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Viruses in Soil Ecosystems: An Unknown Quantity Within an Unexplored Territory

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Abstract

Viral abundance in soils can range from below detection limits in hot deserts to over 1 billion per gram in wetlands. Abundance appears to be strongly influenced by water availability and temperature, but a lack of informational standards creates difficulties for cross-study analysis. Soil viral diversity is severely underestimated and undersampled, although current measures of viral richness are higher for soils than for aquatic ecosystems. Both morphometric and metagenomic analyses have raised questions about the prevalence of nontailed, ssDNA viruses in soils. Soil is complex and critically important to terrestrial biodiversity and human civilization, but impacts of viral activities on soil ecosystem services are poorly understood. While information from aquatic systems and medical microbiology suggests the potential for viral influences on nutrient cycles, food web interactions, gene transfer, and other key processes in soils, very few empirical data are available. To understand the soil virome, much work remains.

INTRODUCTION

The main goal of this review is to synthesize the current state of understanding regarding the ecology of autochthonous viruses in soil ecosystems. In this context, autochthonous viruses are those produced in situ by host organisms that dwell within soils, as opposed to land applied viruses from exogenous sources (e.g., wastewater). The majority of viruses found in soils are believed to be bacteriophages (or phages, viruses that infect bacteria), and much of this review is structured around this assumption. There is emerging evidence that challenges this paradigm, however, and we discuss the implications of those results. Finally, this review emphasizes important knowledge gaps and highlights needs for future research and development with specific regard to the challenges of studying the ecology of viruses in soil systems.

THE SOIL ENVIRONMENT

The term "soil" is deceptive, as it does not describe a single, well-defined system. Soil, rather, refers to a diverse grouping of individual ecosystems that, together, comprise the complex, porous skin of the Earth. It is important to grasp the complexity of the soil habitat because all virus-host interactions in soils occur against this backdrop. In addition to the biological requirements that viruses must contact host cells, replicate, and release progeny, the chemical and physical demands of the soil environment have provided additional evolutionary hammers and anvils that have shaped virus-host interactions in soils over the millennia.

Soils are heterogeneous systems consisting of solid, liquid, and gaseous phases, the proportions and specific components of which can vary considerably over both space and time. Soil solids are made up of varying proportions of gravel, sand (0.05–2.0 mm in diameter), silt (0.002–0.05 mm), clay (<0.002 mm), and organic matter—an amorphous mix of plant- and animal-derived organic compounds in various stages of decomposition. Solids typically constitute only about half of the volume of any given soil. The remaining volume is composed of pore spaces that may be filled by liquids, gases, or a mixture of the two. Within a given soil, it is possible for water-filled, anaerobic pores and gas-filled, aerobic pores to exist simultaneously just tens of micrometers away from each other, giving rise to the microheterogeneity that is characteristic of soils (**Figure 1**). In spite of this, the vast majority of soil microbiology studies have been performed using bulked or composite samples that destroy this microheterogeneity but allow measured properties to be applied as averages, per unit mass. With regard to the soil virome, surface soils (0–10 cm) have been most intensively explored; relatively little is known regarding viruses of the deep terrestrial biosphere.

VIRAL ABUNDANCE

Addressing the seemingly simple question of viral abundance in soils is surprisingly complex and depends on several factors, including extraction and detection methods.

Infectious Counts

Plaque assays have been used since the discovery of bacteriophages to detect and enumerate infectious virus particles from soil samples (e.g., 1). In the plaque assay, an aqueous extract of soil is mixed with broth culture containing target cells and molten agar. The mixture is then poured onto a solid agar plate and allowed to harden. After an appropriate incubation period, the presence and abundance of infectious viruses can be determined by counting plaques, clear areas on the plate where viruses have killed off local bacterial growth. Enrichment of soil samples with broth culture containing target host cells prior to plaque assay tends to increase the success of detecting

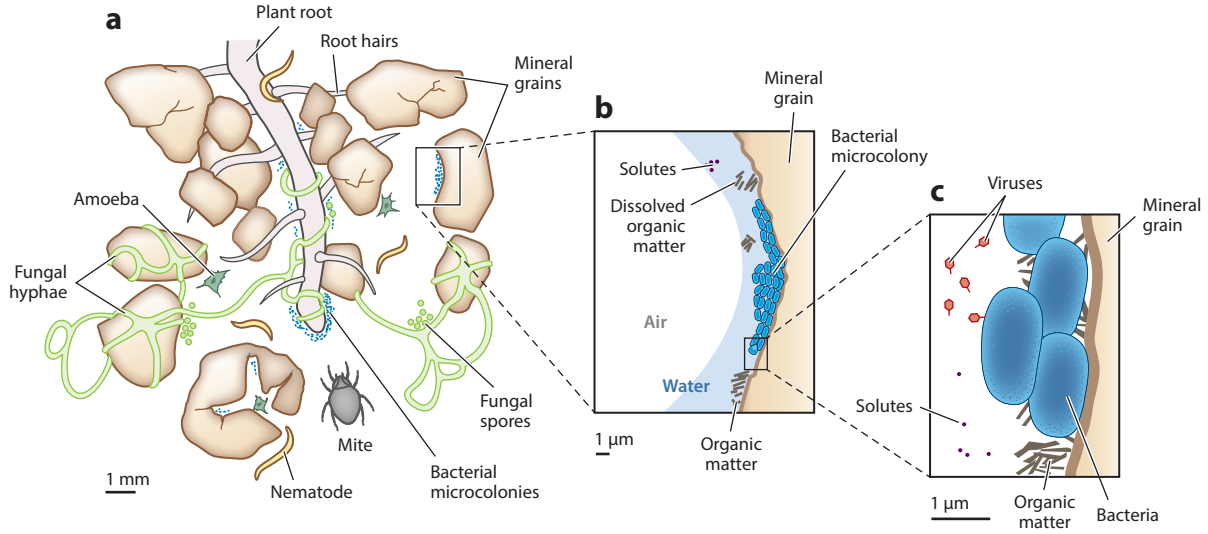

Figure 1

(*a*) Soil aggregate. Mineral grains (sand, silt, clay) are held together by organic matter and microorganisms, including fungal mycelia, plant root exudates, and bacterial exopolymers. (*b*) Increased magnification shows bacterial biofilm attached to mineral grain, organic matter, and air-water interface within the soil pore. (*c*) Increased magnification shows size of typical bacteriophage (50–60-nm capsid diameter) in relation to typical bacterial cell (1–2 µm) within a water film coating the soil pore. Diagrams developed by K.E. Williamson, based on a conceptual diagram of soil structure from the Food and Agriculture Organization of the United Nations.

infectious phages for a particular bacterial host (2). The lower rate of phage detection with direct extraction could be due to phage inactivation during extraction, the low concentration of infectious phages for a given host type in a given soil, or both factors. Infectious count assays also provide a means for the isolation and purification of novel terrestrial viruses. A significant drawback of infectious assays, however, is that viruses can only be detected if they infect the particular host provided in culture, under conditions permissible to viral replication. These limitations rule out estimating total viral abundance in soil samples using infectious assays.

Direct Counts

Unlike infectious assays, direct counts of virus particles can provide reliable estimates of total viral abundance in soil samples. Currently, in situ visualization of virus particles within undisturbed soil samples is not possible. Thus, direct counting methods have relied on a two-step process of extraction and enumeration. Viruses extracted from soil samples have typically been enumerated using transmission electron microscopy (TEM) or epifluorescence microscopy (EFM), although one recent study has used flow cytometry (3). Each of these direct counting procedures requires the efficient dispersion of soil aggregates and extraction of virus particles from the soil matrix into an aqueous carrier solution or eluent. A variety of eluents have been reported, including water (4), phosphate buffer (5, 6), and potassium citrate buffer (7–14). After elution, physical dispersion has been accomplished through bead-beating (3–5), blending (3, 6), or sonication of soils (7–14). A direct comparison of these methods indicated that sonication or blending soil samples in 1% potassium citrate buffer yielded the highest (and assumed most accurate) abundance estimates for a variety of soils (3). Up to nine repeated extractions from the same soil sample indicted that >90% of the total extractable viruses were extracted in the initial extraction (13).

For EFM enumeration, virus suspensions are passed through 0.02-μm filters, and the virus particles, captured on the filter, are stained with a fluorochrome that binds to nucleic acids, such as SYBR Gold (12). Filters are viewed under an appropriate excitation wavelength for the stain, and the emitted fluorescence signals from the stained virus genomes are captured using a digital camera. For TEM enumeration, additional purification such as density gradient centrifugation may be necessary prior to loading samples onto electron microscopy grids, in order to clearly visualize virus particles. Grids are then stained with an electron-dense heavy metal salt (e.g., uranyl acetate), and virus particles are visualized using a transmission electron microscope. An advantage of TEM is that it allows for visualization of viral morphology. However, electron microscopy entails higher operating costs and has lower sample throughput as compared to EFM, as well as poorer capture efficiency, leading to lower abundance estimates (15). EFM is cheaper, with higher sample throughput and much higher capture efficiency, but does not allow for visualization of virion structure. Therefore, care must be taken in processing images to ensure that fluorescence signals from nonviral sources are not included within viral direct count data. The majority of published viral abundance estimates in soils have been obtained using EFM direct counts (**Figure 2*a***).

TRENDS IN VIRAL ABUNDANCE ACROSS SOILS

Despite the critical importance of agricultural soils in feeding humanity, hot deserts are the most highly represented soil type for which viral abundance data are available ($n = 24$), followed by agricultural soils ($n = 13$). Cold deserts ($n = 6$), forest soils ($n = 5$), and wetlands ($n = 5$) are not as well represented in the literature, and fallow or turf systems ($n = 3$) and dune sands ($n = 1$) are poorly represented (**Figure 2*b***). Future studies should aim to examine viral abundance trends in more diverse and underrepresented soil biomes such as boreal forest, unmanaged grasslands, rain forest, coastal dune systems, and, importantly, agricultural soils.

Reported viral abundance in soils ranges from 2.2×10^3 gdw^{-1} (per gram dry weight) in desert sands collected from Saudi Arabia (10) to 5.8×10^9 gdw^{-1} in forest soil collected from eastern Virginia (**Figure 2**) (11). Part of this range can be accounted for by differences in extraction-enumeration methods (3). Bearing in mind these methodological differences, a meta-analysis of the published data indicated that soil type was significantly correlated with viral abundance (Spearman $r = 0.803$, $p < 0.001$). In general, viral abundance appears to be lowest in hot deserts; intermediate in agricultural soils, fields, and cold deserts; and highest in forested and wetland soils (**Figure 2**). Soil viral abundance was significantly positively correlated with bacterial abundance (Spearman $r = 0.647$, $p < 0.001$), which supports the hypotheses that the majority of soil viruses infect bacterial hosts and that host abundance may be a key factor controlling viral abundance in soils.

Meta-analysis of data available in the literature (**Figure 2**) indicated that viral abundance was negatively correlated with soil pH (Spearman $r = -0.352$, $p = 0.009$). Soil pH is an important environmental factor influencing viral attachment to soil surfaces, and therefore viral persistence and extraction efficiency (16, 17). Temperature is also known to influence viral persistence and abundance in soils (18, 19), with lower temperatures likely fostering the persistence of viruses and higher temperatures likely promoting thermal decay of viral particles. Temperature appears to be a key difference that could explain the stark contrast in viral abundance between hot and cold deserts (**Figure 2**), but because soil temperatures are rarely reported in the available studies, meta-analysis could not be conducted to test for a relationship. We strongly suspect that soil water content is a key environmental parameter driving both bacterial and viral abundance in soils, but again, lack of continuity in the existing data set precluded meta-analysis.

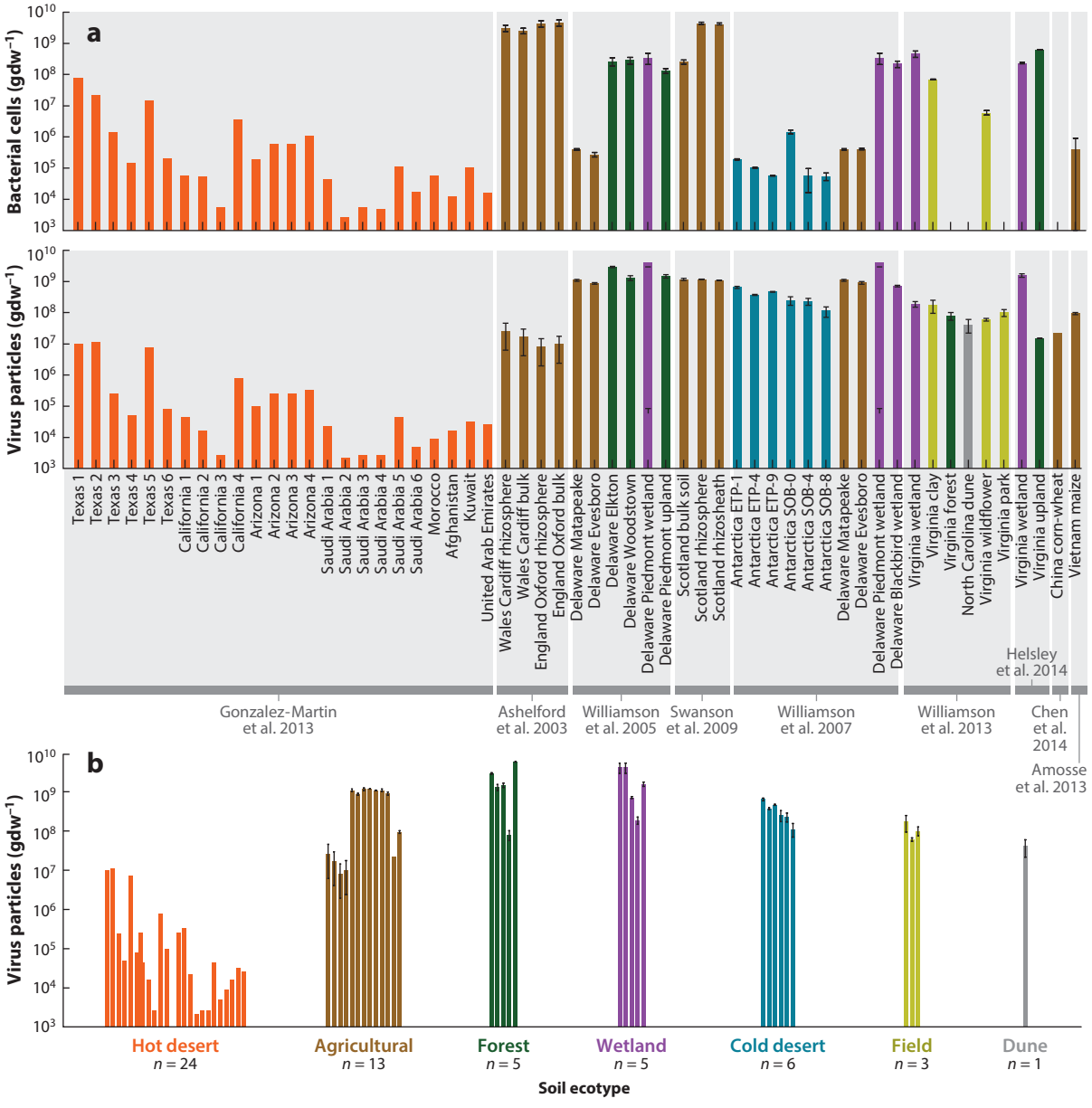

Figure 2

(*a*) Viral abundance and bacterial abundance for all available publications reporting soil viral abundance, grouped by study. (*b*) Viral abundance grouped by soil type. Hot desert soils include samples from the Mojave (United States), Sonoran (United States), Chihuahuan (United States), Saharan (Africa), Namib (Africa), Arabian (Middle East), and Registan (Afghanistan) Deserts (10). Agricultural soils include samples from England (4), Scotland (6), the United States (11, 13, 14), China (8), and Vietnam (7). Forest soils include samples from Delaware (13) and Virginia (3, 11). Wetland soils include samples from Delaware (13, 14) and Virginia (3, 11). Cold desert soils include samples from the McMurdo Dry Valleys, Antarctica (14). Field (fallow) soils include samples from Virginia (3). Dune soils include samples from the Outer Banks, North Carolina (3). Abbreviations: ETP, East Tom Pond (Antarctica); gdw, gram dry weight; SOB, South Obelisk Pond (Antarctica).

Indeed, a major problem we encountered in attempting cross-comparisons between studies was the inconsistent way in which the physical and chemical characteristics of soil samples are reported. A few studies included quantitative details on soil texture, pH, organic matter content, water content at the time of sampling, and other measures, such as cation exchange capacity (e.g., 13). Some studies included information on C and N content (e.g., 7), and others performed analysis of multiple elements (e.g., 10), but almost no studies reported all of these details together. Minimum information standards for reporting environmental metadata that accompany soil viral abundance—or, indeed, other aspects of soil viral ecology—are needed to enable more direct cross-study comparisons in the future. Guidance for such standards could be taken from the genomics research community, where community metadata standards have been adopted for some time (20, 21). A robust set of minimum information standards for soil viral ecology studies would include geography (latitude and longitude); edaphic factors, including soil texture (percentages of sand, silt, and clay), soil class, land use, and vegetation type; and factors most likely to influence biological activity, including pH, soil organic matter content, soil nitrogen, and soil water content at the time of sampling.

All of the viral abundances reported in **Figure 2** are single-time-point measurements. Currently, few data exist regarding change in viral abundance over different timescales, nor have the process rates that govern changes in total viral abundance been assessed. Standard approaches in population ecology would give the equation

$$\Delta N = (\mathrm{VP} - \mathrm{VD}) + (\mathrm{VI} - \mathrm{VE}),$$

where the change in total number of virus particles (ΔN) is given by rates of viral production (VP), viral decay (VD), viral import (VI) through processes such as deposition, and viral export (VE) through processes such as water infiltration. Understanding the interaction of these terms is critical to understanding and predicting trends in viral abundance across soils. For example, in cold desert soils, assuming negligible import/export processes, a low VP rate combined with an even lower VD rate would lead to net accumulation of stored virus particles and a relatively high viral abundance (14). At present, such process rates for viral populations in any natural soil samples are unknown; thus, development of methods to quantify viral process rates is critical to understanding the role of viral infection and lysis within soil microbial communities.

VIRAL DIVERSITY IN SOILS

The diversity of the soil virome is severely underestimated. Although several studies support the idea that the majority of viruses in soils are phages that infect bacteria, many other organisms, including archaea, protists, fungi, nematodes, annelids, arthropods, plants, and burrowing animals, inhabit soil ecosystems and are likely hosts for soil viruses. Bacterial viruses have received the most attention, but with an estimated 10,000 bacterial species g^{-1} soil (22), our present knowledge regarding phage diversity alone still scarcely scratches the surface. The ~2,000 reported bacterial viruses (23) infect hosts within only 11 bacterial and 2 archaeal phyla, with ~85% of these viruses infecting hosts within the 3 phyla *Gammaproteobacteria*, *Firmicutes*, and *Actinobacteria* (24). Using the conservative numbers from the SILVA database (25), there exist 75 bacterial and 28 archaeal phyla, with rarefaction estimates indicating there may be as many as 1,100 bacterial and archaeal phyla combined (26). In short, the diversity of known viruses is highly limited. Considering other host groups within soils, viruses that infect insects (27) and plants (28) have been described, but this knowledge is incomplete and tends to focus on crop pests or viral biological control agents for pests. Almost nothing is known about viruses that infect nonbacterial soil microbes, such as

the archaea, fungi, and soil protozoa. In all, there is yet a tremendous amount of work to be done in characterizing the diversity of the soil virome.

Morphological Diversity

Initial estimates of viral diversity in soils used TEM to assess the morphological diversity of extracted virus particles. In this approach, virus particles are extracted from soil, usually purified through density gradient centrifugation to remove background debris, and deposited onto coated electron microscopy grids for visualization under TEM. Micrographs of observed virus particles are analyzed, typically by measuring capsid dimensions and sorting particles into discrete morphological classes. Virus particles can frequently be sorted based on tail morphology into the International Committee on Taxonomy of Viruses (ICTV)-recognized categories of *Podoviridae* (short, noncontractile tails), *Myoviridae* (long, contractile tails), and *Siphoviridae* (long, noncontractile tails), as well as more general classes of nontailed particles and filamentous structures.

A comparison of viral particle morphologies from six different soils (including agricultural, forested, and wetland soils) revealed distinct viral communities in each soil (13). Whereas one of the agricultural soils was dominated by spherical, nontailed particles (56%), the remaining five soils were dominated by tailed phages (~80%). These observations supported those from previous studies in aquatic systems, suggesting that the majority of viruses found in environmental samples were tailed phages (29–31).

In a separate study focusing on a single agricultural soil, researchers found a dominance of spherical, nontailed particles (~70%), with only 5% of observed particles belonging to the tailed phages (6). While this only brings the total number of soil viral communities analyzed using TEM to seven, the high prevalence of nontailed phages in two of these soils raises questions about the assumed dominance of tailed phages in soils—a point that is revisited in the Metagenomics section, below.

Marker Gene Analysis

The lack of a universally conserved phylogenetic marker, analogous to the prokaryotic 16S rRNA gene, has presented a formidable barrier to constructing a comprehensive view of viral diversity and phylogeny. However, marker genes that are highly conserved within specific viral families may be used to assess phylogenetic relationships within these families.

The most widely used marker gene in soil virome studies is *g23*, a gene encoding the major capsid protein of the T4-like phages. Phage T4 is known to infect the fecal bacterium *Escherichia coli*, but degenerate primers that target more distantly related phages in the T4-like phage family have revealed a surprising degree of genetic diversity within this family and in viral communities from environmental, rather than fecal, sources (32). Since the development of these primers, the *g23* marker gene has been used to assess the genetic diversity of T4-like phages in rice paddy soils in Japan (33–35) and China (36, 37), wetlands in China (38), and upland agricultural fields in China (39). Phylogenetic analysis has indicated that *g23* sequences strongly group by habitat type, with sequences from marine samples forming a sharply defined cluster, sequences from rice paddy soils and freshwaters forming a distinct but more diffuse cluster, and sequences from soils forming a third distinct cluster, but sharing some overlap with the freshwater/paddy grouping (39). This trend suggests that viral genetic diversity is constrained or selected by environmental conditions, with specific viral communities possessing fundamental "marine," "freshwater," or "soil" characteristics. However, viral populations carrying the *g23* gene represent a small fraction of all viral populations within natural environments, an important caveat when attempting to extrapolate the finding of habitat specificity to a broader cross-section of unknown viral populations.

Genomics and Comparative Genomics

The number of viral genome sequencing projects lags behind the number of prokaryotic genome sequencing projects by more than tenfold: An April 2017 search of the National Center for Biotechnology Information (NCBI) Genome database indicated that 96,096 prokaryotic genomes had been sequenced, compared with 7,148 virus genomes. Increasing the number and diversity of sequenced viral genomes for viruses that infect representative soil organisms is essential to improving understanding of the genetic and functional diversity of viruses within soils. The majority of sequenced genomes belong to microorganisms that cause disease in humans, animals, and plants, a population that poorly represents the extant diversity of microorganisms (40). With specific regard to soils, many of the sequenced viral genomes belong to crop pests. Although these viruses are undoubtedly important due to their impacts on plant growth, crop production, and economics, much less is known regarding the genome contents of viruses that infect (micro)organisms living within the soil matrix. Such viruses may yet have important impacts on nutrient availability, soil fertility, and other ecosystem processes.

A key strength of comparative genomic investigations is that they provide insights into patterns within genome organization, functional diversity, and genetic exchange, details that can inform mechanisms underlying the phenotypic and evolutionary diversity within populations. Because individual viruses must be isolated prior to nucleic acid extraction and sequencing, virus genomics can link specific genes and functions with specific viruses. Furthermore, because genome annotation is largely a set of bioinformatically informed predictions, the isolated viruses linked to the annotation can be put through additional bench experiments to test those predictions. Viral genomics has given rise to genetic prospecting, the search for genes with utility in specific applications, e.g., phage lysins as antibacterials (41). Viral genomics has even been used as a pedagogical tool, to teach undergraduate science students basic biology concepts and the fundamentals of scientific research (42, 43).

The requirement of isolation also places a fundamental limitation on genomics approaches, because most microbial hosts are not amenable to cultivation. Thus, any assessment of the genetic diversity of viruses through traditional genomics approaches alone will be incomplete. This problem may be partially solved in the near future through single-virus genomics (44) and single-host-cell genomics approaches (45). Another significant problem with genomics is the so-called bioinformatics bottleneck. Isolation and sequencing of virus genomes can be completed relatively quickly and cheaply; however, rigorous bioinformatics analysis and annotation of the resulting sequence data are laborious and time consuming. Even automated pipelines must still be checked by human operators. There is thus a continuing need for more refined and more efficient bioinformatics tools to identify genes and predict gene function within virus genomes.

An impressive example of comparative genomics applied to the soil viruses lies in the work done with *Mycobacterium smegmatis* strain mc^2 155. More than 1,300 distinct viral genomes (almost 20% of all the virus genomes deposited in the NCBI database) have been sequenced from phages isolated on this single host strain (**http://phagesdb.org/phages/**). Comparative genomics of these phages has revealed a high level of genetic mosaicism, with evidence of not just gene transfer among mycobacteriophages, but of new genes entering the pool from outside sources as well. Although horizontal gene exchange was not so random as to obscure the existence of distinct genetic groupings (clades), its existence highlights the important role phages likely play in genetic exchange and the evolutionary biology of their hosts. Perhaps the greatest overarching lesson to emerge from this analysis is that viral genomes represent the largest pool of possibilities when it comes to solving biological problems, revealing a multitude of different ways to encode proteins that share a particular function.

Metagenomics

By circumventing the requirement for host culture, as well as the limitations of single marker genes, viral metagenomics has allowed researchers to survey the genetic diversity within entire environmental assemblages of viruses. The literature currently contains descriptions of approximately 100 distinct viromes, with at least 55 marine viromes, 20 freshwater viromes, and 17 viromes of other aquatic samples including corals, stromatolites, hot springs, and salterns. Currently only 8 soil viromes and 2 hypolith viromes have been described in the literature (**Table 1**). Soils have been conspicuously undersampled as compared with aquatic environments. The reasons underlying this uneven sampling are complex, but include methodological challenges that soils present to molecular approaches and a bias toward extreme environments such as hot springs and the deep sea, which likely skews our perspective of viral diversity.

Table 1 Soil viral metagenomes

Reference	Soil	Method	MDA[a]	R[b]	*Siphoviridae* (%)[c]	*Podoviridae* (%)[d]	*Myoviridae* (%)[e]	ssDNA (%)[f]	Unaffiliated (%)[g]
106	Joshua Tree National Park, United States	LASL[h]	No	1,000	—	—	—	—	—
	Konza Prairie Long-Term Ecological Research Site, United States	LASL	No	20,000	—	—	—	—	—
	Manu National Park, Peru	LASL	No	1,000,000	—	—	—	—	—
46	Rice paddy, Korea—denatured	LASL	Yes	—	45	24	24	54	64
	Rice paddy, Korea—not denatured	LASL	Yes	—	0	44	22	88	67
107	McMurdo Dry Valleys, Antarctica—soil	Illumina	Yes	15,663	38.3	11	26.1	0.5	58.5
	McMurdo Dry Valleys, Antarctica—hypolith	Illumina	Yes	11,480	52.6	9	21	0.3	81.3
108	Namib Desert, Africa—hypolith	Illumina	No	1,700	48	10	9	7	62.6
5	Machair, Scotland	454	Yes	—	0.18	0.01	0.02	88	87.9
	Brown Earth, Scotland	454	Yes	—	1.3	0.13	0	71.7	97.3
47	Longhill Grove, United States	454	Yes	30,000	4	2	2	85	54.5

[a]Indicates whether multiple displacement amplification (MDA) was used (φ29 polymerase).
[b]Estimated richness.
[c]Percentage of sequences in the library that were affiliated with known *Siphoviridae* sequences in NCBI nr/nt database.
[d]Percentage of sequences in the library that were affiliated with known *Podoviridae* sequences in NCBI nr/nt database.
[e]Percentage of sequences in the library that were affiliated with known *Myoviridae* sequences in NCBI nr/nt database.
[f]Percentage of sequences in the library that were affiliated with known ssDNA virus sequences in NCBI nr/nt database.
[g]Percentage of sequences in the library that were not affiliated with any known virus sequences in NCBI nr/nt database.
[h]Linker-amplified shotgun library.

Of the soil viromes that have been completed, several are dominated by small, ssDNA viruses (5, 46, 47). These findings challenge the dogma that soils are dominated by tailed, dsDNA phages and open the possibility that methodological biases might have led to erroneous conclusions regarding genetic and morphological diversity within the soil virome. For example, inability to distinguish small virus particles that lack tails from background debris would lead to an overrepresentation of more easily identified tailed phages under TEM. In addition, artifacts arising from the use of φ29 polymerase (GenomiPhi), which favors single-stranded templates, may have led to erroneous conclusions regarding metagenomic sequence data (48). However, for one study, both morphological and metagenomic data support the conclusion that the two soils in question were dominated by nontailed viruses (5). These results also underscore the danger in assuming that what is true of one soil virome is true of all. Again, given the spatial and temporal heterogeneity of soils, it is entirely possible for different soils to house completely different viral communities.

Viral metagenomics is a powerful approach for obtaining genetic information about viral populations without the limitation of first isolating a virus in culture. However, the speed and volume with which metagenomic sequences can be generated as compared with the rate at which they can be rigorously analyzed invokes the previously mentioned bioinformatics bottleneck problem (49). An additional problem is that metagenomic data sets often contain a high proportion of reads not affiliated with known sequences. In published soil viromes (**Table 1**), 54.5–97.3% of all reads cannot be matched with a specific taxonomic source, a specific protein function, or both, rendering these reads unusable in most downstream analyses. This problem points back to the necessity of isolating and characterizing individual viruses from soil samples, as sequence data alone are of limited value if they cannot be matched to a known entity.

The estimated number of distinct viral genotypes (richness) in soils varies between 1,000 and 1,000,000 and appears to vary according to soil type (**Table 1**). For comparison, viral richness estimates vary from 532 to 129,000 for marine samples, and from 400 to 40,000 for freshwaters (50). It is interesting to note that both the lower and upper estimates of viral richness in soils are higher than the corresponding values for aquatic systems. This, combined with the high percentage of unaffiliated sequences in soil metagenomes, supports the idea that the soil virome may represent the largest reservoir of genetic diversity on the planet.

IMPACTS OF VIRAL REPLICATION IN SOIL ECOSYSTEMS

Viruses are obligate intracellular parasites, and their replication cycles have important impacts on host populations, community interactions, nutrient cycles, and ecosystem productivity—at least in marine environments (31, 51, 52). It has been assumed that viruses have similar impacts in soils, but robust models that integrate viruses into soil microbial ecology networks, such as those existing for marine systems, have yet to be developed.

Lytic Impacts

Soil viral communities appear to be active and dynamic, responding quickly to changes in the abundance of specific bacterial host species, in both microcosm studies (53–55) and natural field soils (56–58). As such, viruses have the potential to exert significant top-down control on microbial populations, thereby affecting rates of microbially mediated processes within soil nutrient cycles (13, 59, 60); however, the magnitude of these impacts is likely to vary significantly across soil environments (11). In aquatic environments, 10–50% of bacterial mortality is due to viral lysis (30, 61, 62). Lysis products are used by surviving bacteria, stimulating their growth, which results in coupling of virus production and bacterial growth rate (63–66). Most of the C released due to

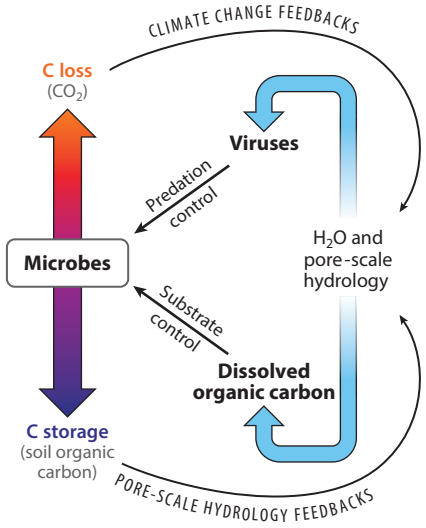

Figure 3

Conceptual model of top-down (viral predation) and bottom-up (physical substrate availability) control of carbon transformation in soil. Both factors are strongly influenced by soil water availability. Model developed by S. Schaeffer; adapted with permission.

viral lysis is cycled through this bacterium–virus–dissolved organic C loop, referred to as a viral shunt (60, 61, 67). This tight cycling of labile C has the effect of retaining dissolved organic C in surface waters that might otherwise be lost to the depths but results in greater loss of CO_2 to the atmosphere (30, 31, 61).

An analogous situation likely exists in soils, with a few important differences. Atmospheric CO_2 fixed by plant photosynthesis provides fresh organic C inputs into soils. Soil microbes decompose this organic C, converting some into biomass with respiratory losses and some into more recalcitrant, mineral-bound forms (68, 69). Through lysis, soil viruses may increase the amount of labile C and reduce the proportion of C going to more stable, mineral-associated or pore-controlled organic matter. Ironically, this increase in labile C from viral lysis should actually serve to increase microbial production and respiration in soils (**Figure 3**).

Several key uncertainties arise when considering this model in terrestrial systems. First, turnover times for soil microbes and viruses are unknown, but are likely longer in soils than in aquatic systems (70). Second, free diffusion and movement of soil bacteria and phages are constrained by soil structure, moisture, and the potential for virus adhesion to soil particles. However, given the colloidal size of viruses, their movement through the soil matrix is likely consistent with that of the rest of the particulate organic C pool. As a soil dries, it is possible that viruses are drawn into very small pores by shrinking water films (**Figure 1**) but can be released upon rewetting, initiating repeated cycles of lytic replication. Developing both lab- and field-scale experiments to test these predictions will lead to better estimates of the proportion of bacterial biomass turned over by viral lysis and the amount of virally mediated nutrient release with subsequent impact on nutrient cycling, soil respiration, and soil fertility.

In addition to carbon, viruses in soil ecosystems likely play important roles in the cycling rates of other nutrients, such as nitrogen (e.g., proteins and nucleic acids), sulfur (e.g., proteins), and phosphorus (e.g., polyphosphate, cellular membranes, and nucleic acids). In particular, the effect of rhizobiophages on the legume-rhizobia symbiosis may impact soil nitrogen flux. The term

rhizobia refers to a broad class of soil bacteria capable of forming stable mutualistic symbioses with leguminous plants, wherein the bacteria reside within specialized nodules on the plant roots. The plant supplies the bacterial symbionts with sugars from photosynthesis, while the rhizobia use the sugars to fix N_2 into NH_4^+, providing the plant host with bioavailable N. Rhizobiophages (phages that infect rhizobia) are indigenous to most soils and infect various rhizobial species (71–73). As such, lytic rhizobiophages can play important roles in rhizobial ecology within soils. For example, application of rhizobiophages to crop soils can reduce nodulation by phage-sensitive rhizobia (74). Rhizobiophages can also influence nodulation competition, especially when phage-resistant rhizobia are used in combination with lytic phages known to be parasitic on competing, phage-sensitive rhizobia (75–77). The outcomes of each of these rhizobiophage-host interactions determine the specific impacts of rhizobial populations within a given soil, including the persistence or loss of effective N-fixing rhizobia strains, success or failure of nodulation events, and, ultimately, symbiotic effectiveness and nitrogen fixation rate. Because of these significant dependencies, establishing estimates of lytic viral impacts on nutrient transformation rates in soils will be essential to developing accurate conceptual models of global nutrient cycles.

Lysogenic Impacts

The sustainability of a lytic strategy depends on the likelihood that progeny viruses of a lytic infection will contact a new host before inactivation in the environment. Soil microheterogeneity imposes certain limitations on lytic virus-host interactions, including (*a*) heterogeneous distribution of host cells, (*b*) oligotrophic conditions and long periods of host dormancy or low activity followed by sporadic pulse events of moisture and nutrient inputs, (*c*) slow host cell growth rates, and (*d*) physical separation and/or restricted access to host bacteria via entrapment in soil micropores or adsorption to particle surfaces. These factors argue against the sustainability of repeated cycles of lytic infection in soils, and would appear to establish strong positive selection for lysogenic replication strategies by temperate phages (78). Temperate phages may integrate their genomes into the host genome, where the phage genome is maintained as a prophage, replicating as the host cell grows and divides (79). This strategy allows temperate viruses to maintain their populations in the face of the specific challenges to sustained lytic viral infection posed by the soil environment. Indeed, available data suggest that lysogeny is widespread among soil bacteria, with up to 40% of terrestrial bacterial populations harboring inducible prophages (14). However, it is important to note that lysogeny has been assessed in very few soils relative to the diversity of soil types on Earth, and the relative importance of lysogenic versus lytic replication is likely to vary by soil type, as well as with space and time within a given soil. Most assessments of lysogeny have been performed using agents that induce host SOS repair systems (e.g., 80), but lysogenic bacteria in soils may also be induced by quorum-sensing compounds such as acyl-homoserine lactones (81), prompting the release of lytically replicating phages under high host densities. Thus, temperate phages in soils may oscillate between lysis and lysogeny on the basis of local environmental conditions, including host availability.

During their residence inside host cells, prophages can have significant impacts on microbial host populations within soils. One such impact is regulation of host metabolism. Temperate phages encode repressors and transcriptional regulatory proteins that silence the expression of phage genes required for lytic replication (79). These same regulatory proteins can also bind to homologous sequences in the host chromosome, downregulating metabolic functions in host bacteria and reducing energy expenditures (82, 83). This phage-controlled suppression of particular metabolic operons directly contributes to host survival when nutrients are scarce or very slowly available. Given that most soils are oligotrophic with patchy distributions of recalcitrant nutrient pools,

there would appear to be strong positive selection for this type of temperate phage regulatory control in soil lysogens. In spite of this evolutionary advantage, specific examples of prophage impacts on host metabolic activities and fitness within soil ecosystems are conspicuously absent from the literature.

In addition to transcriptional repressors, other prophage-borne genes that are expressed during lysogeny may protect bacterial hosts from additional phage infection (superinfection immunity) or change host phenotype (lysogenic conversion), sometimes resulting in significant changes in host fitness. Superinfection immunity can be conferred through a variety of mechanisms, and it not only protects resident prophage against competing phages but also provides a means for bacterial survival in the presence of multiple viral threats (84, 85). Lysogenic conversion is well documented for its role in modulating the severity of human bacterial infections (86). For example, environmental bacteria that cause mild disease symptoms, if any, in human hosts may become extremely virulent due to prophage-encoded toxin proteins that alter the host phenotype, allowing niche expansion and increasing dispersal of the bacterium. This is the case for *Vibrio cholerae* and *Corynebacterium diphtheriae*, the causative agents of cholera and diphtheria, respectively, which have only become clinical pathogens due to lysogenic conversion (87, 88). Not all impacts of lysogenic conversion are this dramatic, however, and many are likely to be subtle, resulting in fitness changes that vary with environmental context (89). Given the microheterogeneity of soil environments, combined with the established prevalence of lysogeny among soil bacteria, it is highly likely that temperate phages impact host population dynamics in multiple ways. For example, temperate phages may alter rhizobial phenotypes by lysogenic conversion, with variable impacts on root nodule colonization, N-fixation efficiency, and crop productivity (90). In spite of the potential importance of these virus-host interactions in microbially mediated processes and soil fertility, very little is known regarding the influence of temperate phages on soil bacteria.

Horizontal Gene Transfer

The movement of genetic material between distantly related species contributes to genetic plasticity in bacteria and provides novel selective advantages in a changing environment. For organisms that reproduce through binary fission, such as bacteria and archaea, phage-mediated horizontal gene transfer (transduction) provides a critical mechanism for diversification and speciation. Transduction has been implicated in the rapid acquisition and spread of antibiotic resistance among bacteria (91, 92) and is likely an important mechanism for transferring gene sets in heterogeneous soil environments. However, whereas earlier microcosm work demonstrated that transduction could be highly efficient in soils (93), very few studies have examined transduction rates in natural soils.

Viruses may also acquire specific host genes that improve viral fitness. A well-documented example lies in the photosystem I and II genes that have been acquired by phages that infect photosynthetic marine cyanobacteria (94). Expression of these genes during infection boosts the photosynthetic output of infected cells and redirects carbon metabolism toward phage protein biosynthesis (95, 96). These "stolen" genes, often referred to as auxiliary metabolic genes (AMGs), generally increase the fitness of viruses that have acquired them and include such functions as carbon metabolism, phosphate regulation, sulfur and lipid metabolism, and biofilm formation (97–99). With specific regard to soils, recent work revealed that phages in atrazine-contaminated soils had acquired the *trz*N gene, which encodes a chlorohydrolase required for atrazine catabolism (100). As with other AMGs, viral acquisition of this particular gene likely improves viral capacity to produce more progeny under resource limitations (e.g., where atrazine may be an alternative or sole C and N source). Beyond this example, however, little is known about viral AMGs in soils, much less how these gene acquisitions influence viral or host populations within soil ecosystems.

With regard to understanding viral impacts on soil ecosystem processes, the common refrain for now is that we simply don't know enough. Scant data are available to inform us about how viruses may alter soil nutrient cycles and interactions within soil food webs, or how significant virus-mediated gene transfer and gene acquisition may be in altering the fitness of soil microbes. Critical next steps should be to establish models and experimental designs to characterize and quantify viral impacts in soil systems. More than this, the breadth and magnitude of viral impacts must be compared across different soils types, because another common refrain of soils is microheterogeneity: What is true of one soil sample may not be readily applied to all.

CONCLUSIONS

Soils represent the greatest reservoir of biodiversity on the planet; prokaryotic diversity in soils is estimated to be three orders of magnitude greater than in all other ecosystems combined (101, 102). Soils are critical to global biogeochemical cycles that support biological diversity within all terrestrial ecosystems. With regard to human civilization, soils provide ecosystem services on a global scale estimated at more than $20 trillion (103), including waste disposal, water purification, and production of food, fiber, and pharmaceuticals (104, 105). A more holistic understanding of soil viral populations and communities is necessary to characterize and quantify viral impacts on the critically important ecosystem services that soils provide. Viral infection of hosts can influence host community capacity to decompose soil organic matter, convert organic phosphorus into plant-available forms, and respire carbon dioxide, to name just a few examples of the myriad ways viruses may influence soil processes. Improving our understanding of the soil virome will also provide critical insights into the roles viruses have played, and continue to play, in shaping host fitness through selection against virus-sensitive genotypes, lysogenic conversion and host metabolic manipulation, and horizontal gene transfer. Soils remain the most poorly understood ecosystems on Earth. At the same time, viruses represent the largest pool of untapped genetic diversity and unexplored sequence space on the planet. In this regard, the soil virome comprises an unknown quantity within an unexplored territory: a vast new frontier, ripe with opportunities for discovery.

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