Title

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Opinion

Diversity in the soil virosphere: to infinity and beyond?

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Viruses are key members of Earth's microbiomes, shaping microbial community composition and metabolism. Here, we describe recent advances in 'soil viromics', that is, virus-focused metagenome and metatranscriptome analyses that offer unprecedented windows into the soil virosphere. Given the emerging picture of high soil viral activity, diversity, and dynamics over short spatiotemporal scales, we then outline key eco-evolutionary processes that we hypothesize are the major diversity drivers for soil viruses. We argue that a community effort is needed to establish a 'global soil virosphere atlas' that can be used to address the roles of viruses in soil microbiomes and terrestrial biogeochemical cycles across spatiotemporal scales.

Highlights

Targeted soil viral metagenomes (viromes) enable in-depth exploration of encapsidated genetic material in soil.

Extra-large-scale combined assemblies can reveal rare viruses otherwise missed from total metagenomes.

RNA-based metatranscriptomes and viromes provide insights into substantial RNA virus diversity in soil.

Viromics combined with laboratory approaches for evaluating viral activity and virus–host interactions, including controlled incubations, cultivation, single-cell approaches, and microscopy, will form the framework for addressing long-standing soil viral ecology questions.

We posit that soils represent the 'perfect storm' of diversity drivers, including spatiotemporal dynamics, physicochemical heterogeneity, and occasional immigrations, resulting in highly diverse and dynamic viral communities.

Metagenomics has transformed viral ecology, but soils present unique challenges

Viruses were discovered 130 years ago, but their diversity and roles across ecosystems have only been actively investigated over the past 30 years. This nascent field of 'viral ecology' was built first on the observation of virus-like particles in different environments, along with laboratory incubations demonstrating substantial turnover in microbial communities caused by viral lysis [1–3]. These community-wide measurements were then complemented by metagenomic approaches, that is, shotgun sequencing of DNA or RNA extracted directly from a sample, which offered a unique window into the extensive genomic diversity of environmental viruses [4–6]. In parallel, studies of cultivated virus–host pairs revealed myriad means by which viruses can control, reprogram, and manipulate their hosts during infection [7,8]. Taken together, viral ecology studies have shown environmental viruses as highly diverse, often very abundant, routinely infecting a significant portion of their microbial host communities, and likely influencing major biogeochemical processes.

Despite this rapid development of viral ecology, the diversity and roles of viruses in soil ecosystems remain poorly constrained. The designation of 'soil' encompasses a broad range of terrestrial environments, including agricultural fields, forests, grasslands, deserts, and many others, each with highly distinct microbiomes [9]. Because of the heterogeneous physicochemical structure of soil, including different hydraulic connectivity, particle and aggregate sizes, and pore spaces, soil samples can be highly variable, even a few millimeters or minutes apart. Meanwhile, amplicon and metagenomic studies both indicate that soil microbiomes are among the most complex on Earth, with high richness and evenness, a diverse 'rare biosphere', and in some soils a substantial amount of 'relic' DNA [9–12]. This makes the reconstruction of microbial and viral genomes from metagenomes less efficient in soil than in most other environments [13–15]. One approach to circumvent this limitation for viral genome recovery is viral metagenomics (viromics), which involves resuspending and enriching viral particles, such that most of the sequencing library is dedicated to viral genomes [6,10]. Soil viromics approaches were established more than a decade ago [16], yet have not been applied as extensively as for other ecosystems [17]. Overall, this translates to a relative paucity of high-throughput and high-resolution genomic data on soil viruses, recently

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designated as an 'unknown quantity' and 'severely underestimated and undersampled' [17]. The soil **virosphere** (see Glossary) was similarly described as 'neglected', with 'omics technologies highlighted as 'particularly underutilized' [18].

In just the past few years however, the soil viral ecology landscape quickly transformed, and many limitations are being overcome. Here we highlight recent advances in soil viromics and how these enable unprecedented insights into global soil viral diversity and dynamics.

Tackling soil viral diversity requires both targeted and extra-large-scale approaches

Soil ecosystems have long been considered one of the major reservoirs of viral diversity [17,19]. This notion was mostly based on the high number of virus-like particles observed in soil samples and on the known diversity of host communities, which was shown to be higher in soils than in any other ecosystem by about one order of magnitude [12,20]. Previous estimates of soil viral richness suggested the presence of 1000 to 1 000 000 genotypes per sample, although there was substantial uncertainty around these estimations due to undersampling [17,21].

Given this predicted high richness of soil viral communities, improvements in sequencing throughput and metagenomic assembly software might have been expected to uncover an extensive soil virosphere in **total metagenomes**, even if these datasets do not specifically target viruses. Although this has been the case for other biomes, such as the human gut [22–24], no such 'explosion' of soil virus sequence space from total metagenomes has yet occurred. In fact, according to the Integrated Microbial Genome/Viral Resource (IMG/VR) v3 database [25], while the number of viral sequences identified per metagenome clearly increased for marine and freshwater samples over the past 5 years, the trend for soil metagenomes remained flat at about 10 to 100 virus sequences per dataset (Figure 1A). This likely reflects technical challenges in assembling complex soil metagenomes, leading to a high number of unassembled reads, short contigs, and ultimately poor recovery of genome-level data. However, several transformative approaches, newly developed for, or now amenable to, soil samples, offer unprecedented opportunities to explore soil viral communities. Here we highlight three such approaches that we consider most promising: (i) soil DNA **viromes**, (ii) combined assembly of total soil DNA metagenomes across multiple samples, and (iii) **metatranscriptomes** and RNA viromes.

Viral metagenomes (viromes), that is, metagenomic sequencing of the 'viral particle' fraction of a sample, can be a powerful approach for comprehensively surveying viruses in a microbiome [4,6,26] (Box 1). Viral fractions have often been considered to be more challenging to generate from soil [27,28], but updated protocols – including improved buffer chemistry to facilitate removal of viral particles from the soil matrix and improved sequencing library preparation – enable viromics to be broadly applied to a variety of soils [10,29–32]. In our opinion, three primary misperceptions or hurdles have precluded a more widespread adoption of viromics for soil ecosystems: (i) an assumption that total metagenomics can offer similar data with greater convenience, (ii) an assumption that viromes offer a skewed view of viral diversity, and (iii) not knowing where to start, in part because of limited centralized resources for soil viromics protocols, guidelines, and discussion, and/or lack of awareness of those that do exist. Yet, while viromes require extra processing steps relative to total metagenomes, they offer a deeper and complementary view of the viral community by enabling a substantially larger proportion of the sequencing effort to be dedicated to virus genomes. Specifically, comparisons of viromes with total metagenomes from agricultural and peat soils confirmed that viromic data enabled the recovery of one to two orders of magnitude more viral sequences than total metagenomes [30,33] (Figure 1B and Box 1). While soil viromics also has an unfortunate reputation for being challenging and requiring soil-specific troubleshooting, our

Glossary

Dormant soil viruses: viruses temporarily inactive but able to resume their replication cycle in favorable conditions. Operationally, these encompass integrated prophages, pseudolysogenized viral genomes stalled in mid-infection inside host cells, and free virions that have been in their respective states (i.e., that have not undergone a complete lytic replication cycle) for at least a week, often longer. This definition of 'dormancy' is thus independent of the infection cycle (e.g., lysogenic, lytic, or chronic) and of the viral genome state (i.e., in a cell or encapsidated), but mainly reflects a significant lag between two cycles of infection and replication.

Encapsidated: contained in, or surrounded by, a protein capsid.

Metatranscriptome: dataset generated via shotgun sequencing of all RNA fragments extracted from a given sample, sometimes following ribosomal RNA depletion. Non-host-associated environmental metatranscriptomes are often primarily composed of microbial ribosomal RNA and transcripts but can include viral transcripts and (RNA) virus genomes.

Total metagenome: dataset generated via shotgun sequencing of all nucleic acids (typically DNA) extracted from a given sample. These datasets are standard shotgun metagenomes, and the designation 'total metagenome' is used here in contrast to viromes (see 'virome' below).

Virion: an encapsidated viral particle, consisting of the viral genome contained in a protein capsid, which is sometimes surrounded by a lipid envelope.

Virome: a viral metagenome, that is, a dataset generated via shotgun sequencing of nucleic acids extracted after viral particle enrichment and/or microbial cell depletion (e.g., via filtration) from the original sample. Viromes are most often based on DNA sequencing, but RNA viromes, that is, the sequencing of RNA extracted from virions, have also been generated.

Virosphere: the viral portion of a microbiome, that is, all viruses globally or in a specific environment, such as the 'soil virosphere'. These viruses can take different forms, including virions, dormant viruses inside host cells, and actively infecting viruses. Different approaches are typically required to

experience is that soil viromics has become reliable, if a bit tedious, that most soil **virions** are likely derived from very recent infections, and that biases from buffer chemistry are likely minimal. Researchers, including ourselves, may have experienced disappointingly low yields of viral DNA, in particular from dry soil samples, yet we are increasingly confident that these are more reflective of real biological patterns, that is, a low number of viral particles in these soils, rather than technical limitations of the soil viromics protocol. Hence, we predict that applying soil viromics to a broad diversity of soil types and locations will greatly accelerate the genomic exploration of soil viral diversity, and we advocate that viromes should be part of any large-scale project aiming to characterize the soil virosphere. Finally, we believe that online platforms for protocol sharing and discussion will help to increase adoption of viromics approaches. In this context, the Emerson laboratory recently added their current soil viromics protocol on protocols.io (https://www.protocols.io/private/F0E5F491BD0F11EC9BD70A58A9FEAC02), and we note that Trubl *et al.* also have a detailed soil viromics protocol available on the Viral Ecology Research and Virtual Exchange network (VERVE Net) space at protocols.io (https://www.protocols.io/view/soil-viral-extraction-protocol-for-ssdna-dsdna-vir-q26g75qklwz1/v3). We encourage the community to leverage and contribute to this resource, along with the associated space for discussions and comments.

Complementary to viromics, approaches to more fully analyze total soil metagenomes are now available and should be more broadly leveraged for soil viral ecogenomics, including especially 'extra-large-scale' combined assemblies. Combined assemblies, that is, reads from multiple related metagenomes assembled together to increase individual genome coverage, have been previously demonstrated to be an efficient way to recover genomes from metagenomes [34], and they can now be directly applied to deeply sequenced, complex metagenomes, thanks to improved computational resources and dedicated tools. For instance, MetaHipMer, a metagenome assembler designed to run on large clusters of compute nodes with distributed memory, was recently used to assemble a 3.34 Terabase soil dataset, yielding a 15.1 Gbp contig set [35]. By improving the recovery of both microbial and viral genomes from total metagenomes, we predict that combined assemblies will substantially expand known soil viral diversity, particularly for viruses that are less likely to be recovered in viromes, such as actively replicating viruses, integrated prophages, giant viruses, and 'jumbo' or 'huge' phages [36,37]. Continuing the development of,

access these different components of the virosphere.

vOTU: a viral 'operational taxonomic unit', typically corresponding to an approximated species-level designation. For soil viromics, vOTUs are most often operationally defined as contiguous DNA sequences ≥10 kbp in length and/or predicted to be ≥50% complete, sharing ≥95% average nucleotide identity across ≥85% of the length of the shorter contig (see [4]).

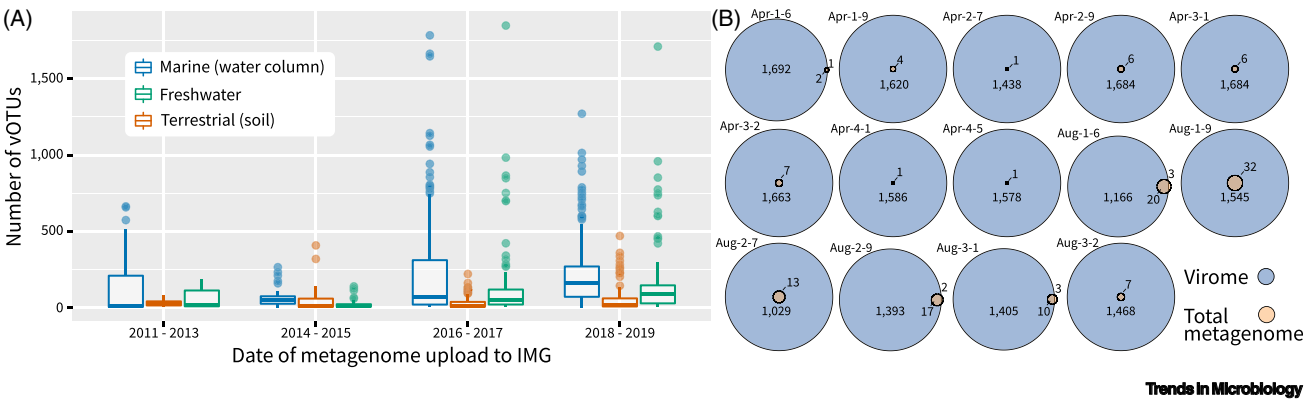

Figure 1. Overview of viral genome recovery from total and virus-targeted soil metagenomes. (A) Number of viral 'operational taxonomic units' (vOTUs) (i.e., nonredundant, approximately species-level viral genomes) identified from published total metagenomes through time, according to the Integrated Microbial Genome/Viral Resource (IMG/VR)v3 database [25], selecting only datasets from three types of biomes: marine, freshwater, and soil. (B) Number and overlap between vOTUs detected in viromes (blue) and total metagenomes (orange) for paired datasets from an agricultural field (14 soil samples, each with a virome and paired total metagenome from the same homogenized soil). Individual samples are designated by a single code with the sampling month concatenated to the column and row of the sampled plot in the field site. Data were extracted from [30].

Box 1. Viral metagenomes (viromes) versus virus genomes from 'total' metagenomes

Exploring viral diversity through shotgun sequencing can involve a variety of different protocols, and here we offer some clarification on two of the primary approaches and suggest some standardized terminology. An important distinction can be made between the study of virus sequences identified in: (i) 'total' metagenomes (i.e., shotgun sequencing of all of the DNA in a sample), which tend to be dominated by sequences from cellular organisms, and (ii) 'virus-targeted' metagenomes (viromes), from which cells are depleted and viral particles enriched, typically by passing the sample through a 0.1–0.45 μm filter prior to shotgun sequencing of the viral fraction. Direct comparisons between soil viromes and total metagenomes are relatively rare, but so far, they suggest a much higher recovery of viral genomes from viromes (about one to two orders of magnitude) and no obvious biases in the types of viruses recovered through viromics, provided that multiple-displacement amplification (MDA) or similar whole-genome amplification methods were not used [30,80]. Based purely on the number of virus sequences recovered, a cost–benefit analysis should thus usually swing in favor of viromics for soil viral community ecology. Nevertheless, viromes and total metagenomes differ in a number of important ways and can capture different subsets of the viral community, with the latter including both viral genomes present inside host cells (e.g., due to lytic or lysogenic infection) and free virions (**encapsidated** viral particles), and the former mostly including virions and underrepresenting, for example, integrated prophages and 'jumbo' or 'huge' phages [36,37]. Hence, given their complementarity, study designs should ideally include both types of datasets to maximize viral genome recovery. One critical question remaining for soil viromes is the extent to which they represent recent successful infections, or whether viral capsids are stable enough in the soil matrix that viromes represent the accumulated outcome of successful infections over a longer time frame. We expect that this question will be addressed in the coming years through temporally resolved sampling and by pairing viromes with activity measurements, such as SIP and metatranscriptomics, but data thus far suggest that viromes tend to capture very recent infections. Regardless, given the presumed extensive global diversity of soil viruses, viromes will be a critically important and complementary approach to total metagenomes for interrogating the soil virosphere.

and facilitating access to, such resources should thus be strongly encouraged, as it would likely benefit both the soil microbial and viral ecogenomics fields.

Finally, RNA-based metagenomes, that is, metatranscriptomes and RNA viromes [38], will also likely reveal a treasure trove of soil viral genomes in the next few years. Metatranscriptomes have typically been used to explore microbial community activity across a broad range of environments, including soils [39], but, until recently, were rarely used for virus discovery [40]. This is rapidly changing however, as recent studies have uncovered tens of thousands of new uncultivated RNA viruses [41,42]. In soil especially, metatranscriptomes have yielded hundreds to thousands of distinct viruses, including bacteriophages and diverse eukaryotic viruses [38,43,44]. Considering the potential host diversity spanning from bacteria and archaea to protists, fungi, nematodes, microfauna, and plants, it is likely that soil environments harbor many novel RNA viruses which RNA-sequencing approaches are now primed to uncover if applied systematically and at large scales.

Taken together, these advances provide an immediate opportunity to comprehensively and thoroughly explore the genomic diversity of soil viral communities. Viral genomes recovered from metagenomes will thus likely form the backbone of soil viral ecogenomics, to be enriched by other emerging approaches, such as long-read metagenomes and single-virus genomics for better access to highly diverse populations [45–49], *in silico* and laboratory methods for linking uncultivated viruses and hosts [50–53] (Box 2), and measures of viral activity, for example, from metatranscriptomics and/or stable isotope probing (SIP) metagenomics [54–56]. If a broad range of soils can be explored through this extended 'omics framework then we are convinced that soil will be confirmed as the largest environmental reservoir of viral diversity on Earth.

A convergence of ecoevolutionary factors likely drives extensive viral diversity in soil

As the true extent of viral diversity in soil is progressively revealed, we suggest that multiple interconnected abiotic and biotic factors be investigated as potential drivers of this diversity in order to gain a more predictive understanding of soil viral ecology and evolution. For example, it will be important to unravel patterns in viral community composition and their underlying drivers over

Box 2. Limits and biases of 'omics for (soil) viral ecology

While 'omics approaches, especially those enabling the recovery of viral genomes from metagenomes, are now coming of age for exploring soil viral diversity [10], they have limits and biases. Here, we highlight three of the most critical challenges and limitations to consider when interpreting 'omics data for soil viral ecology. First, in addition to poor assembly of rare taxa, micro-diverse populations or genomic regions may be missing from metagenomic assemblies, even if highly covered [98]. Long-read sequencing technologies and single-virus genomics can bypass these limitations [45–47], so we expect the recovery of micro-diverse sequences to improve in the coming years, but most current metagenomes are sequenced using short-reads and thus suffer from this limitation. Second, *in silico* host prediction is limited and imperfect. For instance, in the IMG/VR v3 database [25], only ~2% of the soil-derived viral sequences had a host prediction. Improving *in silico* host prediction will likely require: (i) establishing a comprehensive set of new viral cultures from environmentally relevant microbial hosts, (ii) improving *in silico* host prediction tools, and (iii) designing and applying laboratory approaches that connect viruses to hosts without cultivation, for example, by identifying viral and host genomes colocalized in the same cell through proximity ligation, single-cell amplification, or droplet PCR [50,51,53,99,100]. A third major limitation is the paucity of reliable functional annotation for most viral genes. In particular, rigorously interpreting the role of putative virus-encoded metabolic genes (auxiliary metabolic genes, or AMGs) is nearly impossible without precise functional characterization. Taken together, these limitations mean that large portions of soil viral datasets remain uncharacterized and difficult to contextualize, so it may be tempting to apply less stringent cutoffs and a 'kitchen sink' bioinformatics approach to identify more viruses, link more viruses to hosts, and predict more AMGs. However, more is not always better, and permissive cutoffs come with an increased false-positive signal in 'viral' datasets, especially for total metagenomes dominated by nonviral sequences. With technological improvements, more complete sampling of the soil virosphere, and targeted *in vitro* characterization of key virus-encoded genes, we hope that many of these limitations will soon be overcome. Meanwhile, we recommend exercising the utmost caution when interpreting 'omics data in soil viral ecological studies, especially when the reported feature is only observed on a small number of sequences and/or on incomplete genomes, and when no complementary laboratory experiment can be performed to validate the bioinformatics findings.

space, time, environmental conditions, and host metabolic states, and assess how these relate to time scales of viral replication, dispersal, mutation, and recombination to shape viral community assembly. Many of these factors co-vary and/or operate in tandem, but here we have attempted to separate them conceptually.

The most obvious drivers of soil viral biogeography are arguably host ecology, physiology, and soil physicochemical properties, which are themselves tightly coupled. Host communities have already been highlighted as significant drivers of soil viral community composition in multiple studies [57–60]. Consistent with established patterns for other soil microbes [9], viral community composition tends to vary with soil depth, pH, moisture, and across soil compartments, such as between rhizospheres and bulk soils [30,33,43,57,58,61–65]. Early evidence also supports reproducible links between viruses and many components of the terrestrial carbon (C) cycle [33,57,64,66–69]. For instance, viral expression of C-cycling genes to facilitate host viability during infection may explain the frequent detection of virus-encoded glycoside hydrolases and other carbohydrate-active enzymes (CAZymes) in soil [56,57,62,66]. Less is known about viral feedbacks to other biogeochemical cycles, but in agricultural soils, ammonia, nitrate, and total nitrogen (N) concentrations were significantly correlated with viral community composition [30]. We predict that at least some viral feedbacks to biogeochemistry are the direct result of viral lysis and/or viral gene expression during infection, as opposed to simply co-correlation with environmental factors that shape host community composition.

Spatial patterns in soil viral community composition have already been observed at multiple scales, and we predict that substantial spatial differences across short distances (on the scale of meters) will be the paradigm for most soil viral communities. Interestingly, some soil viral communities exhibit significant field-scale distance–decay relationships typically not observed to the same degree in bacterial communities or soil nutrient profiles [30,70], suggesting a partial decoupling of virus–host diversity and dynamics that bears further exploration. Over regional scales, most soil viruses tend to be recovered in sequencing data only from single locations, indicating substantial cross-site and/or cross-habitat differences [38]. At the global scale, 4% of

viral 'operational taxonomic units' **(vOTUs)** were found to be shared between peatlands on different continents, consistent with the existence of a global soil viral 'meta-community', as previously suggested for hot springs [71]. Presumably, this indicates some long-distance atmospheric transport of soil viruses [72] as well as habitat specificity on a global scale. We suspect that omics and imaging data will eventually reveal that travel via hydrologic conduits and non-host biota (e.g., fungal mycelia, plant roots, microfauna [73]) facilitates the majority of subfield-scale phage transport, enhanced by 'leap-frogging' (i.e., the spread of viral populations by chain reactions of host infections over space), with lesser local contributions from dispersal by air [72]. We predict, however, that the scales of soil viral dispersal and mixing of the gene pool are minimal enough to drive the continued emergence of microheterogeneity (genotypic variation) and spatial structuring [70,74], yet substantial enough to preclude allopatric speciation due to spatial isolation as a primary driver of soil viral community biogeography, both locally and globally.

Temporal scales of viral turnover in soil are even less well understood, but several recent studies hint at high turnover rates. On short time scales, near-immediate microbial responses to wet-up are a hallmark of soils that experience long, dry summers and cool, wet winters [75]. In support of a similar viral response to rewetting of dry soil, a sharp increase in viral diversity was observed in biocrust soil metagenomes following laboratory wet-up [61]. Similarly, significant shifts in viral community composition and/or abundance were also observed across months for agricultural fields, forests, grasslands, and peatlands [30,33,56,76,77], contrasting with patterns observed in the oceans, where viral communities can be relatively stable for years with some seasonal fluctuations [78]. To reconcile these differences, we suspect that rates of change in viral community composition relate to the stability of environmental conditions, and thus of host community composition and activity. For example, rapid soil viral community turnover in response to wet-up is likely due to resumption of stalled infections and/or switches from lysogeny to lysis, triggered by changes in host metabolic status, whereas the same time scales in a more environmentally stable ecosystem might show less viral community turnover. Although soils are inherently heterogeneous, they tend to experience similar environmental conditions year to year, according to their local climates. Thus, we predict that longitudinal studies will eventually reveal cyclic patterns of soil viral community composition, currently obscured by extreme subannual temporal variability.

In addition to these rampant spatiotemporal dynamics, other evidence for substantial soil viral activity is mounting. For example, stable isotope probing (SIP) through ^{13}C or $H_2^{18}O$ incorporation identified abundant active viral populations in grassland rhizospheres, agricultural soils, and peatlands [56,64,79], and 58% of viral populations identified in thawing permafrost peat metagenomes were classified as active via detection in metatranscriptomes [57]. Additionally, high viral diversity often remains in soil viromes treated with DNase prior to virion lysis, suggesting that a large portion of the soil viral community can be contained in intact and potentially still infective virions [30,80]. We expect that widespread viral activity will be revealed across many soil types and habitats, such that high soil viral diversity is not simply an accumulation of relic, inert virions, but rather represents dynamic viruses with substantial impacts on microbial processes and biogeochemical cycling.

Given the spatiotemporally heterogeneous nature of soil environments, periods of low soil viral activity also presumably occur under unfavorable conditions for many soil viruses, yet an overarching paradigm that explains viral persistence in soil remains elusive. Several mechanisms have been proposed, and we hypothesize that all of them apply to varying degrees in different soils at different times. Temperate phages, capable of lysogenic replication as prophages integrated in host genomes, have been suggested to represent a larger fraction of viral communities in soil than in other ecosystems, facilitating enhanced survival under changing conditions [10,81].

Consistent with this idea, several molecules were identified as triggers (inducing agents) for (soil) temperate phages to enter the lytic cycle, some of which indicate a coupling between host cell and/or prophage densities and viral replication [81–84]. Pseudolysogeny, that is, the maintenance of a phage genome in a host cell in a 'stalled' infection state, may represent another avenue for viral persistence in soil [19,84]. Finally, the persistence of viruses in soil may also be linked to the survival of infectious viral particles (virions) [17]. Viral decay should, in theory, limit long-term persistence of virions; however, decay rates can be highly variable, depending on environmental conditions, and it is possible that some soil virions remain infectious over long time scales [17,85]. These different persistence mechanisms likely form the basis for 'seed-bank' patterns of **dormancy** and resuscitation [86] in soil viral communities, with dormancy being used here to designate viruses that are not undergoing an active lytic infection and that did not undergo a full infection and replication cycle in the recent past (for soil viral ecology purposes, this likely means within the past week or longer). We posit that these 'seed-bank' dormancy–resuscitation cycles are a major driver of high soil viral diversity, with the 'seeds' being a combination of (pseudo-)lysogenic infections and extracellular virions (Figure 2, Key figure), and that these diverse persistence mechanisms are critical to allow for the recurrence of many viral populations over long time periods as conditions cycle in and out of favor for active replication. Given the complexity of the processes involved, we envision large integrated projects involving a broad combination of experimental scales and techniques applied to the same soil ecosystem(s) as the best path forward for advancing our collective understanding of viral persistence in soil and its associated evolutionary and ecological drivers.

Finally, another likely driver of viral diversity in soil is phage–host coevolutionary dynamics [87]. Many aspects of phage–host ecological and evolutionary dynamics that likely apply in soil have been extensively considered elsewhere, including viral replication strategies [88], the Red Queen, Kill-the-Winner, and Piggyback-the-Winner models [18,89,90], and the diversity and coevolution of phage–host resistance mechanisms [91,92]. However, some of these biotic interactions and processes likely differ in soil environments, relative to aqueous ecosystems, for example, due to less mixing, greater dispersal limitation, fluctuating conditions over time, and the potential for concentration in 'hotspots'. Among these, several factors probably contribute to locally high rates of virus–host interactions in soil [93], including concentration in porewater, along gas–water and mineral surface–water interfaces, and in biofilms [93,94]. Concomitant with high infection rates driving virus–host arms races and viral diversification through mutation, coinfection also seems likely in these concentrated compartments and could promote phage evolution and microdiversity via recombination [95,96]. Such homologous recombination events between coinfecting viruses may be further enhanced by the mosaic organization of many phage genomes, which allows for the lateral transfer of entire functional modules [97], contributing to the elevated genome diversity typically observed for soil viruses.

Concluding remarks

Over the past decade, it appeared as if the transformative approaches, datasets, and insights provided by metagenomics in many environments and microbiomes might never readily translate to soil viral communities. Fortunately, methodological limitations are now being surpassed through improvements in laboratory protocols and analysis tools, such that establishing a global compendium of viral diversity across Earth's soils is now a realistic goal. Given these recent advances and the growing community-oriented resources, both online and in person, to share protocols and expertise among researchers interested in soil viruses, we believe that now is the time for a large-scale, systematic application of 'omics approaches to a broad diversity of soils in order to establish a 'global soil virosphere atlas'. We anticipate that genomic data will confirm the extraordinary diversity of viruses residing in and/or transiting through soils and will form the basis of a framework to

Outstanding questions

Can current methods fully capture soil viral diversity, and what are the remaining 'blind spots', if any?

Under what conditions are viruses most active and dynamic, when are they dormant, and what are the measurable hallmarks of viral activity and dormancy?

Is lysogeny a more common replication strategy in soil than in other environments, and does this trend differ across soils and/or within the same soils over time?

Does soil RNA viral diversity rival or exceed double-stranded (ds)DNA viral diversity, which host(s) do soil RNA viruses primarily infect, and what roles do RNA viruses play in soil?

What is the true relative abundance and contribution of different types of viruses [e.g., single-stranded (ss)DNA, dsDNA, RNA] to the diversity and host impacts of soil viral communities?

What are the spatiotemporal scales of soil viral dispersal, that is, how far do soil viruses usually travel under different physicochemical conditions and over different time scales, and how do the rates of mixing compare with rates of mutation and recombination to drive ecoevolutionary patterns of soil viral diversity? In other words, does dispersal limitation frequently lead to geographic isolation and divergence ('allopatric speciation') of soil viruses?

What is the magnitude of viral impacts on soil biogeochemical cycles locally and globally, and how is this distributed across viral populations, that is, are a few key viruses responsible for most of this impact, or does it result from an accumulation of small effects from a large number of diverse viral infections?

Key figure

Resuscitation of dormant viral 'seed banks' following wet-up of dry soil, or 'purple phage, purple phage, I only can detect you blooming in the pulse of rain'

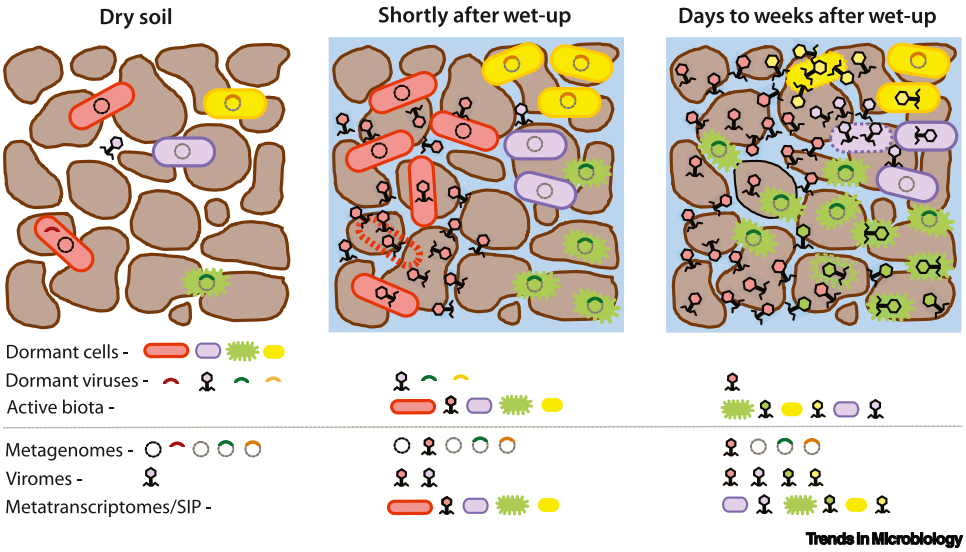

Figure 2. Upon wet-up, previously dormant microbial and viral communities (seed banks) progressively become active and turn over through time, leading to high soil viral diversity detectable through 'omics approaches. The three panels represent three time points: dry soil, shortly after rain or laboratory rewetting, and days to weeks after wet-up. Phages considered as dormant correspond to phages that did not undergo a recent cycle of infection and replication, and are depicted in three ways: (i) contained as genomes inside host cells via lysogeny as integrated prophages (green and orange semicircles) in host genomes (gray circles), (ii) contained as genomes inside host cells stalled in mid-infection via pseudolysogeny (red semicircles are pseudolysogenic viral genomes, black broken line circles are host genomes), or (iii) as free virions. Recently produced phages are also depicted as virions, although virions are considered dormant (purple phages in the leftmost and middle panels, red phages in the rightmost panel) if substantial time has passed since their production, operationally defined here as >1 week, but in order for virions to contribute substantially to viral population persistence, at least some virions would need to be stable for much longer periods of time. Active lytic viral infections are shown diagrammatically as single phages inside host cells. Below each panel are two keys: (i) top key, dormant and active cells and viruses, and (ii) bottom key, the 'omic dataset(s) in which these entities are most likely to be detected, based on a combination of their size fraction, relative abundance in the community, and activity. Adapted with permission from unpublished figure elements generated by Christian Santos-Medellín. Abbreviation: SIP, stable isotope probing.

further characterize soil viral communities. We envision (vir)'omics data as central to the future of soil viral ecology because of their unique ability to capture soil viral diversity with high-resolution and at community scale, and to guide detailed characterization of viral diversity, activity, and host interactions in soil. Importantly, we are convinced that a constant dialogue between 'omics and other techniques, including the isolation of key virus–host pairs, will be required to thoroughly investigate biotic and abiotic drivers of soil viral diversity (see Outstanding questions). Among these drivers, spatiotemporal dynamics in host community composition and activity, physicochemical heterogeneity, locally restricted biotic interactions, the accumulation of viral 'seed banks' through alternating infection strategies, and occasional immigrations from a globally dispersed viral meta-community will likely emerge as combined factors promoting high soil viral diversity. Many of these factors also apply in other microbiomes, but soils seem to represent the 'perfect storm' for incredible diversity to be established and maintained. In that context, 'omics approaches now appear mature

enough to contribute meaningfully to an in-depth characterization of the ecological and evolutionary roles of viruses in soils.

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Declaration of interests

No interests are declared.

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