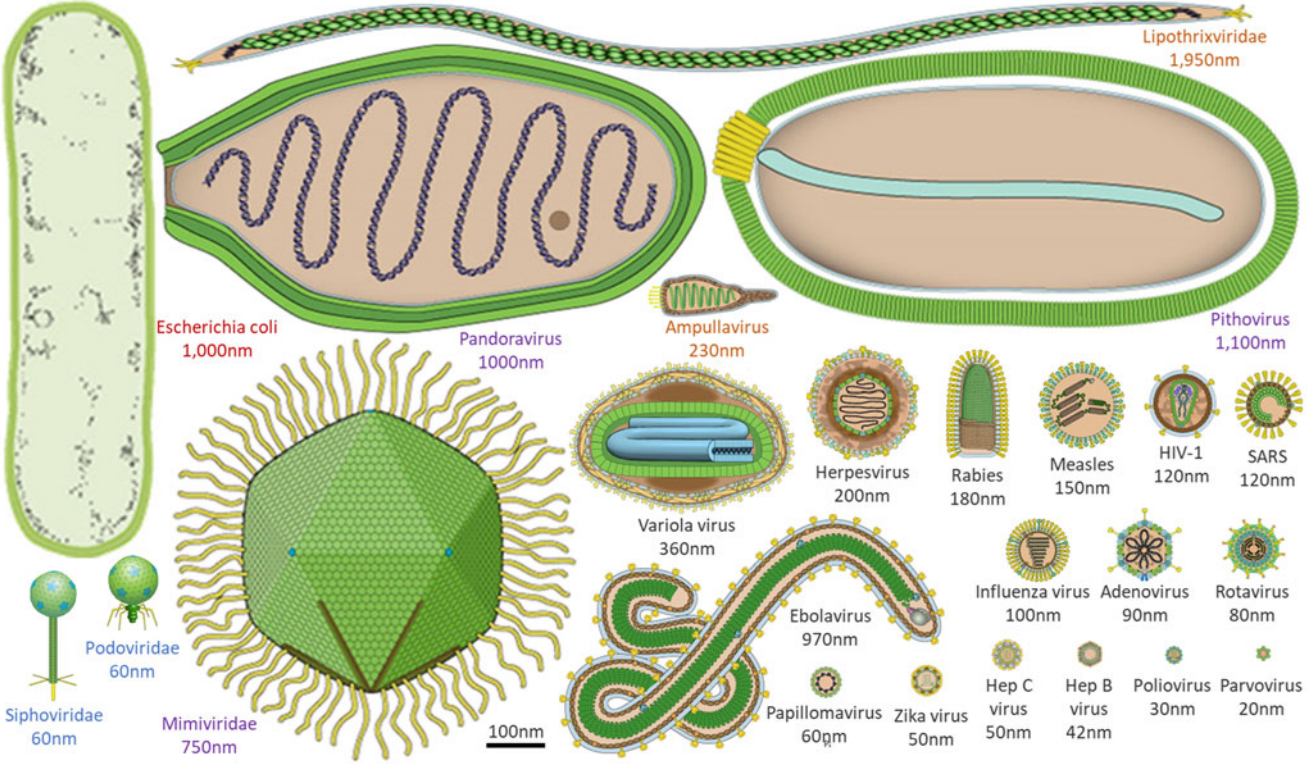


***Figure 1.*** *Morphological comparison of viruses, and a common bacterium at the same scale. Viral morphology and size are highly diverse, ranging from a few to thousands of nanometres. Here, an example bacterium (*Escherichia coli*, red text) is contrasted with example viruses that infect humans (black text), amoebae (purple text), archaea (orange text) and bacteria (blue text). Virus images adapted from ViralZone, Swiss Institute of Bioinformatics.*

2019; Schulz *et al.*, 2020). Viruses can also alter the genetic architecture, phenotypic characteristics, reproduction strategy, infection dynamics and evolution of nearby viruses, which in turn influences how viruses interact with their hosts and ultimately, ecosystems. This spectrum of virus behaviours across all domains of life is also reflected in the productivity of their infected host cells (i.e. modulation of host-cell metabolism and tens to thousands of progeny virus particles per cell) and timing of single-cycle infection (e.g. tens to thousands of minutes). Notably, the timing for one cycle of virus growth correlates with the doubling time of healthy host cells (Jin and Yin 2021).

The diverse morphology of viruses which extends beyond that typically found in cellular life (Fig. 1), and the presence of unique genes for the virus coat or capsid, have already been used as a model for other life: 'capsid-encoding organisms' (Forterre and Prangishvili, 2009). Virus genomes can be comprised of DNA or RNA, double-stranded or single-stranded, in multiple variations of each. Unlike cellular life, there are no universal viral genes to serve as a backbone upon which to build consistent evolutionary trees (Roux *et al.*, 2019; Tisza *et al.*, 2020). The diversity of both virus morphology and viral nucleic acids makes them difficult to search for comprehensively, but also drives expanded toolkits that could be employed in the search for life on other worlds and therefore deserves further investigation.

Virus-like entities may even be detectable on other worlds where life-like processes are only just evolving. Although humans have become explicitly aware of viruses and their effects only in the past few centuries (Loeffler and Frosch, 1897; Beijerinck, 1898), they were likely present as soon as – or even before – the first cell arose on Earth (Moelling and Broecker, 2019). Viral infections have played a key role in the evolution of life on Earth via the exchange of genes between viruses and their hosts, providing genetic diversity, killing dominant hosts thus maintaining balanced populations and possibly even inventing DNA itself (Forterre and Prangishvili, 2009; Enard *et al.*, 2016). Viral genome remnants are present in most, if not all, cellular genomes, including in humans, where viruses play key roles in human physiology (Bannert and Kurth, 2004; Arneth, 2021). Further studies of the history of Earth's viruses may highlight opportunities for detecting emergent life on other worlds.

On Earth, viruses have such a substantial impact that they drive major global biogeochemical cycles, and the same may be true on other worlds. The vast majority of marine viruses infect microbes (Suttle, 2005; Parikka *et al.*, 2017), thus affecting many biological processes, including encoding photosynthesis genes that substantially contribute to atmospheric oxygen ($O_2$), and help regulate global nutrient cycling (Greene and Reid, 2013). Further understanding of the persistence of virus particles and viral impacts on microbial cellular chemistry may therefore help illuminate biosignatures on distant planets, or signatures of past life in the geologic records of other worlds.

Targeting the search for biosignatures should focus on locations that are currently or once were within the environmental ranges compatible with the persistence and replication of information molecules. The more that research is conducted on viral persistence and replication in extreme environments, the more that remarkable feats of persistence and replication may be discovered (e.g. viruses thawed from permafrost could infect a modern-day version of their host; Legendre *et al.*, 2014).

Viral persistence in and response to extreme conditions also has important implications for humans voyaging into space, as recently reviewed by Pavletić *et al.* (2022). However, in addition to viral impacts on human physiology via direct infection and impacts on the microbiome, viral infection of plants or microbes could impact critical life support systems on deep space missions, including algal bioreactors (Matula and Nabity, 2019). Conditions such as microgravity affect fluid dynamics with implications for bacterial biology (as reviewed in Diaz *et al.*, 2019; Acres *et al.*, 2021; Sharma and Curtis, 2022), and presumably also virus–host interactions, but this is still an underdeveloped field.

The diversity of unique viral qualities and their integral role in life on Earth compels us to better understand the potential roles of viruses and virus-like entities in both astrobiology and space biology. There are multiple critical knowledge gaps in our understanding of viruses in the space environment and their potential role in other hypothetical biospheres in our Solar System and beyond.

https://doi.org/10.1017/S1473550423000058 Published online by Cambridge University Press

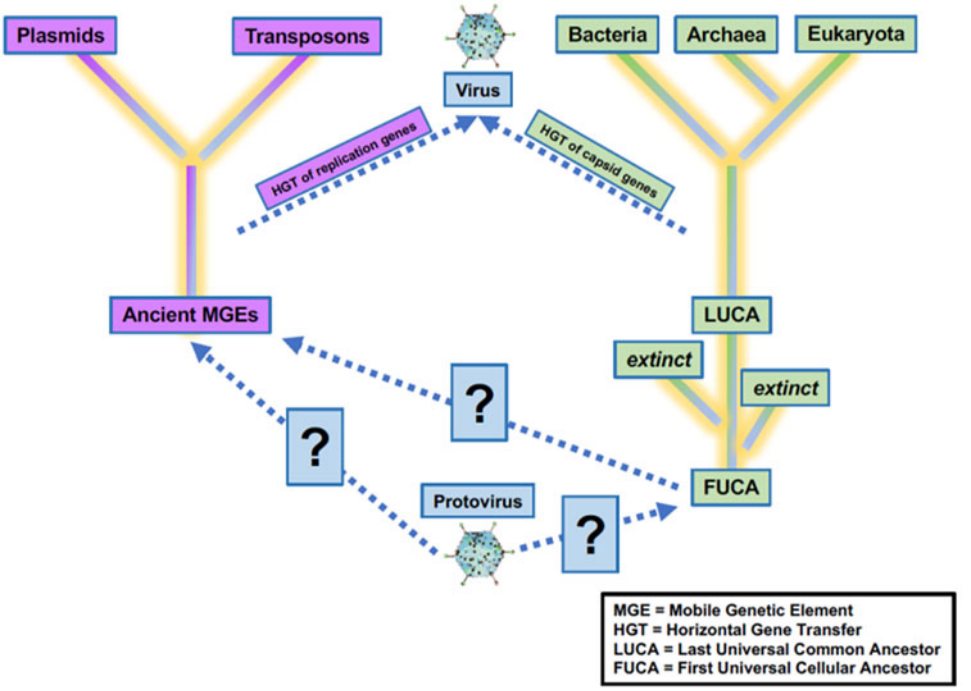


***Figure 2.*** *Uncertainty in the origin(s) and evolution of life on Earth. Viruses play a critical role in the evolution of life as we currently know them, and viruses or precursors of virus-like entities must be considered in origin experiments. Adapted from Harris and Hill (2021).*

In this review, we outline what new virus research here on Earth should investigate to address key scientific questions in planetary science, space biology and astrobiology. These include:

(1) What role(s) did viruses play in the origin and evolution of life on Earth?
(2) What are the environmental limits to the preservation and propagation of virions?
(3) What role(s) could viruses play in potential biospheres elsewhere, and how might those impacts be detectable as biosignatures?
(4) Can the detection of viruses beyond Earth be interpreted as a sign of life?
(5) How do artificial environments affect the human virome?
(6) Can virions be fossilized or otherwise preserved in a recognizable state?

## Potential role(s) of viruses in the early stages of life

Increasing knowledge of the roles of viruses in the origins of life on Earth will benefit the search for life elsewhere. Viruses have and will continue to play a critical role in the evolution of life on Earth. Precursors of virus-like entities may even mark the beginning of life itself, making the virosphere potentially as old and significant as the cellular biosphere (Fig. 2; Janjic, 2018; Moelling and Broecker, 2019). Viral signatures may be pivotal in the search for life elsewhere and in understanding evolutionary mechanics of other biospheres. Theoretical models predict that (depending on the information-transfer properties of the system) parasitic replicators will emerge anywhere in the Universe that life-like processes evolve (Eigen, 1971; Bresch *et al.*, 1980; Vignuzzi and López, 2019; Vlok *et al.*, 2019). Indeed, even viruses themselves fall prey to parasitic replicators in the form of defective virus genomes and virophages that multiply at the expense of fully intact virus genomes (Perrault, 1981; Vignuzzi and López, 2019; Roux *et al.*, 2023). The 'RNA-world' hypothesis characterizes the origin of life with self-replicating RNA, followed by ribonucleoproteins that later

https://doi.org/10.1017/S1473550423000058 Published online by Cambridge University Press

***Table 1.*** *Comparison of cellular and non-cellular entities—cells possess each of these four significant genetic and structural materials*

| | Entity | Example | DNA | RNA | Lipid | Protein |
|---|---|---|---|---|---|---|
| Cellular | Cell | *Escherichia coli* | X | X | X | X |
| Viral | Virus | Herpes simplex virus | X | | X | X |
| | Virus | SARS-CoV-2 | | X | X | X |
| | Virus | Hepatitis B virus | X | X | | X |
| | Virus | Torque teno virus | X | | | X |
| | Virus | Poliovirus | | X | | X |
| Sub-viral | Viroid | Potato spindle tuber viroid | X | | | |
| | Prion | $PrP^{Sc}$ | | | | X |

In the case of viruses and subviral elements, only reduced suites of these key molecules are present. Since non-cellular entities can possess either DNA or non-DNA genomes and may lack lipids, the search for extraterrestrial DNA, RNA or lipid alone would fail to detect some of these entities.

evolved into DNA and larger proteins (Müller *et al.*, 2022). Viruses and virus-like replicators are the only known extant biological entities that contain all types of nucleic acid genomes (Table 1), including single-stranded and double-stranded RNA and DNA or mixtures thereof. RNA viruses serve as models for how RNA and ribonucleoproteins could have propagated via simple self-replicating RNA structures and ribozyme activity (Landweber *et al.*, 1998; Koonin *et al.*, 2006; Tyler, 2008; Durzyńska and Goździcka-Józefiak, 2015; Matsumura *et al.*, 2016; Weinberg *et al.*, 2019). Viruses may have helped support the transition from the early RNA world to the current DNA world (Forterre, 2006; Diemer and Stedman, 2012). Further, the recent acceptance of network-based clustering for virus taxonomy (Jang *et al.*, 2019) in addition to incorporation of three-dimensional (3D) structures (and other viral characteristics) have allowed deep evolutionary inferences that have identified newly proposed phyla of RNA viruses which may be a previously missing link in the transition from the RNA–peptide world to the DNA–RNA–peptide world (Zayed *et al.*, 2022). Further, beyond the origins of life, viruses played a role in the origins of the mitochondrion (Shutt and Gray, 2006) and of the placenta (Mi *et al.*, 2000). Viruses may even have been critical in the evolution of eukaryotes and multicellularity (Forterre and Gaïa, 2016; Lee *et al.*, 2018; Guglielmini *et al.*, 2019). Overall, viruses appear to have played many roles in the origin and evolution of early life.

While uncovering the history of viruses is key to understanding the origin and evolution of life on Earth, an additional benefit is the development of new technologies that are potentially valuable for characterizing the diversity and history of non-terran life. For instance, comparative analysis of 3D atomic structures is a non-genomic method being used to interrogate virus origins that could also be applied to non-terran samples that may lack nucleic acids (Bamford *et al.*, 2005). This method is employed to detect and classify viruses because there are no genes shared by all extant viruses (Roux *et al.*, 2019; Tisza *et al.*, 2020). In comparison, all organismal cells conserve some genomic features such as ribosomal RNA (Table 1). Thus, the search for life elsewhere can tap into such techniques. In particular, we propose the following future research directions.

***Future directions***

(a) Investigate evolutionary relationships between RNA viruses and ribozymes (ribonucleotide enzymes) to evaluate the potential role of viruses in an RNA world, and the transition to a DNA world.
(b) Create tools and methods to enable inferences about early life through comparative analysis of extant viral life.

https://doi.org/10.1017/S1473550423000058 Published online by Cambridge University Press

(c) Reconstruct ancient events to illuminate the origin(s) of viruses on Earth, and their possible roles in the emergence of all life.
(d) Develop phylogenies that consider all biological entities to attempt to connect and better constrain the shared and divergent evolutionary histories of viruses and cellular organisms.

## Habitability, persistence and process limits of viruses and cellular life

Viruses have been known to remain active in a variety of harsh conditions, sometimes influencing the functioning and preservation of their hosts and sometimes reinfecting hosts after being preserved themselves yet still viable. Cellular life can also persist and operate under a variety of extreme conditions on Earth. However, the monitoring of virus–virus and virus–host activity together in extreme environments has been limited, despite these interactions being inherently different than those in milder environments. Therefore, fundamental concepts are not well understood about the types of environmental conditions and processes that are most conducive to the preservation and propagation of information molecules or molecular signatures of life. For example, understanding the upper and lower limits of viruses' persistence and activity under a range of conditions would inform where we might find viruses and other life on both Earth, in space and on other worlds. Additionally, long-term monitoring of viruses in extreme environments could inform their ability to persist under similar conditions on another planetary body. Multiple analytical techniques could be used to better determine viral activity in both dormant and active host environments and to better characterize viruses and low-level host activity as well. While viral process limitations are currently unknown for a range of conditions, viruses persist and interact with their hosts under a wide range of extreme conditions on Earth (Gil *et al*., 2021), including extremes in temperature, pH, pressure and salinity. These conditions manifest in both natural and built environments, and viruses have been observed in a variety of settings (Table 2). Understanding the extreme ranges of conditions in which viruses and their hosts can survive informs the search for life on other worlds, the operational considerations for planetary protection and viral monitoring during human spaceflight.

In a variety of environmental settings, viruses can persist, maintain their integrity and even help preserve their hosts for extended periods of time. For example, viruses that adsorb to clays are protected from inactivation, thus enabling them to persist in soils for longer periods of time in the absence of a host (Bitton, 1975; Lance and Gerba, 1984; Lipson and Stotzky, 1985; Syngouna and Chrysikopoulos, 2010). Virus particles can also be preserved via silicification and can become reactivated (i.e. infectious once again) when the silica is removed (Laidler and Stedman 2010; Laidler *et al*., 2013). Further, upon thawing, viruses preserved in frozen permafrost for ~30 000 years were able to infect modern day versions of their hosts (Legendre *et al*., 2014). Viruses can also influence the preservation of their biological hosts. For example, viruses that infect microbial mats can cause or expedite the microbial mats' fossilization into a stromatolite by (1) acting as a nucleation site for organomineralization, (2) altering microbial host metabolism to promote carbonate precipitation or (3) increasing the production of microbial extracellular substances (Pacton *et al*., 2014; White *et al*., 2021). In summary, viruses can function as key structures for preservation and propagation of biological information, especially in extreme environments. In this context, a better understanding of which environmental and molecular factors drive this long-term persistence will enhance future searches for virus-like elements outside of Earth.

Throughout extreme environmental conditions, viruses can also extend protection to their hosts. For instance, viruses can confer host heat tolerance (Márquez *et al*., 2007), and carry genes for sporulation that may drive their host to form an inactive spore, that is robust to unfavourable conditions (Trubl *et al*., 2018; Van Goethem *et al*., 2019; Pelusi *et al*., 2021; Travers *et al*., 2022). In another instance, viruses allow photosynthesis in cyanobacteria under desiccating conditions (Azua-Bustos *et al*., 2012). Harsh environments, such as polar regions or hydrothermal vents, have a high incidence of temperate viruses, which can reside within their microbial hosts (lysogeny) until conditions are favourable for viral replication (Anderson *et al*., 2014; Brum *et al*., 2016). While in this lysogenic state, viruses may express genes that alter their microbial host's physiology and metabolism, thus increasing the

https://doi.org/10.1017/S1473550423000058 Published online by Cambridge University Press

***Table 2.*** *Example locations where viruses have been observed through isolation or genomic studies*

| Location | Condition | Reference |
|---|---|---|
| Atmosphere | | |
| Droplets suspended in the atmosphere | N/A | Reche *et al.* (2018) |
| Air samples (Korea) | ∼−4 to 20°C, ∼4–15 mb vapour pressure | Whon *et al.* (2012) |
| Freshwater sites | | |
| Lakes, rivers, wetlands (Ontario, Canada) | 3.5–18°C, pH 5.85–9.09 | Kyle and Ferris (2013) |
| Lake Ontario, Lake Erie (Great Lakes) | N/A | Mohiuddin and Schellhorn (2015) |
| Acid mine drainages | | |
| Mine sites (Southern China) | N/A | Gao *et al.* (2022) |
| Sudbury Igneous Complex (Ontario, Canada) | 14°C, pH 2.45–4.00 | Kyle and Ferris (2013) |
| Permafrost-associated soils | | |
| Alaska Peatland Experiment (APEX) site | −1.5°C | Trubl *et al.* (2021) |
| Stordalen Mire field site near Abisko, Sweden | 0–17°C, pH 3.4–6.2 | Trubl *et al.* (2018, 2019) |
| Permafrost thaw gradient (Sweden) | 0–17°C, pH 3.4–6.2 | Emerson *et al.* (2018) |
| Ice cores or cryoconite holes | | |
| Canadian Arctic ice core | −31 to −9°C, 4–6 psu salinity | Wells and Deming (2006a) |
| Arctic nepheloid layer | −12 to 8°C, 50 psu salinity, 200 atm | Wells and Deming (2006b) |
| Cryopeg brine, sea-ice brine, melted sea ice | N/A | Zhong *et al.* (2020) |
| Antarctic cryoconite holes | N/A | Sommers *et al.* (2019) |
| Soda Lakes/solar salterns | | |
| Mono Lake (CA, USA) | ∼5–14°C, pH ∼ 10, 70–85 psu salinity | Brum and Steward (2010) |
| Solar salterns and salt lakes (Sicily, Italy, Thailand, Israel, Slovenia, Spain) | Viruses grown under 37°C, pH 7.2, 30% w/v salt water | Atanasova *et al.* (2012) |
| Magadi and Shala Lakes | Viruses grown under 37°C, pH 9, 5% NaCl | van Zyl *et al.* (2016) |
| Great Salt Plains National Wildlife Refuge (OK, USA) | Virus grown under 15–30°C, pH 6-9, 5–20% NaCl | Seaman and Day (2007) |
| Hot springs | | |
| Obama hot spring sediment (Japan) | 70–90°C, pH 6–9, 0–5.8% NaCl | Nagayoshi *et al.* (2016) |
| Hot Springs (Pozzuoi, Italy) | 87–93°C, pH 1.5 | Haring *et al.* (2005) |
| Yellowstone hot springs (USA) | Bear paw (74°C, pH 7.34); octopus (93°C, pH 8.14) | Schoenfeld *et al.* (2008) |
| Fumarole | | |
| Campi Flegrei volcano (Pozzuoli, Italy) | 81–96°C; pH 1–7 | Baquero *et al.* (2020) |

(*Continued*)

https://doi.org/10.1017/S1473550423000058 Published online by Cambridge University Press

**Table 2.** (*Continued.*)

| Location | Condition | Reference |
|---|---|---|
| Deep-sea sediments | | |
| Deep subsurface sediments of the Peru margin | ~318 mbsf | Engelhardt *et al.* (2013) |
| Subseafloor sediment continental shelf of Peru | 5–159 mbsf | Engelhardt *et al.* (2015) |
| Baltic Sea subseafloor sediments | 37.1–437.1 m depth below water | Cai *et al.* (2019) |
| Deep-sea hydrothermal vents | | |
| Wocan and Tianxiu hydrothermal fields | N/A | Cheng *et al.* (2022) |
| Guaymas Basin, Gulf of CA | 2000 m depth below water; virus isolate grown under 80°C, pH 6.3, 2% NaCl | Thiroux *et al.* (2021) |
| Other | | |
| Chemically harsh conditions | Virus survival in 5–9 M urea | Gupta *et al.* (1995) |
| Chemically harsh conditions | Virus survival in 99% acetonitrile | Olofsson *et al.* (2001) |
| Ice cubes | N/A | Jalava *et al.* (2019) |

This table provides examples, and it is not a comprehensive review of all locations where viruses have been observed/isolated.
psu, practical salinity unit; mbsf, metres below sea floor.

host cell's ability to survive conditions in which resources are limited, including within sea ice, dry soil or acidic environments (Chen *et al.*, 2005; Yu *et al.*, 2015; Howard-Varona *et al.*, 2017; Lee *et al.*, 2021; Wu *et al.*, 2021).

By integrating into hosts that are dormancy capable, viruses may be able to persist through conditions that are incompatible with activity then reactivate when conditions improve. Extreme examples of organisms regaining function after dormancy include: (1) cyanobacteria reviving after a 672 day exposure to space outside of the International Space Station (ISS; Laranjeiro *et al.*, 2021), (2) cyanobacteria in Antarctica metabolizing within a week after rewetting following 20–30 years without stream flow (McKnight *et al.*, 2007), (3) nematodes reviving from 30 000-year-old permafrost in Siberia (Shatilovich *et al.*, 2018) and (4) rotifers in northeastern Siberia reactivating from 24 000-year-old permafrost (Shmakova *et al.*, 2021). Microbes living under low-energy conditions in the South Pacific Gyre are claimed to have even retained their metabolic response after 101.5 million years (Morono *et al.*, 2020). The diverse evidence of microbial and eukaryote dormancy noted above shows the potential for organisms to survive and even grow in space-like environments and planetary analogues. However, not much is known about any hitchhiking viruses, that have accompanied these long-persisting hosts, nor how they may have contributed to long host dormancies. Evaluating the persistence and reactivation of viruses in dormant hosts would hint at the plausibility of their presence in extreme non-terran environments.

The effects of viruses on extremophiles involve virus–host interactions that are fundamentally different relative to ideal environments (Dávila-Ramos *et al.*, 2019). The monitoring of viral persistence and activity in a wide variety of extreme environment types may indicate how viruses survived during early Earth, and what is applicable to alien biospheres. We need expanded experimentation to determine end member limits of viral persistence and activity under conditions like aridity, humidity, low/high $O_2$ content, trapping in amber or soil/sediments, etc. Scoping out the broad viral environmental envelope would point to where virus-like entities might be found on both Earth and other worlds.

We posit that similar virus–host interactions are likely on any planetary body where life exists, thus it is critical to expand our understanding of these interactions on Earth to enable the detection and identification of such phenomena during space missions. For example, it is now feasible to computationally

https://doi.org/10.1017/S1473550423000058 Published online by Cambridge University Press

integrate how functions encoded in virus genomes interact with the material and energy resources of their hosts, thereby predicting the timing and levels of virus growth (Yin and Redovich, 2018). Successfully predicting viral activity in human-support space environments would help assure astronaut health.

Viral activity in low-energy Earth environments can be explored through development of high sensitivity tracer approaches. Bulk tracer methods, such as nucleic acid stable isotope probing (SIP; Radajewski *et al.*, 2000; Manefield *et al.*, 2002), can provide detailed information about the level of stable isotope incorporation by microbes and have been applied to investigate virus activity in complex environments (Pasulka *et al.*, 2018; Lee *et al.*, 2021, 2022; Starr *et al.*, 2021; Trubl *et al.*, 2021). Bulk methods such as SIP integrate over relatively large sample mass (Nuccio *et al.*, 2022), thus requiring more enriched substrate in complex systems. However, investigators have recently developed imaging mass spectrometry approaches that can detect viral replication at the single-particle level based on incorporation of rare stable carbon and nitrogen isotopes (Gates *et al.*, 2018; Pasulka *et al.*, 2018; Mayali *et al.*, 2019). Because virion enrichment has been detected in individual particles using nanometre-scale secondary ion mass spectrometry (NanoSIMS), the approach is clearly equally viable for other very small samples. These studies have demonstrated sensitivity down to 100 nm-diameter virions. Further research is necessary to extend these methods to bacteriophage in the 50 nm-diameter range and experimentally apply them to extreme environmental and space-like conditions. Such powerful techniques could be used to evaluate the resilience of viruses to astrobiologically relevant conditions, thus we propose the following experimental directions.

***Future directions***

(a) Increase of environmental surveys and long-term monitoring of viral persistence and activity in extreme environments that can be used as analogues of planetary bodies and early life on Earth.
(b) Experimental work on the lower and upper limits to viral persistence and activity under a variety of environmental parameters.
(c) Evaluation of the persistence and activity of viruses in dormant and active hosts, combining techniques (e.g. SIP with metagenomics) to better characterize viruses and detect low-level host activity.

## Biogeochemical cycling and biosignature detection

Viruses play critical roles in biogeochemical cycles on Earth. Our understanding of viruses as major players in Earth's biogeochemical cycles has been reshaped by the advent of metagenomic approaches that have enabled the study of uncultivated viruses (Kristensen *et al.*, 2010; Roux *et al.*, 2016, 2019). Marine virology has been particularly intensely studied, highlighting the potential for worlds with liquid oceans, such as Enceladus or Europa, to contain informational molecules such as virus genomes and proteins amongst their organic compounds (Postberg *et al.*, 2018). On Earth, viruses have a huge impact on $O_2$ concentrations by infecting the marine cyanobacteria that are responsible for ~25% of the $O_2$ in Earth's atmosphere. At any moment, about half of marine cyanobacteria are infected, which can lead to either a decrease in $O_2$ production (i.e. cells lysed by viruses) or an increase in the efficiency of their $O_2$ production (Sieradzki *et al.*, 2019). We need an improved understanding of what controls that balance, and how can that be used in biogeochemical modelling. Other work has demonstrated the activity of viruses in cold to sub-freezing temperature soils (Trubl *et al.*, 2021; Wu *et al.*, 2022) and illustrates the potential for viruses in biogeochemical processes of cold terrestrial worlds, such as Mars. Terrestrial worlds, or at least partly rocky bodies, such as comets, may host ice-lidded cryoconite holes in their polar ice caps that could result in the presence of liquid water to support active life (Zawierucha *et al.*, 2017). Could terrestrial worlds with thick mid-deck atmospheres, such as Venus, harbour life and virus-like entities? On Earth, we know that viruses exist within droplets in the atmosphere and can augment iron and sulphur metabolisms (Anantharaman *et al.*, 2014; Bonnain *et al.*, 2016; Roux *et al.*, 2016; Dalcin Martins *et al.*, 2018). Since Venus's clouds contain sulphuric acid, viruses could be influencing biogeochemical processes in the planet's atmosphere as well. More intimately studying the

https://doi.org/10.1017/S1473550423000058 Published online by Cambridge University Press

role of viruses in biogeochemical processes in a variety of Earth analogue environments could inform any potential role viruses might play in biogeochemical processes on other worlds.

The widespread and frequent detection of genes used by viruses to hijack the metabolism of their host cell(s) and manipulate them to produce viral progeny strengthens the need for a conceptual shift towards calling virus-infected cells 'virocells', as this will help emphasize their differences from uninfected cells (Forterre, 2011, 2013). Viral infections can change a microbe's metabolic outputs, impacting the composition and quantity of their biosignatures in ways as profound as the organism's own genome. Combined experimental and *in silico* studies of virus growth on bacterial hosts have shown how the physiological state of the host cell can be reflected in the timing and level of virus production, whereas virus production is intimately linked to the availability of resources for protein synthesis (Mahmoudabadi *et al.*, 2017). Since nutrient availability in the host's environment impacts its ability to produce viruses, the productivity of virus infection can provide a readout of the metabolic demands of the living host (You *et al.*, 2002). Further, computational modelling suggests that viruses evolve to optimally use the finite metabolic energy resources of their host cells (Kim and Yin, 2004). Virus growth may also be linked to host physiology in ways not previously appreciated; studies of viruses that infect bacteria, eukarya and archaea revealed delay times for virus production that correlate with the doubling times of their host cells (Jin and Yin, 2021). Similar virus–host interactions could be at play in other systems, and we must be prepared to identify them.

Virus–host interactions and viral effects on microbial biosignatures can be evaluated through tracking stable isotopic signatures. Autotrophic microbial life preferentially incorporates the lighter isotope available for each biogenic element, a feature often used to identify ancient microbial life and sources of input materials in extant life. Yet the exact signatures of this process depend on which organisms are performing a given metabolic process, how many enzymatic reactions take place in relevant metabolic pathways and how the enzyme(s) work. For example, fractionation factors are different for denitrification via fungi versus bacteria (Ostrom and Ostrom, 2017), or for methanogenesis depending on the initial substrate and the organisms involved (Whiticar, 1999; Hornibrook *et al.*, 2000; Penning *et al.*, 2006). When a virus infects a microbial host, it redirects its metabolism, thus possibly impacting these isotopic values. Such impact could be more dramatic if a virus were to encode an auxiliary metabolic gene that has a different fractionation factor than the host version of that gene. For example, kinetic measurements of virus and host versions of the same enzyme have revealed that the virus enzyme had a significantly lower $k_{cat}/K_M$ value than the host enzyme (Thompson *et al.*, 2011). Such viral influences can lead to large variations in isotopic signatures, leading to uncertainty when distinguishing between abiotic and biological processes and in the utility of a particular biosignature (Schwieterman *et al.*, 2018). While complex and potentially difficult for distinguishing biotic from abiotic processes, isotopic fractionation has the potential to reveal fundamental characteristics of biosphere metabolism, including the impact and contribution of viruses.

Isotopic fractionation measurements have long been applied to the geologic record of Earth to infer characteristics of the metabolisms of past biospheres and changes over time (Johnson *et al.*, 2021). The isotope history of marine ecosystems throughout Earth's history is well-studied (Zerkle and Mikhail, 2017; Krissansen-Totton *et al.*, 2015). However, the isotopic composition of icy environments through time on Earth is less well-characterized but may provide useful analogue environments applicable to other planetary bodies such as Mars (Havig and Hamilton, 2019). Distinguishing viral influences on both present isotopic signatures and on their variation over multiple timescales may therefore provide a reference for interpreting isotopic biosignatures of past life on other worlds. To better understand the role of viruses in biogeochemical cycling on Earth in order to interpret and model geochemical cycles elsewhere, we propose the following experimental directions.

***Future directions***

(a) Improve understanding of how different viruses hijack their host's cellular machinery.
(b) Further explore the broad range of virus–host interactions and dynamics in various ecosystems.

https://doi.org/10.1017/S1473550423000058 Published online by Cambridge University Press

(c) Quantitatively estimate the role and impact(s) of virocell metabolism on Earth's different biogeochemical cycles.
(d) Improve understanding of the extent and distribution of viral impacts on biosignatures for major Earth biogeochemical cycles, including the potential magnitude of such effects.
(e) Search for the existence of any generalizable biosignatures associated with viral metabolic reprogramming to reduce uncertainty associated with biosignatures for life detection.

## Impact of viruses on human space exploration

Human spaceflight continues to evolve, and many new technologies and tests are being developed for the safety of crew members and planetary protection for future human-landed missions. However, many studies exclude viruses, despite their threats (and potential benefits) to many types of cellular life. Thus, questions remain as to how viruses interact with other viruses and their hosts in space environments. For example, how do natural versus artificial environments drive changes in the virome and how are viruses impacted and shaped by their environment? As human spaceflight expands to sustained missions beyond low Earth orbit (LEO), nearly every aspect of the mission (including ECLSS, human health and performance, lander operations and extravehicular activities (EVA)) must consider the impact of virus–host interactions. Otherwise, the impacts may prove to be analogous to the current concern about volatile contamination on the ISS where current instrument capabilities and measurements are less sensitive or erroneous due to environmental conditions untested prior to flight (Regberg *et al.*, 2022).

### *Viral response to spaceflight environments*

Viral studies in microgravity have been very limited, with most microbiology investigations focused on bacteria. Those have shown a variety of species-specific and even strain-specific morphological and physiological responses to the low fluid shear force and lack of liquid media convection in microgravity (as reviewed in Diaz *et al.*, 2019; Acres *et al.*, 2021; Sharma and Curtis, 2022). Without externally applied forces, nearly everything in a microgravity environment can remain suspended or quiescent. Brownian motion dominates, thus reducing the ability for host cells to gather nutrients (Zea *et al.*, 2016), and thereby influencing the size, structure and organization of these host cells. Additionally, the physical limitations of microgravity could potentially modify the infiltration capabilities of viruses. Further, the lack of buoyancy effects may also influence viral dispersal and host encounter rates in both the spacecraft cabin atmosphere and quiescent fluid systems. Another response to microgravity is aggregation (Zea *et al.*, 2018; Domnin *et al.*, 2022), which might limit the ability of viruses outside an aggregate to encounter a host surface or increase host encounter rate once one host in an aggregate is lysed. Even with external forces (fans, pumps, etc.) moving liquid and gas loops, it is still unknown how well viruses can adhere to encountered surfaces. Simulated microgravity has been associated with a thicker cell membrane envelope in *Escherichia coli* (Zea *et al.*, 2018), which could inhibit the ability of viruses to infect hosts. In contrast, lower membrane integrity was observed in *Vibrio fischeri* (now known as *Aliivibrio fischeri*; Vroom *et al.*, 2021), which could make hosts more susceptible to membrane-disrupting events.

The effects of microgravity on virus dynamics can be measured through environmental sequencing of surfaces aboard space stations and crewmember microbiomes, yet there is a dismaying lack of virus-centric metagenomic analyses (according to Mora *et al.*, 2019, there has been a single study). Although there may be limitations in sampling size, frequency and depth of coverage due to the technical and logistical challenges of obtaining spacecraft samples, several data sets from the ISS are already available for reanalysis (Be *et al.*, 2017; Singh *et al.*, 2018; Urbaniak *et al.*, 2018, 2022; Checinska Sielaff *et al.*, 2019; Avila-Herrera *et al.*, 2020). In future studies, the virus-to-host ratio for known virus–host pairs is one statistic that can be estimated, and its distribution explored spatially, temporally and in response to natural or experimental perturbations. Targeted and untargeted metagenomic sequencing

https://doi.org/10.1017/S1473550423000058 Published online by Cambridge University Press

of microgravity samples would have complementary strengths allowing for comprehensive analyses that include data from broad surveys at the species and genus taxonomic levels and to narrowly focused studies concerning specific strain and variant population dynamics. Viral dispersal dynamics, host adsorption, infection and progeny productivity in spaceflight environments are extremely understudied and deserve significant further work.

### *Environmental control and life support systems*

ECLSS are imperative for supporting human spaceflight. These flight-proven technologies on the ISS control air composition and temperature, food and water and waste remediation. However, long-duration missions beyond LEO will require robust alternatives that do not rely on frequent resupply missions (Anderson *et al*., 2019). Closing the carbon loop through bioregenerative technologies is one approach for providing ECLSS. Algal photobioreactors can remove carbon dioxide ($CO_2$), liberate $O_2$, remove or alter waste and produce edible biomass (Matula and Nabity, 2019; Fahrion *et al*., 2021). These photobioreactors can withstand the dynamic temperature environment experienced within the ISS thermal control loops (Matula and Nabity, 2021; Matula *et al.*, 2021). Preliminary spaceflight studies using algae for ECLSS observed thriving cultures (Helisch *et al*., 2020; Poughon *et al*., 2020). Likewise, extremophilic algae, lichen, cyanobacteria and fungi included in experiments mounted on the outside of the ISS and Space Shuttle survived weeks to months-long missions (de Vera *et al*., 2019; Malavasi *et al*., 2020). However, these studies did not characterize the full microbiome within the non-axenic cultures. This current lack is extremely important. For example, virophages can have profound implications for microbial nutrient cycling, often referred to as the microbial loop. Predator–prey simulation models indicate that the presence of virophages regulates helper virus and algal host dynamics altering carbon flux through the microbial loop in aquatic ecosystems (Yau *et al*., 2011). To survive unfavourable conditions (e.g. microgravity, viral infection and antimicrobials), some microbes can form protective barriers such as biofilms. Bacteria exude a variety of organic matter dubbed extracellular polymeric substances, which forms the biofilm and acts like glue securing and protecting the bacteria allowing them to remain hydrated, control local pH and other services. Biofilms can benefit certain systems such as the rhizosphere (plants root zones) aiding in food production. However, biofilms can also be detrimental by corroding or clogging machinery that supports ECLSS, or preventing wound healing in skin abrasions, to name just a couple of examples (Landry *et al*., 2020). Recently, highlighted in Justiniano *et al*. (2023) are the knowledge gaps in our understanding of microbiomes (including viruses) and biofilm formation in space, although we do know that viruses can adapt new strategies to prevent bacterial growth or kill organisms within biofilms. Understanding potential biome or virome shifts within these systems over long durations may elucidate the need for specific algal species selection, causes of viral or bacterial-based system failures and operational considerations (Fig. 3; Matula and Nabity, 2019).

### *Bioregenerative ECLSS*

Efforts on the ISS have focused on plants for $CO_2$ removal and $O_2$ production, as a source of multivitamins, for urine reuse and to promote psychological well-being (Dzhos *et al*., 2021). On Earth, plant viruses can be a threat to agriculture, and disease management relies on rapid and accurate viral identification (Rubio *et al*., 2020). Many plants have been grown under microgravity, including flower-producing species, herbs and vegetables. However, due to the stress of spaceflight, viral infection of these plants can be hugely influential on functionality. A recent study found that spaceflight factors significantly affect tomato plants (Dzhos *et al*., 2021), increasing the productivity of the plants and the concentration of some vitamins such as carotenoids, which are important for crew members on long-term space missions. Importantly, plants grown from seeds that were in space for half a year prior to germination were resistant to viral infection. Moreover, seed resilience of many plant species (amongst other organisms) was tested by keeping them outside the ISS for 558 or 682 days, thus

https://doi.org/10.1017/S1473550423000058 Published online by Cambridge University Press

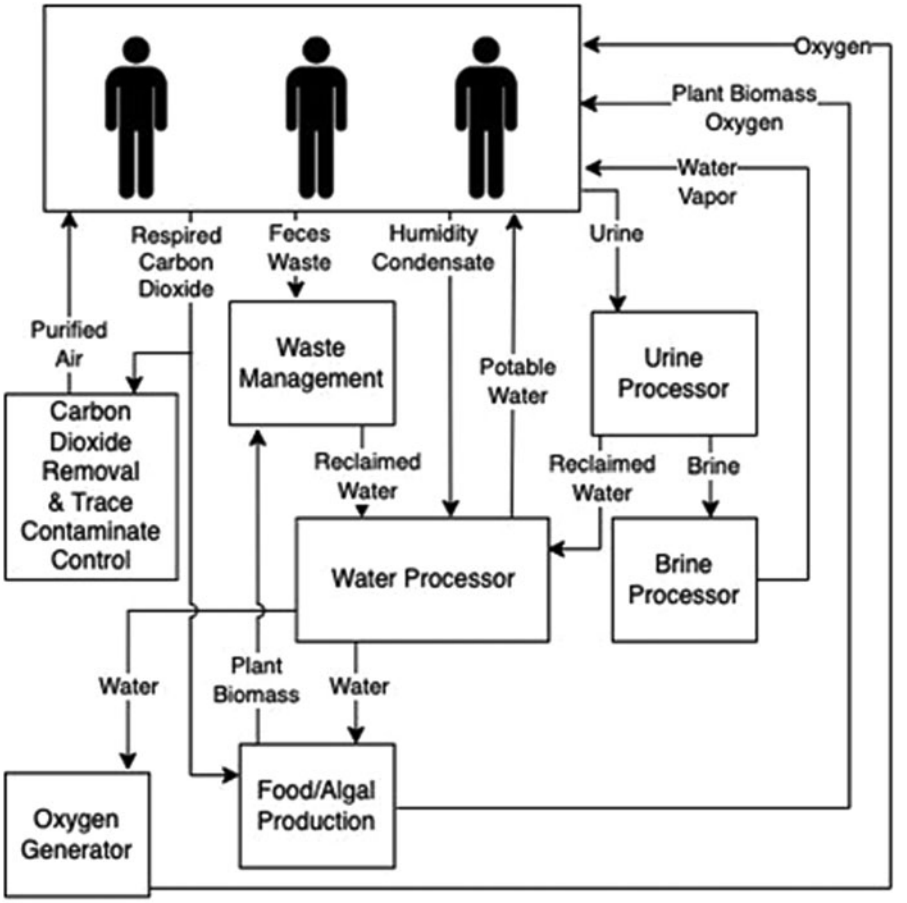


***Figure 3.*** *Review of spaceflight ECLSS potentially impacted by viruses. A simplified overview of environmentally controlled life-support systems for spacecraft and surface habitats that could be positively or negatively impacted by viruses.*

exposing them to high levels of radiation (Tepfer and Leach, 2017). All seeds were able to germinate after the shorter flight, but only seeds with a stronger coat could survive the longer radiation exposure. While these studies show promise for cultivating plants on long-term voyages, our understanding of plant–virus interactions on short flights is extremely limited and we have no information for long-term flights.

### *Human physiology and the viral ecology of crewed spacecraft*

In space-bound crewmembers, latent viruses are shown to reactivate more than in matched controls. Sometimes this reactivation occurs before leaving Earth and is presumably stress related. However, there are several plausible reactivation triggers associated with spaceflight itself, such as ultraviolet or ionizing radiation (Pavletíc *et al.*, 2022), but precisely which mechanisms might be responsible for increased virus reactivation in crewmembers have not been determined. Reactivation has included herpes simplex virus, Epstein–Barr virus and varicella zoster virus (Stowe *et al.*, 2000; Mehta *et al.*, 2004; Pierson et al., 2005; Rooney *et al.*, 2019). After 6–12 months in space, crewmembers experienced significant changes in their microbiome (both internal and external) that led to rashes and hypersensitivity episodes (Voorhies *et al.*, 2019). These symptoms warrant further investigation into the

https://doi.org/10.1017/S1473550423000058 Published online by Cambridge University Press

effects of long-term space environment exposure on humans combined with their accompanying microbes and viruses.

The community composition of viruses and other organisms on the ISS is mostly comprised of viruses that only infect non-human cells, such as bacteriophages. This composition differs from that of terrestrial space analogues (e.g. MDRS and HI-SEAS), thus pointing towards the space environment as the likely trigger (Mora *et al*., 2019). Although these viruses do not infect humans, they can be transported back to Earth and released into Earth environments via plants, humans and other entities that can harbour them. Continued efforts are needed for quarantining people within space stations for long-term space travel and increased screening for crewmembers returning to Earth. A viable way to test viral transmission and educate humans, especially those preparing for space travel is through simulations that consider epidemiology parameters (Patel *et al*., 2021). Although it might be tempting to contemplate elimination of viruses from spacecraft wherever possible, we should be sensitive to the unanticipated positive roles viruses may play and the unreasonable resources that viral elimination would entail. As well, a virus's presence can be beneficial by sometimes warding off more pathogenic relatives by cross-protection or superinfection exclusion (Folimonova *et al*., 2020). Immunologic 'eustress' can promote a healthy organism, as is seen by comparison to the physiological deficits of germ-free animals (Round and Mazmanian, 2009). Indeed, in some cases, viruses can be symbiotic with their host organisms (Roosinck, 2011). As such, viruses are key players in crewmember health and safety and in the ecology of crewed environments.

### *Spaceflight operational impacts*

Given the differences in viral dispersal patterns observed in low-gravity environments and the extreme conditions under which viruses persist and interact with hosts (see Section 'Viral response to spaceflight environments'), even surfaces often considered sterile, or at least clean, must be investigated. Some bacterial species – for example, *Bacillus pumilis* strain SAFR-032 (Tirumalai et al., 2013) – are refractory to pre-flight sterilization, and some could be tolerant as well given the extreme diversity of viral morphotypes (including lipid-free viruses, see Fig. 1). Even with perfect sterilization of equipment (which is essentially unobtainable), it is not currently possible to achieve a virus-free environment on a manned space mission because of post-launch viral shedding by crewmembers (*vide supra*). The frequency of sampling, maintaining and cleaning life support systems will impact day-to-day spaceflight operations, during both cruise phase and orbital or surface sustained missions. EVA and associated planetary protection protocols must also be accommodated: for example, protecting areas with high-scientific value from contamination (e.g. Rummel *et al*., 2014). Modifications of operations may entail landing further from a planetary surface sample site, designing new vehicles, requiring more stringent cleaning protocols or considering entirely new sample sites (e.g. Meyer *et al*., 2019). Additionally, contamination restrictions during EVA will narrow the design of space suit systems (Willson *et al*., 2014). Attempts to dictate planetary protection protocols without fully understanding viral dispersal and survivability will potentially result in overly restrictive constraints that ultimately hinder scientific discovery or alternatively inadequate protocols that do not achieve proper containment.

To understand the impact of viruses on human space exploration, we suggest that the following experiments be prioritized.

### *Future directions*

(a) Characterize differences in dispersal, aggregation and adsorption processes of viruses in both fluid and air microgravity environments.
(b) Compare the ability of viruses to adsorb to and infect hosts during and after microgravity-induced cellular changes.
(c) Investigate virus–host interactions, especially in response to perturbation effects on virus-to-host ratios.
(d) Develop enhanced capabilities for onboard biosurveillance.

https://doi.org/10.1017/S1473550423000058 Published online by Cambridge University Press

(e) Investigate viromes in built environments relevant to space, including the ISS.
(f) Explore virus–host dynamics for both human- and plant-associated microbiomes in space.
(g) Assess space-associated triggers that cause latent viral infections to become virulent.
(h) Quantitatively estimate the role and impact(s) of virocell metabolism in spaceflight environments.
(i) Identify viral loading impacts significant to both planetary protection requirements, and preservation of human health. Explore desirable modifications to spaceflight operations (travel, landing and EVA) that could ameliorate such loading impacts.
(j) Characterize viral influence on microbial biofilm composition and growth dynamics in human, algal and plant systems under various space-related conditions.

## Detection of viruses on Earth and elsewhere

A variety of instrumentation exists that can detect both microbial biomarkers and viruses. These types of techniques have often been used on Earth to study viruses (Sommers *et al.*, 2021; Vincent and Vardi, 2023). However, these same techniques can be difficult to conduct with automated systems in space and have yet to be designed specifically for space missions. Therefore, it is currently unclear if and how informational molecules can be reliably detected in environmental samples beyond Earth. Engineering investigations must be paired with science goals to further develop innovative, flight-ready technologies to detect and characterize viruses, virus-like particles and virus genomes efficiently and accurately. Once developed, such technologies could then be applied in Earth analogue systems to test performance and limitations for detecting viruses and virus-like particles. Additionally, these instruments and protocols can be incorporated into standard operating procedures for planetary sample missions.

While autonomous virus-detection technology is not yet ready, the future is promising. For example, solid-state nanopore-based biosensors are currently being designed for spaceflight and have proven capability for evaluating different biomarkers (i.e. DNA/RNA, proteins and whole viral particles – spanning from a few nanometres to over 100 nanometres in diameter). Solid-state nanopore sensors would be particularly useful in space missions because they also can measure particle-size distributions within virus populations and discriminate between viral particles by monitoring the change in electrical current as particles pass through an electrically biased pore or by measuring the mass density of viruses (Zhou *et al.*, 2011; Arjmandi *et al.*, 2014; McMullen *et al.*, 2014; Akpinar and Yin, 2015; Wu *et al.*, 2016). Researchers have adapted flow cytometry to the scale of virus particles; specifically, flow virometry employs more powerful lasers, reduced diameter fluid flows, wider-angle sampling of scattered light and fluorescent labelling of particles (Bhat *et al.*, 2022). Additionally, microscopy has been used to evaluate microbial and viral populations on Earth. Microscopy technologies paired with spectroscopic techniques, can inform the physical shapes and boundaries belonging to certain chemical or mineralogic compositions (Zhang *et al.*, 2013). While an atomic force microscope was used during the Phoenix Mars Lander to investigate Martian soils (Pike *et al.*, 2011) and recently, a scanning electron microscope was added to the ISS (Own *et al.*, 2018) higher performance complex space-qualified microscopes have yet to be developed that can reliably detect virus-like particles. Another technique that could be applied to space virology is NanoSIMS, which performs *in situ* quantitative trace sample analysis with exceptional sensitivity and spatial resolution (Mayali, 2020; Pett-Ridge and Weber, 2022). NanoSIMS has been used to detect viruses (Gates *et al.*, 2018) and map their elemental and isotopic distributions in complex communities and in mineralized samples (Pacton *et al.*, 2014). Also, the Network for Life Detection is currently using NanoSIMS to discern between abiotic and biological signatures based on the differences in elemental and isotopic patterns of cellular organisms versus minerals and other precipitates. These technologies complement spectroscopic methods by providing orthogonal evidence for viral and non-viral life (Zhang *et al.*, 2013). Overall, there are multiple instrument designs either waiting to be developed or currently being developed for detecting viruses and life beyond Earth.

Environmental virology techniques could also complement life detection instrumentation. In conjunction with other previously described instruments, high fidelity sequencing methods and high-

https://doi.org/10.1017/S1473550423000058 Published online by Cambridge University Press

resolution structure analysis could also confirm the presence of viruses on other worlds (Bamford *et al*., 2005). Moreover, to reduce signal-to-noise problems, relic or environmental DNA can be removed from a system using propidium monoazide to enhance detectability of intact viruses and microbes (Wagner *et al*., 2008; Weinmaier *et al*., 2015). Thus, methods of environmental virology could also be necessary for life detection and returned samples (Janjic, 2018; Ricciardi *et al*., 2022; Table 3). Much work remains to integrate both engineering and science perspectives. While some instrumentation, such as light, electron and atomic force microscopy, has been used on Mars and in space on the ISS (Pike *et al*., 2011; Own *et al*., 2018), instrumentation in development must also be tested in extreme analogue environments to explore and overcome limitations. Moreover, for planetary protection purposes, utilizing instrumentation for detecting viruses should be incorporated into standard operating procedures for flight and sample return missions. To fully understand the presence of viruses in space environments, new technologies need to be developed and include the following.

***Future directions***

(a) Develop innovative autonomous flight-ready technologies for efficient and accurate detection and characterization of virus genomes.
(b) Apply such technologies to Earth analogue systems to explore prospects and limitations for detecting viruses and virus-like particles.
(c) Incorporate measurements of viruses in standard operating procedures for sample return missions and subsequent examinations.

## Conclusion

For effective pursuit of astrobiology questions, it is critical to understand how life on Earth functions, as it is currently the only place where life is confirmed to exist. Viruses are key contributors to Earth's ecosystems; however, much remains unknown regarding their influence on cellular life, role in evolutionary history and their fundamental physical interactions with the Earth system. Basic ecological factors, such as persistence and decay under various scenarios, also remain underexplored. Likewise, safe and effective crewed deep space travel will require a thorough understanding of the human microbiome in space, including viruses. Here, we have outlined ways in which focused astrovirological investigations can broadly advance the goals of space science. Across the diverse disciplines that make up astrobiology and space biology, we highlight several classes of investigations which cannot afford to neglect viruses. Most of the diversity of life on Earth is comprised of viruses, whether diversity is defined by number of species, type of genetic information, number of individuals (Breitbart *et al*., 2002; Roossinck, 2012; Paez-Espino *et al*., 2016; Parikka *et al*., 2017; Mushegian, 2020), absence of any universally present gene, mode of replication or number of unique (i.e. non-homologous) genes (Koonin and Wolf, 2012). Viruses should be explicitly considered in organism-level astrobiological investigations. The ability to detect virus-like entities must be a point of assessment for instruments and missions to directly detect extraterrestrial organisms for planetary protection, human spaceflight safety and sample return (Janjic, 2018; Ricciardi *et al*., 2022). When organisms are used as model systems, viruses should be adequately represented to reduce biases and increase utility of diverse astrobiological studies.

> 'Whether viruses are alive or not may be a moot question, but if a virion (or virus-like particle) were to be unequivocally detected in an extraterrestrial sample, very few people would claim that this would not be evidence for life – wherever that sample was from'.
> – Berliner *et al*. (2018)

**Financial support.** The work of G.T., J.P-R., N.M. and P.K.W. was supported by the US Department of Energy (DOE) Office of Science, Office of Biological and Environmental Research Genomic Science programme award SCW1632, and by Lawrence Livermore National Laboratory LDRD award 21-LW-060, under the auspices of the DOE under contract DE-AC52-07NA27344. The work conducted by S.R. was supported by the U.S. Department of Energy Joint Genome

***Table 3.*** *Possible virus detection methods: how can we detect extraterrestrial viruses?*

| Virus detection method | Advantages | Challenges |
|---|---|---|
| Infection | Highly specific for virus identification and host response | 1. Requires correct host<br>2. Requires suitable conditions |
| Virion detection | Unique structures enable direct detection | 1. Virions may not represent active viruses<br>2. Could produce false positives |
| Metagenome sequencing | Very broad net theoretically allowing any virus to be identified | 1. Sequences may not resemble known viruses<br>2. Nucleic acid may be hard to purify or amplify<br>3. Exoviruses may not contain nucleic acids |
| Virus effects on hosts | May be detectable remotely | 1. May be difficult to differentiate between infected and uninfected hosts<br>2. No detectable hosts In environment |
| Microscopy indicators (SEM, TEM, AFM) | Provide potential indication of viruses to be confirmed by methods above | 1. Typical virion size is small<br>2. Very high resolution is necessary<br>3. Morphologies may be indistinct |

Of the currently known methods for virus detection, those suitable for astrobiology applications are challenging to imagine but we can leverage off the suite of methods already in use on Earth.

Institute (https://ror.org/04xm1d337), DOE Office of Science User Facility and the Office of Science of the U.S. Department of Energy operated under contract no. DE-AC02-05CH11231. Work by K.M.S. was supported by the U.S. National Science Foundation, MCB-1929273 and MCB-2025305. Work by J.Y. was supported by the U.S. National Science Foundation (MCB-2029281 and CBET-2030750). Work in the Kaelber lab was supported in part by the U.S. National Institutes of Health, R21GM140345.

**Conflict of interest.** The authors report no conflict of interest.

## References

Acheson NH (2011) *Fundamentals of Molecular Virology* (No. Ed. 2). Hoboken, New Jersey: John Wiley & Sons, Inc.

Acres JM, Youngapelian MJ and Nadeau J (2021) The influence of spaceflight and simulated microgravity on bacterial motility and chemotaxis. *npj Microgravity* **7**, 7.

Akpinar F and Yin J (2015) Characterization of vesicular stomatitis virus populations by tunable resistive pulse sensing. *Journal of Virological Methods* **218**, 71–76.

Anantharaman K, Duhaime MB, Breier JA, Wendt KA, Toner BM and Dick GJ (2014) Sulfur oxidation genes in diverse deep-sea viruses. *Science* **344**, 757–760.

Anderson RE, Sogin ML and Baross JA (2014) Evolutionary strategies of viruses, bacteria and archaea in hydrothermal vent ecosystems revealed through metagenomics. *PLoS ONE* **9**, e109696.

Anderson M, Sargusingh M, Gatens R, Perry J, Schneider W, Macatangay A, Toomarian N, McKinley M and Shaw L (2019) NASA environmental control and life support technology development and maturation for exploration: 2018 to 2019 overview. 49th International Conference on Environmental Systems.

https://doi.org/10.1017/S1473550423000058 Published online by Cambridge University Press

Arjmandi N, Van Roy W and Lagae L (2014) Measuring mass of nanoparticles and viruses in liquids with nanometer-scale pores. *Analytical Chemistry* **86**, 4637–4641.
Arneth B (2021) Leftovers of viruses in human physiology. *Brain Structure and Function* **226**, 1649–1658.
Atanasova NS, Roine E, Oren A, Bamford DH and Oksanen HM (2012) Global network of specific virus–host interactions in hypersaline environments. *Environmental Microbiology* **14**, 426–440.
Avila-Herrera A, Thissen J, Urbaniak C, Be NA, Smith DJ, Karouia F, Mehta S, Venkateswaran K and Jaing C (2020) Crewmember microbiome may influence microbial composition of ISS habitable surfaces. *PLoS ONE* **15**, e0231838.
Azua-Bustos A, González-Silva C, Arenas-Fajardo C and Vicuña R (2012) Extreme environments as potential drivers of convergent evolution by exaptation: the Atacama Desert coastal range case. *Frontiers in Microbiology* **3**, 426.
Backstrom D, Yutin N, Jorgensen SL, Dharamshi J, Homa F, Zaremba-Niedwiedzka K, Spang A, Wolf YI, Koonin EV and Ettema TJ (2019) Virus genomes from deep sea sediments expand the ocean megavirome and support independent origins of viral gigantism. *mBio* **10**, e02497–e0218.
Bamford DH, Grimes JM and Stuart DI (2005) What does structure tell us about virus evolution? *Current Opinion in Structural Biology* **15**, 655–663.
Bannert N and Kurth R (2004) Retroelements and the human genome: new perspectives on an old relation. *Proceedings of the National Academy of Sciences* **101**(suppl_2), 14572–14579.
Baquero DP, Contursi P, Piochi M, Bartolucci S, Liu Y, Cvirkaite-Krupovic V, Prangishvili D and Krupovic M (2020) New virus isolates from Italian hydrothermal environments underscore the biogeographic pattern in archaeal virus communities. *The ISME Journal* **14**, 1821–1833.
Be NA, Avila-Herrera A, Allen JE, Singh N, Checinska Sielaff A, Jaing C and Venkateswaran K (2017) Whole metagenome profiles of particulates collected from the International Space Station. *Microbiome* **5**, 1–19.
Beijerinck MW (1898) Ueber ein Contagium vivum fluidum als Ursache der Fleckenkrankheit der Tabaksblatter.
Berliner AJ, Mochizuki T and Stedman KM (2018) Astrovirology: viruses at large in the Universe. *Astrobiology* **18**, 207–223.
Bhat T, Cao A and Yin J (2022) Virus-like particles: measures and biological functions. *Viruses* **14**, 383.
Bitton G (1975) Adsorption of viruses onto surfaces in soil and water. *Water Research* **9**, 473–484.
Bonnain C, Breitbart M and Buck KN (2016) The Ferrojan Horse hypothesis: iron-virus interactions in the ocean. *Frontiers in Marine Science* **3**, 25.
Breitbart M, Salamon P, Andresen B, Mahaffy JM, Segall AM, Mead D, Azam F and Rohwer F (2002) Genomic analysis of uncultured marine viral communities. *Proceedings of the National Academy of Sciences* **99**, 14250–14255.
Bresch C, Niesert U and Harnasch D (1980) Hypercycles, parasites and packages. *Journal of Theoretical Biology* **85**, 399–405.
Brum JR and Steward GF (2010) Morphological characterization of viruses in the stratified water column of alkaline, hypersaline Mono Lake. *Microbial Ecology* **60**, 636–643.
Brum JR, Hurwitz BL, Schofield O, Ducklow HW and Sullivan MB (2016) Seasonal time bombs: dominant temperate viruses affect Southern Ocean microbial dynamics. *The ISME Journal* **10**, 437–449.
Cai L, Jørgensen BB, Suttle CA, He M, Cragg BA, Jiao N and Zhang R (2019) Active and diverse viruses persist in the deep sub-seafloor sediments over thousands of years. *The ISME Journal* **13**, 1857–1864.
Castelán-Sánchez HG, Lopéz-Rosas I, García-Suastegui WA, Peralta R, Dobson AD, Batista-García RA and Dávila-Ramos S (2019) Extremophile deep-sea viral communities from hydrothermal vents: structural and functional analysis. *Marine Genomics* **46**, 16–28.
Checinska Sielaff A, Urbaniak C, Mohan GBM, Stepanov VG, Tran Q, Wood JM, Minich J, McDonald D, Mayer T, Knight R and Karouia F (2019) Characterization of the total and viable bacterial and fungal communities associated with the International Space Station surfaces. *Microbiome* **7**, 1–21.
Chen Y, Golding I, Sawai S, Guo L and Cox EC (2005) Population fitness and the regulation of *Escherichia coli* genes by bacterial viruses. *PLoS Biology* **3**, e229.
Cheng R, Li X, Jiang L, Gong L, Geslin C and Shao Z (2022) Virus diversity and interactions with hosts in deep-sea hydrothermal vents. *Microbiome* **10**, 1–17.
Dalcin Martins P, Danczak RE, Roux S, Frank J, Borton MA, Wolfe RA, Burris MN and Wilkins MJ (2018) Viral and metabolic controls on high rates of microbial sulfur and carbon cycling in wetland ecosystems. *Microbiome* **6**, 222.
De La Higuera I and Lázaro E (2022) Viruses in astrobiology. *Frontiers in Microbiology* **13**, 4268.
De Vera JP, Alawi M, Backhaus T, Baqué M, Billi D, Böttger U, Berger T, Bohmeier M, Cockell C, Demets R and De la Torre Noetzel R (2019) Limits of life and the habitability of Mars: the ESA space experiment BIOMEX on the ISS. *Astrobiology* **19**, 145–157.
Diaz A, Li W, Irwin T, Calle L and Callahan M (2019) Investigation of Biofilm Formation and Control for Spacecraft – An Early Literature Review, 49th International Conference on Environmental Systems, Boston, MA.
Diemer GS and Stedman KM (2012) A novel virus genome discovered in an extreme environment suggests recombination between unrelated groups of RNA and DNA viruses. *Biology Direct* **7**, 1–14.
Domnin PA, Parfenov VA, Kononikhin AS, Petrov SV, Shevlyagina NV, Arkhipova AY, Koudan EV, Nezhurina EK, Brzhozovskiy AG, Bugrova AE, Moysenovich AM, Domnin PA, Parfenov VA, Kononikhin AS, Petrov SV, Shevlyagina NV, Arkhipova AY, Koudan EV, Nezhurina EK, Brzhozovskiy AG, Bugrova AE and Moysenovich AM (2022) Combined impact of magnetic force and spaceflight conditions on *Escherichia coli* physiology. *International Journal of Molecular Sciences* **23**, 1837.

https://doi.org/10.1017/S1473550423000058 Published online by Cambridge University Press

Durzyńska J and Goździcka-Józefiak A (2015) Viruses and cells intertwined since the dawn of evolution. *Virology Journal* **12**, 673.

Dzhos E, Golubkina N, Antoshkina M, Kondratyeva I, Koshevarov A, Shkaplerov A, Zavarykina T, Nechitailo G and Caruso G (2021) Effect of Spaceflight on Tomato Seed Quality and Biochemical Characteristics of Mature Plants. *Horticulturae* **7**, 89.

Eigen M (1971) Selforganization of matter and the evolution of biological macromolecules. *Die Naturwissenschaften* **58**, 465–523.

Emerson JB, Roux S, Brum JR, Bolduc B, Woodcroft BJ, Jang HB, Singleton CM, Solden LM, Naas AE, Boyd JA and Hodgkins SB (2018) Host-linked soil viral ecology along a permafrost thaw gradient. *Nature Microbiology* **3**, 870–880.

Enard D, Cai L, Gwennap C and Petrov DA (2016) Viruses are a dominant driver of protein adaptation in mammals. *eLife* **5**, e12469.

Engelhardt T, Sahlberg M, Cypionka H and Engelen B (2013) Biogeography of *Rhizobium radiobacter* and distribution of associated temperate phages in deep subseafloor sediments. *The ISME Journal* **7**, 199–209.

Engelhardt T, Orsi WD and Jørgensen BB (2015) Viral activities and life cycles in deep subseafloor sediments. *Environmental Microbiology Reports* **7**, 868–873.

Fahrion J, Mastroleo F, Dussap CG and Leys N (2021) Use of photobioreactors in regenerative life support systems for human space exploration. *Frontiers in Microbiology* **12**, 1748.

Folimonova SY, Achor D and Bar-Joseph M (2020) Walking together: cross-protection, genome conservation, and the replication machinery of *Citrus tristeza* virus. *Viruses* **12**, 1353.

Forterre P (2006) Three RNA cells for ribosomal lineages and three DNA viruses to replicate their genomes: A hypothesis for the origin of cellular domain. *Proceedings of the National Academy of Sciences* **103**, 3669–3674.

Forterre P (2011) Manipulation of cellular syntheses and the nature of viruses: The virocell concept. *Comptes Rendus Chimie* **14**, 392–399.

Forterre P (2013) The virocell concept and environmental microbiology. *The ISME Journal* **7**, 233–236.

Forterre P and Gaïa M (2016) Giant viruses and the origin of modern eukaryotes. *Current Opinion in Microbiology* **31**, 44–49.

Forterre P and Prangishvili D (2009) The great billion-year war between ribosome-and capsid-encoding organisms (cells and viruses) as the major source of evolutionary novelties. *Annals of the New York Academy of Sciences* **1178**, 65–77.

Gao S, Paez-Espino D, Li J, Ai H, Liang J, Luo Z, Zheng J, Chen H, Shu W and Huang L (2022) Patterns and ecological drivers of viral communities in acid mine drainage sediments across Southern China. *Nature Communications* **13**, 2389.

Gates SD, Condit RC, Moussatche N, Stewart BJ, Malkin AJ and Weber PK (2018) High initial sputter rate found for vaccinia virions using isotopic labeling, NanoSIMS, and AFM. *Analytical Chemistry* **90**, 1613–1620.

Gil JF, Mesa V, Estrada-Ortiz N, Lopez-Obando M, Gómez A and Plácido J (2021) Viruses in extreme environments, current overview, and biotechnological potential. *Viruses* **13**, 81.

Greene SE and Reid A (2013) Viruses throughout life & time: friends, foes, change agents: A report on an American Academy of Microbiology Colloquium San Francisco, July 2013. Washington, DC: American Society for Microbiology.

Guglielmini J, Woo AC, Krupovic M, Forterre P and Gaia M (2019) Diversification of giant and large eukaryotic dsDNA viruses predated the origin of modern eukaryotes. *Proceedings of the National Academy of Sciences* **116**, 19585–19592.

Gupta K, Lee Y and Yin J (1995) Extremo-phage: in vitro selection of tolerance to a hostile environment. *Journal of Molecular Evolution* **41**, 113–114.

Häring M, Rachel R, Peng X, Garrett RA and Prangishvili D (2005) Viral diversity in hot springs of Pozzuoli, Italy, and characterization of a unique archaeal virus, *Acidianus* bottle-shaped virus, from a new family, the Ampullaviridae. *Journal of Virology* **79**, 9904–9911.

Harris HM and Hill C (2021) A place for viruses on the tree of life. *Frontiers in Microbiology* **11**, 604048.

Havig JR and Hamilton TL (2019) Snow algae drive productivity and weathering at volcanic rock-hosted glaciers. *Geochimica et Cosmochimica Acta* **247**, 220–242.

Helisch H, Keppler J, Detrell G, Belz S, Ewald R, Fasoulas S and Heyer AG (2020) High density long-term cultivation of *Chlorella vulgaris* SAG 211-12 in a novel microgravity-capable membrane raceway photobioreactor for future bioregenerative life support in SPACE. *Life Sciences in Space Research* **24**, 91–107.

Hornibrook ERC, Longstaffe FJ and Fyfe WS (2000) Evolution of stable carbon isotope compositions for methane and carbon dioxide in freshwater wetlands and other anaerobic environments. *Geochimica et Cosmochimica Acta* **64**, 1013–1027.

Howard-Varona C, Hargreaves KR, Abedon ST and Sullivan MB (2017) Lysogeny in nature: mechanisms, impact and ecology of temperate phages. *The ISME Journal* **11**, 1511–1520.

Jalava K, Kauppinen A, Al-Hello H and Räsänen S (2019) An outbreak of norovirus infection caused by ice cubes and a leaking air ventilation valve. *Epidemiology & Infection* **147**, e57.

Jang H-B, Bolduc B, Zablocki O, Kuhn JH, Roux S, Adriaenssens EM, Brister JR, Kropinski AM, Krupovic M, Lavigne R and Turner D (2019) Taxonomic assignment of uncultivated prokaryotic virus genomes is enabled by gene-sharing networks. *Nature Biotechnology* **37**, 632–639.

Janjic A (2018) The need for including virus detection methods in future Mars Missions. *Astrobiology* **18**, 1611–1614.

Jin T and Yin J (2021) Patterns of virus growth across the diversity of life. *Integrative Biology* **13**, 44–59.

Johnson PA, Johnson JC and Mardon AA (2021) Where to Look Next: Extant Life Niches and Biomarkers on Mars. In 52nd Lunar and Planetary Science Conference (No. 2548, p. 1081).

Justiniano YAV, Goeres DM, Sandvik EL, Kjellerup BV, Sysoeva TA, Harris JS, Warnat S, McGlennen M, Foreman CM, Yang J and Li W (2023) Mitigation and use of biofilms in space for the benefit of human space exploration. *Biofilm* **5**, 100102.

Kim H and Yin J (2004) Energy-efficient growth of phage Q Beta in *Escherichia coli*. *Biotechnology and Bioengineering* **88**, 148–156.

Koonin EV and Wolf YI (2012) Evolution of microbes and viruses: a paradigm shift in evolutionary biology? *Frontiers in Cellular and Infection Microbiology* **2**, 119.

Koonin EV, Senkevich TG and Dolja VV (2006). The ancient Virus World and evolution of cells. *Biology Direct* **1**, 1–27.

Krissansen-Totton J, Buick R and Catling DC (2015) A statistical analysis of the carbon isotope record from the Archean to Phanerozoic and implications for the rise of oxygen. *American Journal of Science* **315**, 275–316.

Kristensen DM, Mushegian AR, Dolja VV and Koonin EV (2010) New dimensions of the virus world discovered through metagenomics. *Trends in Microbiology* **18**, 11–19.

Kyle JE and Ferris FG (2013) Geochemistry of virus–prokaryote interactions in freshwater and acid mine drainage environments, Ontario, Canada. *Geomicrobiology Journal* **30**, 769–778.

Laidler JR and Stedman KM (2010) Virus silicification under simulated hot spring conditions. *Astrobiology* **10**, 569–576.

Laidler JR, Shugart JA, Cady SL, Bahjat KS and Stedman KM (2013) Reversible inactivation and desiccation tolerance of silicified viruses. *Journal of Virology* **87**, 13927–13929.

Lance JC and Gerba CP (1984) Virus movement in soil during saturated and unsaturated flow. *Applied and Environmental Microbiology* **47**, 335–337.

Landry KS, Morey JM, Bharat B, Haney NM and Panesar SS (2020) Biofilms – impacts on human health and its relevance to space travel. *Microorganisms* **8**, 998.

Landweber LF, Simon PJ and Wagner TA (1998) Ribozyme engineering and early evolution. *BioScience* **48**, 94–103.

Laranjeiro R, Harinath G, Pollard AK, Gaffney CJ, Deane CS, Vanapalli SA, Etheridge T, Szewczyk NJ and Driscoll M (2021) Spaceflight affects neuronal morphology and alters transcellular degradation of neuronal debris in adult *Caenorhabditis elegans*. *iScience* **24**, 102105.

Lee G, Sherer NA, Kim NH, Rajic E, Kaur D, Urriola N, Martini KM, Xue C, Goldenfeld N and Kuhlman TE (2018) Testing the retroelement invasion hypothesis for the emergence of the ancestral eukaryotic cell. *Proceedings of the National Academy of Sciences* **115**, 12465–12470.

Lee S, Sieradzki ET, Nicolas AM, Walker RL, Firestone MK, Hazard C and Nicol GW (2021) Methane-derived carbon flows into host–virus networks at different trophic levels in soil. *Proceedings of the National Academy of Sciences* **118**, e2105124118.

Lee S, Sieradzki ET, Hazard C and Nicol GW (2022) Propagation of viral genomes by replicating ammonia-oxidising archaea during soil nitrification. *The ISME Journal* **17**, 309–314.

Legendre M, Bartoli J, Shmakova L, Jeudy S, Labadie K, Adrait A, Lescot M, Poirot O, Bertaux L, Bruley C and Couté Y (2014) Thirty-thousand-year-old distant relative of giant icosahedral DNA viruses with a pandoravirus morphology. *Proceedings of the National Academy of Sciences* **111**, 4274–4279.

Lipson SM and Stotzky G (1985) Specificity of virus adsorption to clay minerals. *Canadian Journal of Microbiology* **31**, 50–53.

Loeffler F and Frosch P (1897) Summarischer bericht uber die ergebnisse der untersuchungen zur erforschung der maul-und klauenseuche. *Zentbl Bakteriol Parasitenkd Infektionskr Hyg Abt I* **22**, 257–259.

Luria SE, Darnell Jr JE, Baltimore D and Campbell A (1978). In Hadar J (ed). *General Virology*, 3rd Edn. New York: John Wiley & Sons, Inc.

Mahmoudabadi G, Milo R and Phillips R (2017) Energetic cost of building a virus. *Proceedings of the National Academy of Sciences* **114**, E4324–E4333.

Malavasi V, Soru S and Cao G (2020) Extremophile microalgae: the potential for biotechnological application. *Journal of Phycology* **56**, 559–573.

Manefield M, Whiteley AS, Griffiths RI and Bailey MJ (2002) RNA Stable isotope probing, a novel means of linking microbial community function to phylogeny. *Applied and Environmental Microbiology* **68**, 5367–5373.

Márquez LM, Redman RS, Rodriguez RJ and Roossinck MJ (2007) A virus in a fungus in a plant: three-way symbiosis required for thermal tolerance. *Science* **315**, 513–515.

Matsumura S, Kun Á, Ryckelynck M, Coldren F, Szilágyi A, Jossinet F, Rick C, Nghe P, Szathmáry E and Griffiths AD (2016) Transient compartmentalization of RNA replicators prevents extinction due to parasites. *Science* **354**, 1293–1296.

Matula EE and Nabity JA (2019) Failure modes, causes, and effects of algal photobioreactors used to control a spacecraft environment. *Life Sciences in Space Research* **20**, 35–52.

Matula EE and Nabity JA (2021) Effects of stepwise changes in dissolved carbon dioxide concentrations on metabolic activity in Chlorella for spaceflight applications. *Life Sciences in Space Research* **29**, 73–84.

Matula EE, Nabity JA and McKnight DM (2021) Supporting simultaneous air revitalization and thermal control in a crewed habitat with temperate *Chlorella vulgaris* and eurythermic Antarctic Chlorophyta. *Frontiers in Microbiology* **12**, 2348.

Mayali X (2020) NanoSIMS: microscale quantification of biogeochemical activity with large-scale impacts. *Annual Review of Marine Science* **12**, 449–467.

Mayali X, Weber PK, Nuccio E, Lietard J, Somoza M, Blazewicz SJ and Pett-Ridge J (2019) Chip-SIP: stable isotope probing analyzed with rRNA-targeted microarrays and NanoSIMS. In Dumont MG and Hernández García M (eds). *Stable Isotope Probing*. New York, NY: Humana, pp. 71–87.

McKnight DM, Tate CM, Andrews ED, Niyogi DK, Cozzetto K, Welch K, Lyons WB and Capone DG (2007) Reactivation of a cryptobiotic stream ecosystem in the McMurdo Dry valleys, Antarctica: a long-term geomorphological experiment. *Geomorphology* **89**, 186–204.

McMullen A, De Haan HW, Tang JX and Stein D (2014) Stiff filamentous virus translocations through solid-state nanopores. *Nature Communications* **5**, 1–10.

Mehta SK, Cohrs RJ, Forghani B, Zerbe G, Gilden DH and Pierson DL (2004) Stress-induced subclinical reactivation of varicella zoster virus in astronauts. *Journal of Medical Virology* **72**, 174–179.

Meyer M, Bakermans C, Beaty D, Bernard D, Boston P, Chevrier V, Conley C, Feustel I, Gough R, Glotch T, Hays L, Junge K, Lindberg R, Mellon M, Mischna M, Neal CR, Pugel B, Quinn R, Raulin F, Rennó N, Rummel J, Schulte M, Spry A, Stabekis P, Wang A and Yee N (2019) Report of the joint workshop on induced special regions. *Life Sciences in Space Research* **23**, 50–59.

Mi S, Lee X, Li X-P, Veldman GM, Finnerty H, Racie L, LaVallie E, Tang X-Y, Edouard P, Howes S, Keith JC and McCoy JM (2000) Syncytin is a captive retroviral envelope protein involved in human placental morphogenesis. *Nature* **403**, 785–789.

Moelling K and Broecker F (2019) Viruses and evolution – Viruses first? A personal perspective. *Frontiers in Microbiology* **10**, 14542.

Mohiuddin M and Schellhorn HE (2015) Spatial and temporal dynamics of virus occurrence in two freshwater lakes captured through metagenomic analysis. *Frontiers in Microbiology* **6**, 960.

Mora M, Wink L, Kögler I, Mahnert A, Rettberg P, Schwendner P, Demets R, Cockell C, Alekhova T, Klingl A, Krause R, Zolotariof A, Alexandrova A and Moissl-Eichinger C (2019) Space station conditions are selective but do not alter microbial characteristics relevant to human health. *Nature Communications* **10**, 3990.

Morono Y, Ito M, Hoshino T, Terada T, Hori T, Ikehara M, D'Hondt S and Inagaki F (2020) Aerobic microbial life persists in oxic marine sediment as old as 101.5 million years. *Nature Communications* **11**, 1–9.

Müller F, Escobar L, Xu F, Węgrzyn E, Nainytė M, Amatov T, Chan CY, Pichler A and Carell T (2022) A prebiotically plausible scenario of an RNA–peptide world. *Nature* **605**, 279–284.

Mushegian AR (2020) Are there 1031 virus particles on earth, or more, or fewer? *Journal of Bacteriology* **202**, e00052–20.

Nagayoshi Y, Kumagae K, Mori K, Tashiro K, Nakamura A, Fujino Y, Hiromasa Y, Iwamoto T, Kuhara S, Ohshima T and Doi KT (2016) Physiological properties and genome structure of the hyperthermophilic filamentous phage ϕOH3 which infects thermus thermophilus HB8. *Frontiers in Microbiology* **7**, 50.

Nuccio EE, Blazewicz SJ, Lafler M, Campbell AN, Kakouridis A, Kimbrel JA, Wollard J, Vyshenska D, Riley R, Tomatsu A and Hestrin R (2022) HT-SIP: a semi-automated stable isotope probing pipeline identifies cross-kingdom interactions in the hyphosphere of arbuscular mycorrhizal fungi. *Microbiome* **10**, 199.

Olofsson L, Ankarloo J, Andersson PO and Nicholls IA (2001) Filamentous bacteriophage stability in non-aqueous media. *Chemistry & Biology* **8**, 661–671.

Ostrom NE and Ostrom PH (2017) Mining the isotopic complexity of nitrous oxide: a review of challenges and opportunities. *Biogeochemistry* **132**, 359–372.

Own CS, Martinez J, Cushing J, DeRego T, Own LS, Weppelman G, Thomas-Keprta KT, Rahman Z and Pettit DR (2018) Portable electron microscopy for ISS and beyond. In ISSR&D Conference 2018 (No. JSC-E-DAA-TN57264).

Pacton M, Wacey D, Corinaldesi C, Tangherlini M, Kilburn MR, Gorin GE, Danovaro R and Vasconcelos C (2014) Viruses as new agents of organomineralization in the geological record. *Nature Communications* **5**, 1–9.

Paez-Espino D, Eloe-Fadrosh EA, Pavlopoulos GA, Thomas AD, Huntemann M, Mikhailova N, Rubin E, Ivanova NN and Kyrpides NC (2016) Uncovering Earth's virome. *Nature* **536**, 425–430.

Parikka KJ, Le Romancer M, Wauters N and Jacquet S (2017) Deciphering the virus-to-prokaryote ratio (VPR): insights into virus–host relationships in a variety of ecosystems. *Biological Reviews* **92**, 1081–1100.

Pasulka AL, Thamatrakoln K, Kopf SH, Guan Y, Poulos B, Moradian A, Sweredoski MJ, Hess S, Sullivan MB, Bidle KD and Orphan VJ (2018) Interrogating marine virus–host interactions and elemental transfer with BONCAT and NanoSIMS-based methods. *Environmental Microbiology* **20**, 671–692.

Patel A, Sharma P, Reddy N, Princy R, Tsaramirsis G, Pavlopoulou A, Koçer ZA and Piromalis D (2021) Bio-virus spread simulation in real 3D space using augmented reality. *Engineered Science* **16**, 319–330.

Pavletić B, Runzheimer K, Siems K, Koch S, Cortesão M, Ramos-Nascimento A and Moeller R (2022) Spaceflight virology: what do we know about viral threats in the spaceflight environment? *Astrobiology* **22**, 210–224.

Pelusi A, De Luca P, Manfellotto F, Thamatrakoln K, Bidle KD and Montresor M (2021) Virus-induced spore formation as a defense mechanism in marine diatoms. *New Phytologist* **229**, 2251–2259.

Penning H, Claus P, Casper P and Conrad R (2006) Carbon isotope fractionation during acetoclastic methanogenesis by *Methanosaeta concilii* in culture and a lake sediment. *Applied and Environmental Microbiology* **72**, 5648–5652.

Perrault J (1981) Origin and replication of defective interfering particles. In: Shatkin AJ (ed). *Initiation Signals in Viral Gene Expression. Current Topics in Microbiology and Immunology*, vol 93. Berlin, Heidelberg: Springer. https://doi.org/10.1007/978-3-642-68123-3_7

Pett-Ridge J and Weber PK (2022) NanoSIP: NanoSIMS applications for microbial biology. In Navid A (ed). *Microbial Systems Biology*. New York, NY: Humana, pp. 91–136.

Pierson DL, Stowe RP, Phillips TM, Lugg DJ and Mehta SK (2005) Epstein–Barr virus shedding by astronauts during space flight☆. *Brain, Behavior, and Immunity* **19**, 235–242.

Pike WT, Staufer U, Hecht MH, Goetz W, Parrat D, Sykulska-Lawrence H, Vijendran S and Madsen MB (2011) Quantification of the dry history of the Martian soil inferred from in situ microscopy. *Geophysical Research Letters* **38**, 24.

Postberg F, Khawaja N, Abel B, Choblet G, Glein CR, Gudipati MS, Henderson BL, Hsu H-W, Kempf S, Klenner F, Moragas-Klostermeyer G, Magee B, Nölle L, Perry M, Reviol R, Schmidt J, Srama R, Stolz F, Tobie G, Trieloff M and Waite JH (2018) Macromolecular organic compounds from the depths of Enceladus. *Nature* **558**, 564–568.

Poughon L, Laroche C, Creuly C, Dussap CG, Paille C, Lasseur C, Monsieurs P, Heylen W, Coninx I, Mastroleo F and Leys N (2020) *Limnospira indica* PCC8005 growth in photobioreactor: model and simulation of the ISS and ground experiments. *Life Sciences in Space Research* **25**, 53–65.

Radajewski S, Ineson P, Parekh NR and Murrell JC (2000) Stable-isotope probing as a tool in microbial ecology. *Nature* **403**, 646–649.

Reche I, D'Orta G, Mladenov N, Winget DM and Suttle CA (2018) Deposition rates of viruses and bacteria above the atmospheric boundary layer. *The ISME Journal* **12**, 1154–1162.

Regberg A, Bell MS, Davis R, Roeschel J, Rucker M, Tschirschwitz M and Wallace S (2022) ISS External Microorganisms: A Payload to Close Planetary Protection Knowledge Gaps for Crewed Missions, 44th COSPAR Scientific Assembly. https://www.govinfo.gov/content/pkg/FR-2022-11-17/pdf/2022-24999.pdf.

Ricciardi A, Cassey P, Leuko S and Woolnough AP (2022) Planetary biosecurity: applying invasion science to prevent biological contamination from space travel. *BioScience* **72**, 247–253.

Rooney BV, Crucian BE, Pierson DL, Laudenslager ML and Mehta SK (2019) Herpes virus reactivation in astronauts during spaceflight and its application on earth. *Frontiers in Microbiology* **16**, 16.

Roosinck MJ (2011) The good viruses: viral mutualistic symbioses. *Nature Reviews Microbiology* **9**, 99–108.

Roossinck MJ (2012) Plant virus metagenomics: biodiversity and ecology. *Annual Review of Genetics* **46**, 359–369.

Round JL and Mazmanian SK (2009) The gut microbiota shapes intestinal immune responses during health and disease. *Nature Reviews Immunology* **9**, 313–323.

Roux S, Brum JR, Dutilh BE, Sunagawa S, Duhaime MB, Loy A, Poulos BT, Solonenko N, Lara E, Poulain J, Pesant S, Kandels-Lewis S, Dimier C, Picheral M, Searson S, Cruaud C, Alberti A, Duarte CM, Gasol JM., Vaqué D, Bork P, Acinas SG, Wincker P and Sullivan MB (2016) Ecogenomics and potential biogeochemical impacts of globally abundant ocean viruses. *Nature* **537**, 689–693.

Roux S, Chan LK, Egan R, Malmstrom RR, McMahon KD and Sullivan MB (2017) Ecogenomics of virophages and their giant virus hosts assessed through time series metagenomics. *Nature Communications* **8**, 858.

Roux S, Adriaenssens EM, Dutilh BE, Koonin EV, Kropinski AM, Krupovic M, Kuhn JH, Lavigne R, Brister JR, Varsani A and Amid C (2019) Minimum information about an uncultivated virus genome (MIUViG). *Nature Biotechnology* **37**, 29–37.

Roux S, Fischer MG, Hackl T, Katz LA, Schulz F and Yutin N (2023) Updated virophage taxonomy and distinction from polinton-like viruses. *Biomolecules* **13**, 204.

Rubio L, Galipienso L and Ferriol I (2020) Detection of plant viruses and disease management: relevance of genetic diversity and evolution. *Frontiers in Plant Science* **11**, 1604–1607.

Rummel JD, Beaty DW, Jones MA, Bakermans C, Barlow NG, Boston PJ, Chevrier VF, Clark BC, de Vera JPP, Gough RV, Hallsworth JE, Head JW, Hipkin VJ, Kieft TL, McEwen AS, Mellon MT, Mikucki JA, Nicholson WL, Omelon CR, Peterson R, Roden EE, Sherwood Lollar B, Tanaka KL, Viola D and Wray JJ (2014) A new analysis of Mars 'special regions': findings of the second MEPAG Special Regions Science Analysis Group (SR-SAG2). *Astrobiology* **14**, 887–968.

Schoenfeld T, Patterson M, Richardson PM, Wommack KE, Young M and Mead D (2008) Assembly of viral metagenomes from yellowstone hot springs. *Applied and Environmental Microbiology* **74**, 4164–4174.

Schulz F, Roux S, Paez-Espino D, Jungbluth S, Walsh DA, Denef VJ, McMahon KD, Konstantinidis KT, Eloe-Fadrosh EA, Kyrpides NC and Woyke T (2020) Giant virus diversity and host interactions through global metagenomics. *Nature* **578**, 432–436.

Schwieterman EW, Kiang NY, Parenteau MN, Harman CE, DasSarma S, Fisher TM, Arney GN, Hartnett HE, Reinhard CT, Olson SL, Meadows VS, Cockell CS, Walker SI, Grenfell JL, Hegde S, Rugheimer S, Hu R and Lyons TW (2018) Exoplanet biosignatures: A review of remotely detectable signs of life. *Astrobiology* **18**, 663–708.

Seaman PF and Day MJ (2007) Isolation and characterization of a bacteriophage with an unusually large genome from the great salt plains national wildlife refuge, Oklahoma, USA. *FEMS Microbiology Ecology* **60**, 1–13.

Sharma G and Curtis PD (2022) The impacts of microgravity on bacterial metabolism. *Life* **12**, 774.

Shatilovich AV, Tchesunov AV, Neretina TV, Grabarnik IP, Gubin SV, Vishnivetskaya TA, Onstott TC and Rivkina EM (2018) Viable nematodes from late Pleistocene permafrost of the Kolyma river lowland. In Gabibov AG (ed). *Doklady Biological Sciences*, vol. **480**, pp. 100–102. New York, NY: Pleiades Publishing.

Shmakova L, Malavin S, Iakovenko N, Vishnivetskaya T, Shain D, Plewka M and Rivkina E (2021) A living *Bdelloid rotifer* from 24,000-year-old Arctic permafrost. *Current Biology* **31**, R712–R713.

Shutt T and Gray M (2006) Bacteriophage origins of mitochondrial replication and transcription proteins. *Trends in Genetics* **22**, 90–95.

Sieradzki ET, Ignacio-Espinoza JC, Needham DM, Fichot EB and Fuhrman JA (2019) Dynamic marine viral infections and major contribution to photosynthetic processes shown by spatiotemporal picoplankton metatranscriptomes. *Nature Communications* **10**, 60.

Singh NK, Wood JM, Karouia F and Venkateswaran K (2018) Succession and persistence of microbial communities and antimicrobial resistance genes associated with international space station environmental surfaces. *Microbiome* **6**, 1–23.
Sommers P, Fontenele RS, Kringen T, Kraberger S, Porazinska DL, Darcy JL, Schmidt SK and Varsani A (2019) Single-stranded DNA viruses in Antarctic cryoconite holes. *Viruses* **11**, 1022.
Sommers P, Chatterjee A, Varsani A and Trubl G (2021) Integrating viral metagenomics into an ecological framework. *Annual Review of Virology* **8**, 133–1588.
Starr EP, Shi S, Blazewicz SJ, Koch BJ, Probst AJ, Hungate BA, Pett-Ridge J, Firestone MK and Banfield JF (2021) Stable-isotope-informed, genome-resolved metagenomics uncovers potential cross-kingdom interactions in rhizosphere soil. *Msphere* **6**, e00085–21.
Stowe RP, Pierson DL, Feeback DL and Barrett AD (2000) Stress-induced reactivation of Epstein–Barr virus in astronauts. *Neuroimmunomodulation* **8**, 51–58.
Suttle CA (2005) Viruses in the sea. *Nature* **437**, 356–361.
Syngouna VI and Chrysikopoulos CV (2010) Interaction between viruses and clays in static and dynamic batch systems. *Environmental Science & Technology* **44**, 4539–4544.
Tepfer D and Leach S (2017) Survival and DNA damage in plant seeds exposed for 558 and 682 days outside the International Space Station. *Astrobiology* **17**, 205–215.
Thiroux S, Dupont S, Nesbø CL, Bienvenu N, Krupovic M, L'Haridon S, Marie D, Forterre P, Godfroy A and Geslin C (2021) The first head-tailed virus, MFTV1, infecting hyperthermophilic methanogenic deep-sea archaea. *Environmental Microbiology* **23**, 3614–3626.
Thompson LR, Zeng Q, Kelly L, Huang KH, Singer AU, Stubbe J and Chisholm SW (2011) Phage auxiliary metabolic genes and the redirection of cyanobacterial host carbon metabolism. *Proceedings of the National Academy of Sciences* **108**. http://dx.doi.org/10.1073/pnas.1102164108.
Tirumalai MR, Rastogi R, Zamani N, O'Bryant Williams E, Allen S, Diouf F, Kwende S, Weinstock GM, Venkateswaran KJ, Fox GE and Setlow P (2013) Candidate Genes That May Be Responsible for the Unusual Resistances Exhibited by Bacillus pumilus SAFR-032 Spores. *PLoS ONE* **8**, e66012.
Tisza MJ, Pastrana DV, Welch NL, Stewart B, Peretti A, Starrett GJ, Pang Y-YS, Krishnamurthy SR, Pesavento PA, McDermott DH, Murphy PM, Whited JL, Miller B, Brenchley J, Rosshart SP, Rehermann B, Doorbar J, Ta'ala BA, Pletnikova O, Troncoso JC, Resnick SM, Bolduc B, Sullivan MB, Varsani A, Segall AM and Buck CB (2020) Discovery of several thousand highly diverse circular DNA viruses. *eLife* **9**, 2470.
Travers Cook TJ, Skirgaila C, Martin OY and Buser CC (2022) Infection by dsRNA viruses is associated with enhanced sporulation efficiency in *Saccharomyces cerevisiae*. *Ecology and Evolution* **12**, e8558.
Trubl G, Jang HB, Roux S, Emerson JB, Solonenko N, Vik DR, Solden L, Ellenbogen J, Runyon AT, Bolduc B and Woodcroft BJ (2018) Soil viruses are underexplored players in ecosystem carbon processing. *mSystems* **3**, e00076-18.
Trubl G, Roux S, Solonenko N, Li Y-F, Bolduc B, Rodríguez-Ramos J, Eloe-Fadrosh EA, Rich VI and Sullivan MB (2019) Towards optimized viral metagenomes for double-stranded and single-stranded DNA viruses from challenging soils. *PeerJ* **7**, e7265.
Trubl G, Kimbrel JA, Liquet-Gonzalez J, Nuccio EE, Weber PK, Pett-Ridge J, Jansson JK, Waldrop MP and Blazewicz SJ (2021) Active virus–host interactions at sub-freezing temperatures in Arctic peat soil. *Microbiome* **9**, 1–15.
Tyler KL (2008) Segmented double-stranded RNA viruses: structure and molecular biology. *The Lancet Infectious Diseases* **8**, 224.
Urbaniak C, Sielaff AC, Frey KG, Allen JE, Singh N, Jaing C, Wheeler K and Venkateswaran K (2018) Detection of antimicrobial resistance genes associated with the international space station environmental surfaces. *Scientific Reports* **8**, 1–13.
Urbaniak C, Morrison MD, Thissen JB, Karouia F, Smith DJ, Mehta S, Jaing C and Venkateswaran K (2022) Microbial tracking-2, a metagenomics analysis of bacteria and fungi onboard the international space station. *Microbiome* **10**, 1–19.
Van Goethem MW, Swenson TL, Trubl G, Roux S and Northen TR (2019) Characteristics of wetting-induced bacteriophage blooms in biological soil crust. *MBio* **10**, e02287–19.
Van Zyl LJ, Nemavhulani S, Cass J, Cowan DA and Trindade M (2016) Three novel bacteriophages isolated from the east African rift valley soda lakes. *Virology Journal* **13**, 1–14.
Vignuzzi M and López CB (2019) Defective viral genomes are key drivers of the virus–host interaction. *Nature Microbiology* **4**, 1075–1087.
Vincent F and Vardi A (2023) Viral infection in the ocean – a journey across scales. *PLoS Biology* **21**, e3001966.
Vlok M, Lang AS and Suttle CA (2019) Application of a sequence-based taxonomic classification method to uncultured and unclassified marine single-stranded RNA viruses in the order Picornavirales. *Virus Evolution* **5**, 10.
Voorhies AA, Mark Ott C, Mehta S, Pierson DL, Crucian BE, Feiveson A, Oubre CM, Torralba M, Moncera K, Zhang Y and Zurek E (2019) Study of the impact of long-duration space missions at the international space station on the astronaut microbiome. *Scientific Reports* **9**, 1–17.
Vroom MM, Rodriguez-Ocasio Y, Lynch JB, Ruby EG and Foster JS (2021) Modeled microgravity alters lipopolysaccharide and outer membrane vesicle production of the beneficial symbiont *Vibrio fischeri*. *npj Microgravity* **7**, 8.
Wagner AO, Malin C, Knapp BA and Illmer P (2008) Removal of free extracellular DNA from environmental samples by ethidium monoazide and propidium monoazide. *Applied and Environmental Microbiology* **74**, 2537–2539.

Weinberg CE, Weinberg Z and Hammann C (2019) Novel ribozymes: discovery, catalytic mechanisms, and the quest to understand biological function. *Nucleic Acids Research* **47**, 9480–9494.
Weinmaier T, Probst AJ, La Duc MT, Ciobanu D, Cheng J-F, Ivanova N, Rattei T and Vaishampayan P (2015) A viability-linked metagenomic analysis of cleanroom environments: eukarya, prokaryotes, and viruses. *Microbiome* **3**, 1.
Wells LE and Deming JW (2006a) Modelled and measured dynamics of viruses in Arctic winter sea-ice brines. *Environmental Microbiology* **8**, 1115–1121.
Wells LE and Deming JW (2006b) Characterization of a cold-active bacteriophage on two psychrophilic marine hosts. *Aquatic Microbial Ecology* **45**, 15–29.
White III RA, Visscher PT and Burns BP (2021) Between a rock and a soft place: the role of viruses in lithification of modern microbial mats. *Trends in Microbiology* **29**, 204–213.
Whiticar MJ (1999) Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane. *Chemical Geology* **161**, 291–314.
Whon TW, Kim MS, Roh SW, Shin NR, Lee HW and Bae JW (2012) Metagenomic characterization of airborne viral DNA diversity in the near-surface atmosphere. *Journal of Virology* **86**, 8221–8231.
Willson D, Rask JC, George SC, deLeon P, Bonaccors R, Blank J, Slocombe J, Silburn K, Steele H, Gargarno M and McKay CP (2014) The performance of field scientists undertaking observations of early life fossils while in simulated space suit. *Acta Astronautica* **93**, 193–206.
Wu H, Chen Y, Zhou Q, Wang R, Xia B, Ma D, Luo K and Liu Q (2016) Translocation of rigid rod-shaped virus through various solid-state nanopores. *Analytical Chemistry* **88**, 2502–2510.
Wu R, Davison MR, Gao Y, Nicora CD, Mcdermott JE, Burnum-Johnson KE, Hofmockel KS and Jansson JK (2021) Moisture modulates soil reservoirs of active DNA and RNA viruses. *Communications Biology* **4**, 1–11.
Wu R, Bottos EM, Danna VG, Stegen JC, Jansson JK and Davison MR (2022) RNA viruses linked to eukaryotic hosts in thawed permafrost. *mSystems* **7**, e00582-22.
Yau S, Lauro FM, DeMaere MZ, Brown MV, Thomas T, Raftery MJ, Andrews-Pfannkoch C, Lewis M, Hoffman JM, Gibson JA and Cavicchioli R (2011) Virophage control of Antarctic algal host–virus dynamics. *PNAS* **108**, 6163–6168.
Yin J and Redovich J (2018) Kinetic modeling of virus growth in cells. *Microbiology and Molecular Biology Reviews* **82**, e00066–17.
You L, Suthers PF and Yin J (2002) Effects of *Escherichia coli* physiology on growth of phage T7 in vivo and in silico. *Journal of Bacteriology* **184**, 1888–1894.
Yu ZC, Chen XL, Shen QT, Zhao DL, Tang BL, Su HN, Wu ZY, Qin QL, Xie BB, Zhang XY and Yu Y (2015) Filamentous phages prevalent in *Pseudoalteromonas* spp. confer properties advantageous to host survival in Arctic sea ice. *The ISME Journal* **9**, 871–881.
Zawierucha K, Ostrowska M and Kolicka M (2017) Applicability of cryoconite consortia of microorganisms and glacier-dwelling animals in astrobiological studies. *Contemporary Trends in Geoscience* **6**, 1–10.
Zayed AA, Wainaina JM, Dominguez-Huerta G, Pelletier E, Guo J, Mohssen M, Tian F, Pratama AA, Bolduc B, Zablocki O and Cronin D (2022) Cryptic and abundant marine viruses at the evolutionary origins of Earth's RNA virome. *Science* **376**, 156–162.
Zea L, Nisar Z, Rubin P, Cortesão M, Luo J, McBride SA, Moeller R, Klaus D, Müller D, Varanasi KK, Muecklich F and Stodieck L (2018) Design of a spaceflight biofilm experiment. *Acta Astronautica* **148**, 294–300.
Zea L, Prasad N, Levy SE, Stodieck L, Jones A, Shrestha S, *et al*. (2016) A molecular genetic basis explaining altered bacterial behavior in space. *PLoS ONE* **11**, e0164359.
Zerkle AL and Mikhail S (2017) The geobiological nitrogen cycle: From microbes to the mantle. *Geobiology* **15**, 343–352.
Zhang Y, Hung T, Song J and He J (2013) Electron microscopy: essentials for viral structure, morphogenesis and rapid diagnosis. *Science China Life Sciences* **56**, 421–430.
Zhong ZP, Rapp JZ, Wainaina JM, Solonenko NE, Maughan H, Carpenter SD, Cooper ZS, Jang HB, Bolduc B, Deming JW and Sullivan MB (2020) Viral ecogenomics of Arctic cryopeg brine and sea ice. *mSystems* **5**, e00246-20.
Zhou K, Li L, Tan Z, Zlotnick A and Jacobson SC (2011) Characterization of hepatitis B virus capsids by resistive-pulse sensing. *Journal of the American Chemical Society* **133**, 1618–1621.