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Opinion

The Role of Rhizosphere Bacteriophages in Plant Health

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Microbiomes and their hosts influence each other; for instance, the microbiome improves host fitness, whereas the host supports microbiome nutrition. Most studies on this topic have focused on the role of bacteria and fungi, although research on viruses that infect bacteria, known as 'bacteriophages' (phages), has gained importance due to the potential role bacteriophages play in the resilience and functionality of the gut microbiome. Like the gut microbiome, the rhizosphere harbors a complex microbiome, but little is known about the role of phages in this ecosystem. In this opinion, we extend the knowledge gained in human gut virus metagenomics (viromics) to disentangle the potential role of phages in driving the resilience and functionality of the rhizosphere microbiome. We propose that future comparative studies across environments are necessary to unravel the underlying mechanisms through which phages drive the composition and functionality of the rhizosphere microbiome and its interaction with the plant host. Importantly, such understanding might generate strategies to improve plant resistance and resilience in the context of climate change.

Highlights

Viruses influence host fitness through direct or indirect interactions. The latter occur mostly through bacteriophages that regulate host-associated bacterial communities.

Bacteriophages play an important role in the ecology and evolution of the host-associated microbiome and are directly linked to host fitness.

The composition and diversity of viruses associated with the soil rhizosphere are largely unexplored.

The rhizosphere virome might alleviate plant responses to abiotic stress, thereby influencing plant resistance and resilience to climate change scenarios.

Introduction

As a nonmotile organism, plants rely on their roots for growth. Nearly half of total photosynthetically assimilated carbon is transported belowground, supporting the build-up of root biomass and the metabolic costs of a functional and balanced root system [1]. As a consequence, the soil layer surrounding the root system, also known as the **rhizosphere** (see Glossary), receives a huge input in C-rich molecules through **rhizodeposition** and contrasts greatly with the soil situated as close as 2–5 mm from the roots [2]. This elevated energetic investment orchestrated by plants modulates the composition and activity of residing microbes and represents a hot spot of microbial activity and diversity, generating what is known as the '**rhizosphere effect**' or '**rhizosphere priming**' [3]. Importantly, imbalanced rhizosphere microbiomes lead to a decrease in plant growth and fitness [4].

Microbiomes housed in the rhizosphere have recently been subjected to broad comparative studies, revealing general patterns in the structure, composition, dynamics, and functioning across different host species, in addition to the role of the microbiome in plant nutrition and growth (Box 1). Despite the overwhelming number of publications on microbiome–host interactions, viruses – the most abundant entity on the planet – have been left out of the equation. Viruses infect three domains of life: Bacteria, Archaea, and Eukarya. Whereas plant and animal viruses directly influence their hosts, **bacteriophages** or **phages** influence the host indirectly by interacting with the bacterial component of the microbiome.

Phages are ubiquitous, being found across ecosystems, such as marine, terrestrial, and human gut [5–7], but due to methodological limitations, most currently available knowledge on virus

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metagenomes (**viromes**) comes from the marine environment. More recently, the relevance of viromes has reached studies focusing on the human gut, which is expected to contain about 81 000 viruses and has low virus-to-microbe ratio, indicating that **lysogenic phages** are favorable in high-density systems such as the human gut [8] (Box 2). Although the number of identified viruses in soils is much lower (27 000), the fact that soils harbor the highest microbial diversity (up to 10^{10} bacterial species/g soil) indicates that the viral abundance and diversity in soils remains largely untapped [8–10].

In this opinion paper, we argue that phages play a critical role in influencing the dynamics of the rhizosphere microbiome. Given the lack of information on the soil virome, we borrow results obtained in the human virome to build our case. The similarities between the rhizosphere and gut systems have been elegantly addressed recently [11–14] and highlight the suitability in transposing the concepts related to the microbiome structure and function between plants and animals (Figure 1 and Box 3). We start by discussing the current knowledge on bacteriophages in the gut and rhizosphere microbiomes, their relative contribution to bacterial resilience in the context of gut dysbiosis, and how this knowledge can be transposed to plant–microbiome interactions. We summarize by proposing experimental approaches that can generate insights on the role of phages in the stabilization of the rhizosphere soil bacterial communities.

Bacteriophages in the Rhizosphere and the Human Gut: Abundance and Diversity

Gut bacteriophages significantly shape the structure and function of the gut microbiome, thereby contributing to human health [15,16]. It is estimated that the gut virome consists of more than 10^{12} **virus-like particles (VLPs)** residing in the human colon and 10^9 per gram in fecal samples [17]. Moreover, *in vitro* study has demonstrated phage transcytosis across epithelial cell layers from different tissues and has revealed active phages within membrane-bound vesicles,

Glossary

Auxiliary metabolic genes (AMGs): phage genes that affect host metabolism. They are not essential for phage replication and reproduction.

Bacteriophages or phages: bacteriophages are viral particles that infect bacterial cells, which are used as machinery to replicate viral particles.

Core microbiome: convergent set of microbial species that have been named as a concept useful to define the prevailing microbial genotypes in a given microbiome and unusually adapted to define which species must be found in an equilibrated or optimal microbiome.

Darwin's paradox: condition in which high diversity is observed in low-input nutrient environment.

Lysogenic conversion: changes posed by temperate phage integration into the host's phenotype.

Lysogenic phages: a virus of bacteria that can integrate into a bacterial genome and, upon receiving a cue, can excise and propagate.

Rhizodeposition: release of photosynthetically assimilated carbon by the roots.

Rhizosphere: layer of soil tightly attached to the roots, where the diversity and abundance of molecules released through rhizodeposits are involved in the structure and activity of microbial communities by selectively recruiting populations of the soil microbiome.

Rhizosphere effect/rhizosphere priming: plant effort in building a beneficial environment whereby plant-beneficial microorganisms contribute to both nutrient availability and phytosanitary status.

Viral shunt: viral shunting is a process in which viral lysis releases readily available nutrients (i.e., dissolved organic matter and inorganic nutrients) to other neighboring microorganisms.

Viromes: viral microbiomes.

Viromics: viral metagenomics.

Virus-like particles (VLPs): structures resembling viruses; not necessarily infectious, as they might contain no viral genetic material.
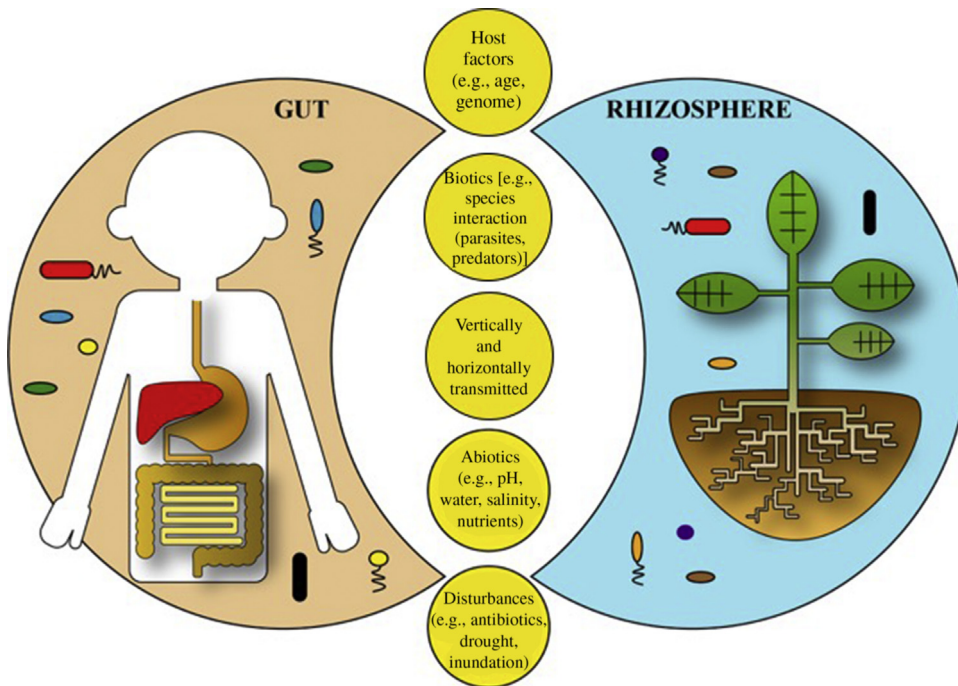
Trends in Microbiology

Figure 1. Parallels between the Microbiome Observed in the Human Gut and the Plant Rhizosphere. Both the gut and rhizosphere microbiomes are driven by host factors, such as age, as well as by biotic and abiotic interactions. Moreover, both play significant roles in host fitness and resistance/resilience to disturbances. Adapted from [15].

evidencing its access to the cytosol of host cells [18]. It has been estimated that the average number of human phages transcytosing across the gut epithelial cells is about 31 billion particles per day, implying the putative role of phages in modulating host fitness [19].

Box 3. The Rhizosphere and the Gut Microbiome – Physiologically Distinct Systems With Parallel Functions

A comparative analysis of the gut of animals within the rhizosphere of higher plants reveals several functional and organizational parallels. In both organisms, nutrition is functionally supported by microbial communities in a cross-regulatory network that also involves third-party influences (physical, chemical, and biological) [11]. Gut microbiomes are structured similarly to the rhizosphere microbiome, considering the variations in microbiome composition in different compartments (ileum, cecum, midcolon, and rectum), as observed in plants (primary and secondary roots, branching points, root tips, and root hairs). Multicellular heterotrophic eukaryotes such as humans have developed a complex digestive system to deal with the organism's nutrition, comprising different structures, such as the esophagus, stomach, small and large intestines, and colon. After food ingestion, the gut microbiome aids the digestive system to break down and transform food into small molecules that follow to absorption by specialized cells laying on the gastrointestinal mucosa. Plants, on the other hand, absorb smaller molecules dissolved in the soil solution and generate their own energy though photosynthesis, while the rhizosphere microbiome can break down and transform inaccessible nutrients from the organic matter, the soil matrix, or even the atmosphere into soluble and assimilable compounds. Despite cellular and physiological differences in how nutrients are assimilated and transported, both the human gut and plant roots act as main nutrient providers for the organism's cells and tissues, thus exerting great control over human and plant metabolism, respectively. Similarly to the rhizosphere, the amount of energy dispensed to gut tissues to assure the supply of the organism's nutritional demands can be proportionally higher than observed for other body tissues, accounting for up to 25% of whole-body energy consumption [71]. Like the rhizodeposits, a gel-like mucus layer rich in highly glycosylated proteins, named 'mucins,' is produced and exuded into the gut lumen, whose major functions are related to the protection of the gastrointestinal tract against pathogens [72]. Nevertheless, recent studies have found that the constitution of host-specific glycan structures shape the gut microbiome by selecting bacteria able to use such carbohydrates as an energy source; in counterpart, the gut microbiome regulates the expression of genes involved with immune and metabolic functions in the gut epithelial cells [73,74].

The study of **viromics** has contributed a wealth of information and changed the perspective on viral diversity, increasing the discovered number of novel viral populations up to 16-fold [20]. Prior to the viromics era, culture-based isolation of gut phages limited the scope of the studies to phages of model/pathogenic organisms (e.g., *Escherichia coli*), generally used as indicator bacteria of the presence of bacteriophages. However, as the most abundant bacteria in the gut microbiome belong to the Firmicutes and Bacteroidetes phyla, the use of *Escherichia coli* hindered true phage diversity in the human gut [21]. For instance, members of the bacteriophage crAssphage family, predicted to infect diverse Bacteroidetes, comprise up to 90% of reads obtained from the human gut virome [22–25]. By contrast to the human gut (and other ecosystems such as marine), the abundance and diversity of the soil virome are far less explored, mainly because of the high heterogeneity of soils, which poses challenges such as spatiotemporal sampling efforts. This aspect, together with the lack of optimization of the current techniques, contributes to our limited understanding of the life cycles and activities of phages in soil [8,9].

As mentioned earlier, the high diversity and abundance of bacterial species in rhizosphere soils are expected to be echoed in the respective abundance and diversity of the soil virome. Direct microscopy counts of VLPs across soil systems showed viral abundance around 10^9 VLPs per gram dry weight (gdw) of soil [26], varying greatly between different soil ecosystems: The lowest is observed in hot deserts (~10^3–10^7 gdw^{-1}), followed by intermediate abundance in agricultural soils (10^6–10^8 gdw^{-1}), fields (10^7–10^8 gdw^{-1}), and cold deserts (10^8 gdw^{-1}), whereas the highest viral abundance is found in forests (5.8×10^9 gdw^{-1}) and wetland soils (10^9 gdw^{-1}) [9]. Unlike other systems where the most abundant phage family is known (i.e., crAssphages and SAR11 phages in the human gut [22] and ocean [27], respectively), the most abundant phage from soil systems has yet to be discovered. It is estimated that tailed phages (i.e., *Myoviridae, Podoviridae,* and *Siphoviridae*) are the most abundant phages in the soil virome, even though their respective diversity is still underestimated. Despite the variety of methodologies that have been used to characterize soil viral diversity, such as (i) viral morphology [26], (ii) phage marker gene [28], (iii) viral 'hallmark' genes, (iv) viral proteomics phylogeny [29], (v) viral gene content [30], and (vi) gene-sharing network [31], soil viruses remain the largest untapped genetic pool on Earth [8–10,32,33]. Moreover, current metagenomic approaches are flawed in the study of soil viromes both upstream (e.g., viral separation from soil matrices, nucleic acid extraction) and downstream (e.g., available information in databanks, predictive bioinformatic tools), producing a biased outcome of the actual soil virome composition [34].

The Role of Phages in Bacterial Community Ecology

Regardless of the ecosystem, the ecoevolutionary roles of phages are linked to the biotic and abiotic interactions associated with their lytic and lysogenic lifestyles [35,36] (Box 2). Taking the well-documented marine ecosystems as an example, phage-induced lysis controls bacterial populations, being responsible for half of the daily mortality of marine bacteria [37,38]. Moreover, viral lysis/**viral shunt** breakdown releases readily available labile nutrients to retrofeed the bacterial community while providing a biological pump that supplies organic carbon to the deep ocean [39]. In poor nutrient environments such as in tropical coral reefs, viral shunting creates an ocean biodiversity hot spot, known as **Darwin's paradox** [40]. The lysogenic lifestyle influences the ecological and evolutionary aspects of bacterial communities due to phage-directed genomic changes that 'reprogram' the host metabolism by providing phage-encoding **auxiliary metabolic genes (AMGs)** through **lysogenic conversion** [41].

Different from the marine ecosystem, microbial interactions in both the human gut and the rhizosphere are more complex due to the involvement of host genomic factors. Despite obvious genomic differences between humans and plants, several functional and physiological parallels can be

drawn between the gut and the rhizosphere, including the ecology of their microbiome (Box 3 and Figure 1). Both are highly dynamic and continuously exposed to the influence of direct factors, such as abiotic and biotic stresses, or indirect forces that lead to changes in the microbiome in response to their direct effects on the host fitness [42]. Variations in the gut microbiome have been consistently related to lifestyle in many species beyond humans, where diet and exposure to chemicals and antibiotics have major roles [43]. Likewise, the structure and composition of the rhizosphere microbiome is dynamic, varying in response to environmental (e.g., nutrient availability, abiotic stress), biotic (e.g., invasive species), and genomic (host) signals, throughout the plant life cycle [13,44]. Similar to the marine environment, the dynamics of the host-associated bacterial communities are likely subjected to the effect of phages, with viral lysis contributing to the turnover in bacterial community composition as well as serving as a source of nutrients to the remaining community, whereas lysogenic lifestyle could act as a source of genetic novelty.

The Gut Virome and Human Health

From a human health perspective, several studies have revealed the importance of taking into account the dynamics of the gut virome [16]. It has been shown that healthy individuals from different countries share about 23 bacteriophages, a putative core virome of healthy gut phages, which is significantly lower in patients with Crohn disease and ulcerative colitis [15]. Indeed, the role of the virome in bacterial dysbiosis associated with gut diseases [e.g., intestinal inflammation/inflammatory bowel disease (IBD), type 1 diabetes, myalgic encephalomyelitis/chronic fatigue syndrome, colorectal cancer] has become more evident [45–48]. Two very interesting conclusions have been drawn from these studies. First, changes in the diversity and structure of intestinal phages, and not the eukaryotic viruses, can trigger human autoimmune diseases such as type 1 diabetes [45]. Second, viromic changes precede alterations in the gut bacteriome, as shown for patients with IBD (increased richness of Caudovirales phages and decreased diversity in the gut bacteriome) [48,49].

Dysbiosis in the gut ecosystem induced by external perturbation, such as antibiotic treatment, has been shown to have phage-induced mechanisms such as an increase in the flow of genes associated with bacterial resistance and an increase in bacteria–phage interactions, leading to high lateral gene transfer [50]. Thus, in the long term, antibiotic therapy stimulates the role of phages in providing antibiotic resistance to the gut microbiome [51], but contrary to the significant shifts in composition and diversity of bacterial communities upon antibiotic treatments, gut phage communities apparently showed low to moderate changes (Figure 2). Manrique *et al.* [52] proposed that this response was due to the conservation in phage diversity, activation of prophages, phage persistence, and gene mobilization. Although the exact underlying mechanisms of how bacteriophages influence the structure of the human gut microbiome are unknown, the studies mentioned above have provided strong evidence that the phage community plays an important role in the adaptive capacity (i.e., resistance and resilience) of the gut microbiome to external perturbations, where these interactions were shown to preserve the functionality of the gut microbiome during antibiotic stress [50]. Overall, it is becoming clear not only that phages significantly contribute to the ecology of the gut bacterial community but also that understanding viral ecological dynamics might be crucial for understanding and applying the concept of the microbiome in personal medicine.

The Virome and Plant Health

Given the functional and structural similarities between the gut and rhizosphere microbiomes, one would expect an equivalent response of the phage community to the rhizosphere microbiome and plant health [53]. Rhizosphere phages can potentially modulate soil bacterial community structure and organic matter cycling; hence; they are closely involved in soil and rhizosphere functioning. Specifically, diverse and active viral populations were reported in deep terrestrial ecosystems, where they played a critical role in driving both microbial community structure and nutrient
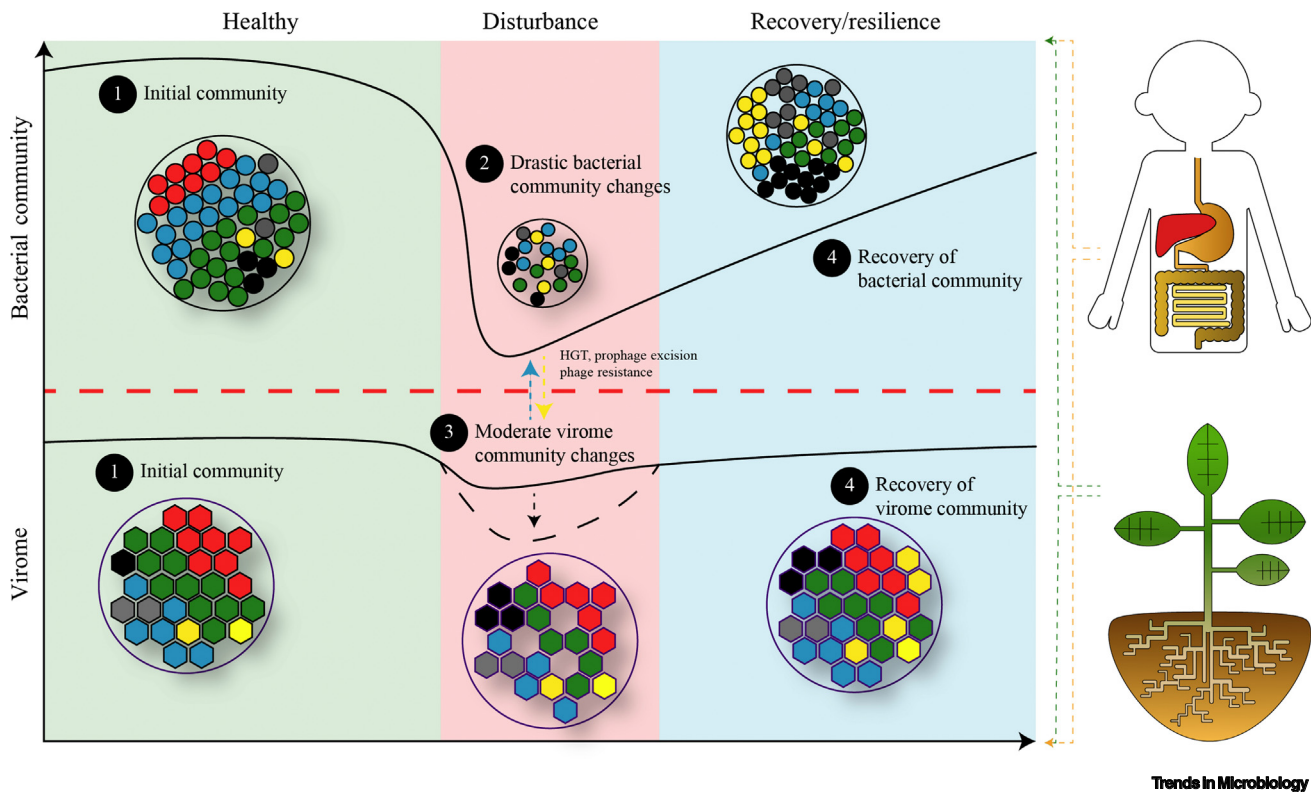
Figure 2. Potential Temporal Trajectory of Host-Associated Bacterial and Viral Communities upon Disturbance. Both the bacterial and viral communities associated with the gut microbiome of healthy individuals are diverse (1), but they respond differently to disturbances (e.g., antibiotic treatment). Whereas bacterial communities are drastically affected (2), the virome seems to be resistant to or less impacted by the treatment (3), due to phage persistence, the activation of prophages, or horizontal gene transfer (HGT). While both communities might change again once treatment ends, it is unclear whether the bacterial communities return to their original composition or reach an alternative stable state that can influence host fitness (4). Although these patterns have been observed for the gut microbiome, similarities in the ecological and evolutionary drivers of the gut and rhizosphere microbiome suggest that comparable responses could be observed in the rhizosphere microbiome in response to abiotic stress, such as drought or salinity. Adapted from [52].

turnover [53,54]. The importance of soil phages in soil functioning is further supported by studies done in permafrost soil that identified several AMGs in viral populations, which were involved in C cycling [10,33]. Finally, VLPs may directly and indirectly influence plant nutrient availability through potential effects on the soil food web, such as interference with the diversity and structure of bacterial communities and by driving mutations or regulating gene expression in selected microbial phylotypes [32,55,56]. Importantly, these effects can potentially generate plant–soil feedback by influencing nutrient inputs (from organic matter deposition to the cycling of soil nutrients such as N, P, and S) and the qualitative/quantitative modifications on rhizodeposits [57,58]. Nevertheless, it has recently been argued that viral predation does not constitute an important factor affecting the soil bacterial community [59]. Previous studies have shown that the abundance of VLPs was less than that of the rhizosphere bacteria, suggesting its lower susceptibility to phages [26]. Alternatively, similar ratios could indicate that the viromic community is mainly lysogenic due to the high abundance of bacterial hosts [8,9]. From a broader ecological perspective, it is likely that the virome plays a role in the resilience of the rhizosphere microbiome in response to biotic and abiotic pressures, as observed for the dysbiosis in the gut microbiome.

Here we propose a simple experimental setup to elucidate the effects of the viromes on the rhizosphere microbiome (Figure 3). The first experimental approach aims at disentangling the
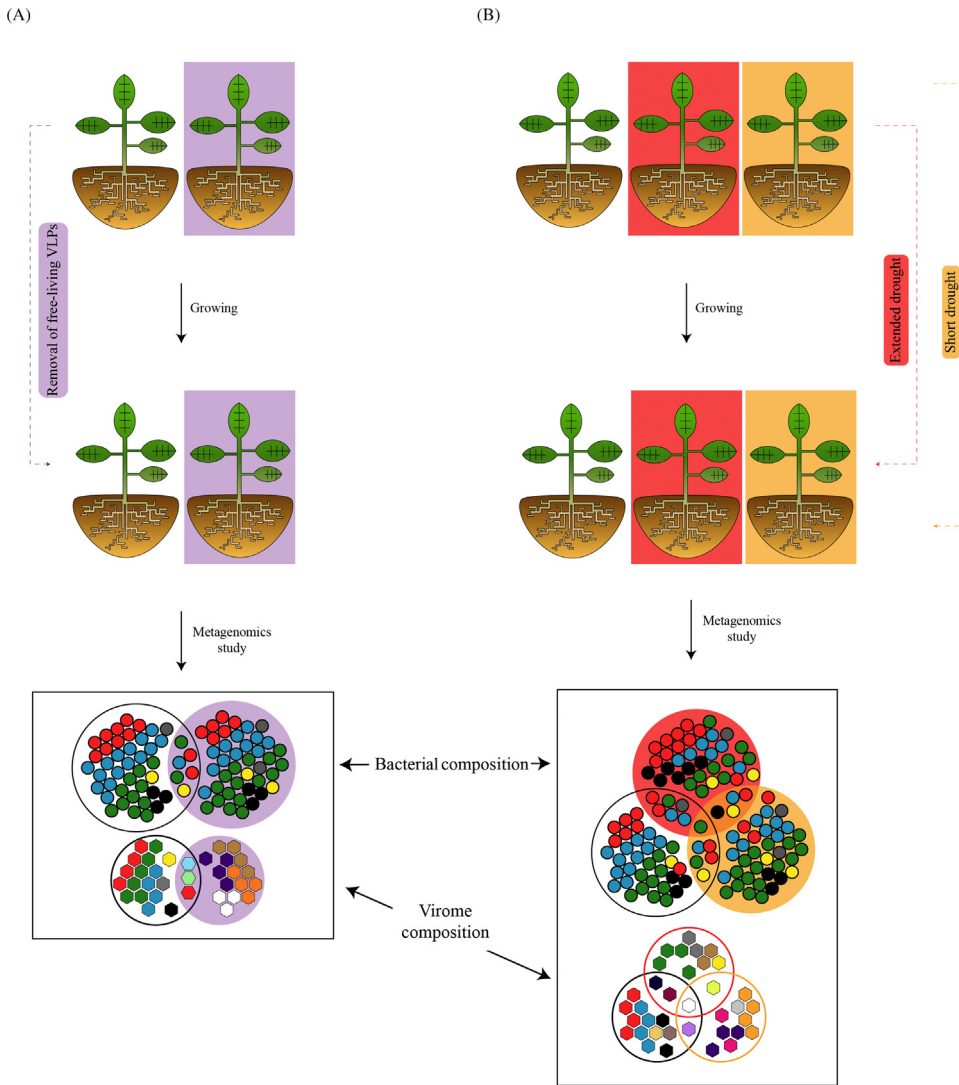
Figure 3. Manipulative Experiments to Infer the Role of Phages in Determining the Structure of Bacterial and Viral Communities in the Rhizosphere and How These Changes Influence Host Response to Disturbances. (A) In order to infer how viral communities influence the structure and functioning of the bacterial communities associated with the rhizosphere (A), we propose to grow plants in a microcosm setup, in which the soil communities are previously manipulated, to remove virus. Briefly, free virus-like particles (VLPs) can be removed through serial filtration of soil suspensions. The cells recovered during the filtration are pooled and reinoculated into sterile soil for recolonization. When soils reach stable microbial abundances, plants are introduced, and the development of the rhizosphere microbiome can be followed by using shotgun metagenomic approaches and viromics. (B) To infer whether the virome, including bacteriophages, is influenced by abiotic stress such as drought (i) in a similar fashion observed for bacterial communities and (ii) whether the resilience of the bacterial communities is dependent on the presence of phages, we propose to expose the rhizosphere communities generated in (A) to disturbances that vary in strength and duration.

importance of free VLPs in structuring the composition and functioning of the rhizosphere microbiome. This could be tested in microcosm experiments in which sterile soil is inoculated with a soil suspension from a native community [60]. The removal of free VLPs could be achieved by several filtration steps in which soil particles and associated microbiome are separated from viruses. Upon re-establishment of the soil microbial communities, plants would be introduced

and further sampled for their rhizosphere microbiome after several weeks. The role of virus in shaping the structure and functioning of the rhizosphere microbiome would be achieved by performing a multivariate comparative analysis of plant traits (e.g., biomass production, nutritional status), soil quality (nutrient status, enzymatic activities), and shotgun metagenomics [61] and viromics [53] of the DNA extracted from microcosms that received natural soil or free VLP suspension. From a virus perspective, such an approach could define, among others, the role of specific viral taxa, VLP density, and target bacterial group imprints in the rhizosphere microbiome. The data generated by shotgun metagenomics would provide evidence of shifts in microbial composition and function due to the presence of viruses, while the integration of plant traits and soil quality would provide clues to the complex interplay between plants, microorganisms, and viruses in the functioning of soil ecosystems.

A second follow-up experiment could verify whether the virome can alleviate dysbiosis induced by abiotic stress in the rhizosphere microbiome. Using the same approach as previously, planted microcosms could be exposed to several stressors, such as drought (Figure 3B), and plant fitness as well as the rhizosphere microbiome being followed over time using the same metagenomic techniques (Figure 3A). Here, we could test the hypothesis that in the absence of VLPs, the rhizosphere microbiome will not be resilient to changes imposed by the stress, due to the reduction in microbiome dynamics. We therefore expect that drought will have a stronger negative effect on the rhizosphere microbiome and plant health in the absence of VLPs. Comparative analysis of rhizosphere microbiomes over an extended period and/or at different levels of stress would reflect the impact of stress on the microbiomes and the putative role of phages in microbiome resilience. Furthermore, in soils harboring viruses (control), it would be interesting to verify whether the sensitivity of the rhizosphere microbiome to abiotic stress is greater than that of the virome, as observed for the gut microbiome [52] (Figure 2). To infer the consequences of VLP-driven changes in the rhizosphere microbiome for the plants in both experiments, several plant traits, such as above- and belowground biomass and photosynthetic rates, would provide straightforward measurements, whereas measuring plant gene expression and or metabolomics could provide a mechanistic interpretation of the effect of VLPs on plant functioning. Likewise, observed changes in soil nutrient content (abiotic factors) and enzymatic activities (soil processes such as mineralization, nitrification, denitrification, and phosphate mineralization) would establish a causal link between VLP-driven changes in the rhizosphere and the functioning and quality of soils.

Concluding Remarks
The soil microbiome plays an important role in regulating biogeochemical cycles and global climate as well as in sustaining plant growth. However, our current knowledge of the soil microbiome is strongly biased against the soil virome [9]. Here, we borrowed data from different environments, such as marine ecosystems and the human gut microbiome, to establish the possible effects of the virome on the ecological dynamics of the rhizosphere microbiome and host health. Future experimental approaches should provide a framework to investigate the significant role of phages in resilience of rhizosphere microbiomes and the mechanisms leading to these responses (see Outstanding Questions). This knowledge is key to providing an integrated view of the ecological and evolutionary consequences of the microbiome for host fitness. For instance, bacteriophages have been shown to be an effective biocontrol agent of bacterial crop disease [62], such as *Dickeya solani* [63]. A recent paper by Wang *et al.* [64] showed that the increased number of phage combinations has effectively decreased *Ralstonia solanacearum* infection in tomato up to 80%. In an ecological context, we propose that exploring the microbiome across different hosts and environments can help us develop a better understanding of potential common mechanisms underlying shifts in microbiome composition and how these shifts

Outstanding Questions
What is the diversity and abundance of the rhizosphere virome?

To what extent do virus lifestyle and host range play a role in the rhizosphere microbiome?

Does the diversity of bacteriophages in the rhizosphere respond to soil and plant type, similar to the bacterial and fungal components of the plant-associated microbiome?

How does the rhizosphere virome withstand biotic and abiotic stresses? To what extent can the rhizosphere virome contribute to plant resistance and resilience?

What factors and mechanisms drive the resilience of the rhizosphere virome? Are they similar to those observed in the human gut microbiome?

Does rhizosphere microbiome complexity influence viromic resilience?

influence the host. From a practical, agronomic perspective, as the rhizosphere microbiome plays an important role in plant development and health, determining the impact of phages on microbiome regulation might generate strategies to improve plant production.

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