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Viruses in soil: Nano-scale undead drivers of microbial life, biogeochemical turnover and ecosystem functions

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ABSTRACT

Viruses are ubiquitous in nature and have various ecological functions. Despite their very high abundance (up to $10^{10}\,g^{-1}$), viruses in soil remain disregarded with just a few, mostly descriptive studies published to date. With this review we focus on the probable functioning of viruses in soil and the consequences for microbial life and turnover, related mechanisms of biogeochemical cycling of carbon (C) and nutrients, and long-term C stabilization. We draw on the limited literature for soil, the richer knowledge from aquatic ecosystems and sediments, and evidence from pure culture studies.

Evidence from soil and other ecosystems indicate that the vast majority of soil bacteria are infected by phages at any time. Consequently, we introduce and adapt five concepts for the role of phages in soil: (1) *Viral Shunt*, (2) '*Forever Young*', (3) *Viral Regulatory Gate of EXOMET*, (4) *C sequestration by microbial necromass stabilization*, and (5) *Microscale divergence of C/N/P stoichiometry*.

The '*Viral Shunt*' accounts for the short-circuiting of trophic C and nutrient transfers by virus-induced mortality. Phages (even without soil animals – the *Microbial Loop* concept) explain bacterial death rates and the release of easily available C and nutrients, consequently accelerating biogeochemical cycles in soil. The concept '*Forever Young*' postulates that viral infection maintains the active bacterial population at a young age, because infection and lysis lead to a short life expectancy. We controversially discuss this concept in relation to hypotheses such as (1) the dormancy of most soil microorganisms, (2) maintenance energy for microorganisms, and (3) their high C use efficiency. We unify the previously suggested but unexplained concepts of '*Regulatory Gate*' and '*EXOMET*' through the lytic release of intracellular enzymes and metabolites. Due to the elemental composition of phages, infection results in *Stoichiometric C/N/P Imbalances* at the microscale, with consequences for P limitation at macroscales. The recently developed concept of '*C Sequestration by Necromass Stabilization*', i.e., that C sequestered in soil derives from microbial necromass, is discussed from the new perspective of viral lysis and "entombment" of cell fragments in nano-pores.

Very few studies have investigated viruses in soil, so all research directions are open, important and fascinating. The most urgent are those elucidating virus functions, their consequences for microbial life, and ecological relevance, and to confirm (or to reject) the proposed concepts. Functioning very fast at the nano-scale, the undead drivers govern microbial life in soil and biogeochemical turnover from micro to ecosystem scales.

1. Introduction: why viruses?

Viruses are ubiquitous in the environment and greatly influence ecosystem structure and functions (Fuhrman, 1999; Leendertz et al., 2006; Roossinck, 2015). Viruses that infect bacteria – bacteriophages – are a major cause of bacterial mortality in marine ecosystems, and thereby dramatically alter carbon (C) and nutrient cycling (Jover et al., 2014). In the oceans, viruses lyse 20% of microorganisms per day, with 10^{23} viral infections ongoing per second and $10^{4}\,Mg\,C\,s^{-1}$ released from cells by their lysis (Suttle, 2007). Viruses are possibly the most abundant biological entities in the world. Despite very high viral abundance in soil (up to $10^{10}\,g^{-1}$; Williamson et al., 2017), their importance for soil life remains completely unknown and generally disregarded (Emerson et al., 2018). Only a few studies have reported descriptive investigations of viral abundance and DNA or RNA sequences. Existing methods for direct assessment of viral abundance are based on extraction and direct counts by transmission electron or epifluorescence microscopy (Williamson et al., 2013). Because *in situ* visualization of viruses in soil is not yet possible, the counts (reviewed by Williamson et al., 2017) are greatly underestimated. Hence, the true number of *free*

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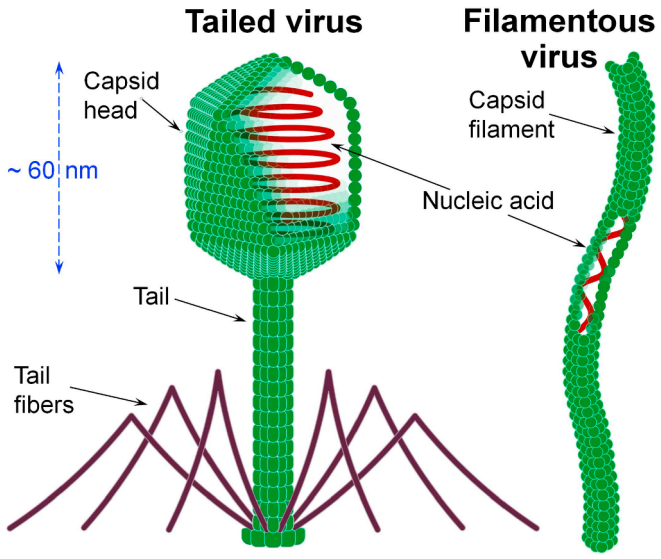

Fig. 1. Two common forms of viruses in soil: Tailed and filamentous. The tail fibers and capsid consist mainly of proteins. Some viruses also have outer lipid membranes – the viral envelope.

virions in soil is comparable and probably higher than that of bacterial cells (10^7–10^{12} cells g^{-1}, Watt et al., 2006). The total number of viruses (including intracellular viruses inside bacteria) is probably 1-2 orders of magnitude higher than the bacterial populations (Emerson et al., 2018). The abundance of viruses in marine ecosystems exceeds that of bacteria and archaea by ~15-fold, but because of extremely small size, viral biomass is only ~5% as large as prokaryotic biomass (Suttle, 2007). This small share of biomass belies a disproportionate influence on microbial ecology.

Knowledge about viruses in soil, and especially their functional consequences, lags far behind research in other environments (waters, plants, animals). With this review, we focus on the functioning of viruses in soil, in particular bacteriophages, and the consequences for microbial life and biogeochemical cycling, enzyme activities, C and nutrient turnover, C/N/P stoichiometry, as well as related mechanisms of long-term C stabilization. We do not limit the scope to the existing soil science literature, but also draw on research in other ecosystems that can provide insights into viral roles in microbial life and death.

2. Virus morphology and life cycles

2.1. Virus morphology

When outside a host cell, a phage exists as a virion, consisting of viral genomic material (DNA or RNA) encapsulated in a protein structure termed the capsid (Fig. 1) (see Table 1 for definitions of terms). Aside from this similarity, virion structure is diverse. The majority of characterized phages also have a protein tail attached to the capsid, involved in host recognition and infection. A few have an outer lipid membrane, the *viral envelope*. Virion size is related to the size of the genome (Cui et al., 2014), and both vary greatly. The MS2 phage of *Escherichia coli* has a virion diameter of 26 nm and a single-stranded RNA genome of only 3569 nucleotides, carrying just four genes, whereas a 160 nm-diameter capsid carries the *Bacillus megaterium* phage

Table 1
Terminology used in the review (modified from Madigan et al., 2017).

Term	Explanation
Virus	Genetic element that cannot replicate independently of a living (host) cell.
Virion	The infectious virus particle; the nucleic acid (DNA or RNA) genome surrounded by a protein coat (in some cases with other components).
Phage (bacteriophage)	A virus that infects bacteria.
Infection	Sequence of processes by which a phage attaches to a host cell and introduces its genetic material into the host cytoplasm, initiating either lysogenic or lytic pathways.
Prophage	Phage genome integrated into the bacterial genome.
Lysogen	A bacterium containing a prophage.
Lytic cycle/pathway	A part of the life cycle of a phage after host infection, resulting in the destruction (lysis) of the infected cell and its membrane.
Lysogenic cycle/pathway	A part of the life cycle of a phage after the host infection not resulting in the destruction (lysis) of the infected cell but remaining as a prophage.
Burst size	Average number of virions released from one infected host cell after lysis (lytic pathway).

Table 2
Main properties of bacteriophages.[a]

Properties	Common values	References
Morphology		
Form	Spherical/polyhedral capsid, often with tail; filamentous	Reavy et al., 2015; Swanson et al., 2009
Diameter, nm	30–80 nm; filamentous longer	King et al., 2011; Weinbauer, 2004
Volume, μm^3	0.00003–0.0005	
C content, fg	0.05–0.2	Suttle, 2005, 2007
C/N/P	17/5/1	Clasen and Elser, 2008
Protein:Nucleic acid	1:1–16:1	Guttman et al., 2005; King et al., 2011
Genome		
Nucleic acids	DNA or RNA	King et al., 2011
Strandedness	Single or double	
Arrangement	Linear or circular	
Base pairs, kbp	3.5 – > 200	Campbell et al., 1995; King et al., 2011; Prigent et al., 2005; Yuan and Gao, 2017
Life style/cycle		
Infection	Lytic (virulent), Lysogenic (genome or plasmid)	
Burst size	10 (oligotrophic conditions, slow-growing hosts) – 200 (large, fast-growing hosts) (5–500)	Campbell et al., 1995; Parada et al., 2006; Weinbauer and Höfle, 1998; Wommack and Colwell, 2000

[a] The table does not pretend to the complete description of properties, but offers an overview of the typical properties of bacteriophages in nature. For further details and specifics see text, the references as well as reviews by King et al. (2011); Weinbauer (2004); Kimura et al. (2008).

G genome of 497 kilo-base pairs of double-stranded DNA (Yuan and Gao, 2017), larger than some bacterial genomes. The capsids of characterized phages are most commonly around 60 nm in diameter (Ackermann, 1998; Williamson et al., 2017) consistent with most observations of viruses in aquatic environments (predominantly phages; Kimura et al., 2008) which have diameters of 30–80 nm (Weinbauer, 2004). Reports from soil also fall in or around this range (Reavy et al., 2015; Williamson et al., 2005). Consequently, phages are on average 20–50 times smaller than bacterial cells (1–5 μm) (Fig. 2). This means their volume is 8000–125,000 times smaller than the volume of bacterial cells (Fig. 2, inset table).

The nature of the genomic material is variable between species (Table 2). Both DNA and RNA phages are known, with both single and double-stranded nucleic acids, in linear or circular arrangements (King et al., 2011). Tailed phages (the order Caudovirales), which have been best characterized, are approximately half protein and half nucleic acid by mass (Guttman et al., 2005), but this does not apply to all viral orders. Filamentous inoviruses have only 6–14% nucleic acid (King et al., 2011). For specifics of virus morphology, genetics and life cycles, the reader is referred to excellent reviews presenting these topics in detail for aquatic environments (Weinbauer, 2004) and soils (Kimura et al., 2008).

host cell and introducing its genetic material into the host's cytoplasm. The viral genomic material hijacks the host's metabolism and redirects it to serve the purposes of viral replication.

Knowledge of phage life cycles is most complete for the tailed phages. When such a phage encounters a suitable host, its tail first binds to specific receptors on the surface of the bacterium. For example, some phages exploit their hosts' iron-uptake mechanisms: Iron complexes at the ends of the phage tail fibers bind strongly to membrane-bound iron receptors, providing a point of access to the cytoplasm, as articulated in the "*Ferrojan Horse Hypothesis*" (Bonnain et al., 2016). For many phages, the tail enzymatically degrades the cell wall to allow it contact with the cell membrane. Genomic material is introduced through the membrane into the host, sometimes with certain associated phage proteins (Guttman et al., 2005). This process sequence is termed *infection* (Table 1). The rest of the virion remains outside the host cell (Zhao et al., 2013) (Fig. 3). The infection can be directly followed by replication, transcription and translation of the phage genes, largely or entirely by the host's biosynthetic machinery. During this first stage after infection (the eclipse period), the host appears normal under the microscope, but the resulting gene products are radically disrupting host metabolism and shifting resources toward replication of the viral genome and synthesis of new phage proteins. After the eclipse period, the infection enters the maturation period, when new virions are as-

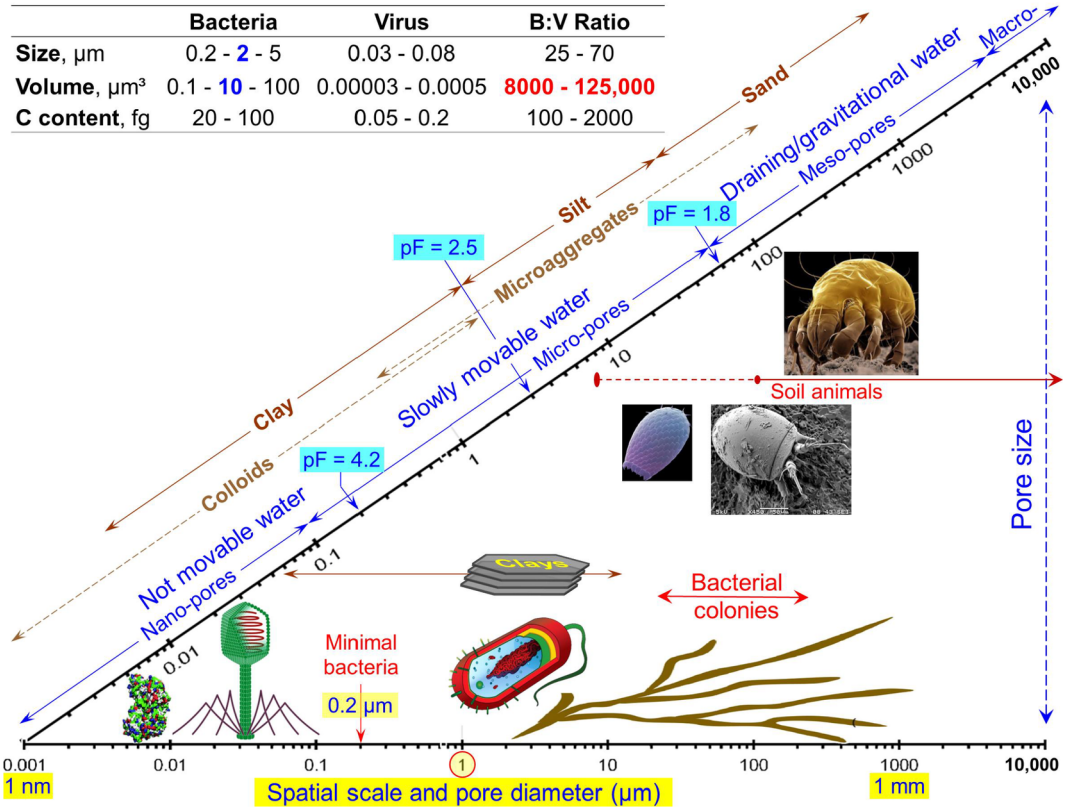

Fig. 2. Schematic representation of the physical properties (upper sloped scale) and biological occupation (x scale) of pores in soil. Note, the logarithmic scales reflect that the pore size changes across the scale by a factor of 10^8. Pore sizes and water properties are presented in blue (Ψ is soil water potential). The biotic characteristics are presented in red. The size of the mineral particles (clay, silt, sand) and colloids is presented in brown. Note that the sizes and properties are presented for visualization and do not cover the whole range of variability. The water content between pF 1.8 or 2.5 (free draining or gravitational water, depending on soil texture) and 4.2 (wilting point) reflects the plant available water.
The inset table (top left) reflects the size, volume and C content of one bacterial cell, one virus particle and the bacteria-to-virus ratio. Two values in a cell reflect the range of the parameter. Three values reflect the minimum, median (blue, bold) and maximum range.

2.2. Life cycle of viruses: between living and dead

A phage, like any virus, has no independent metabolism and lacks essential cellular machinery, such as ribosomes (Guttman et al., 2005). Phage reproduction relies entirely on encountering a suitable bacterial

sembled intracellularly. Only during this short period (~20 min for marine phages; Barylski et al., 2014) the infection becomes visible (Fig. 3). Finally, the host cell is lysed (burst), releasing the phage progeny. The average number of virions released per lysed host cell is termed the *burst size* (Table 1) and depends on host and phage species

and on growth conditions, such as the nutritional status of the host. The data for ocean phages show burst sizes from 20 to 100 (Parada et al., 2006; Wommack and Colwell, 2000). Simple calculation shows that, because of very small virion size, virions occupy much less than 10% of the host cell volume before lysis, even for the maximal burst sizes.

Viruses that only engage in such lytic infections are termed *virulent* (Table 1). However, many phages are also capable of *lysogenic infection*, involving the integration of their genome into the host genome, or sometimes carried as a plasmid. In lysogeny, most of the viral genome is silenced, no virions are produced, and viral genome replication is directly tied to normal cell division by the host. A virus in this state is termed a prophage, and lysogenic infections can persist for many generations of the host (Clokie et al., 2011). Lysogeny is more prevalent in locations and times of low host abundance and productivity (Breitbart, 2012; Howard-Varona et al., 2017), low temperature, and oligotrophic conditions (Paul, 2008). Lysogeny presents a survival advantage by allowing phages to "hide out" during unfavorable conditions (Paul, 2008; Erez et al., 2017). Importantly, the prophage retains the ability to initiate a lytic cycle, which occurs spontaneously at a low frequency or in response to changed environmental conditions, such as host stress, seasonal blooms (Brum et al., 2016) or the development of resource hotspots. Consequently, bacterial death due to changed environmental conditions is not (only) because they cannot survive the stress, but possibly because prophages are triggered to initiate the lytic cycle. In contrast, chronic infection without mortal consequences is common for eukaryotic viruses, e.g. for mycoviruses, which infect fungi in soil (Vainio et al., 2017).

2.3. Virus dissemination, propagation and distribution in soil

Current understanding of the physico-chemical interactions between phage virions and the soil is limited. Capsid surfaces are negatively charged at typical soil pH, due to ionizable groups on their protein coats (Kimura et al., 2008; Michen and Graule, 2010). Electrostatic repulsion can therefore be expected with the most common soil minerals and organic matter, and likely contributes to faster viral dissemination. Electrostatic adsorption will occur only through relatively labile divalent cation bridges (Bales et al., 1991), formed when a divalent cation such as Ca^{2+} bridges between two negative charges (e.g., a virus and a clay surface) (Bales et al., 1991; Sobeck and Higgins, 2002). Hydrophobic interactions with soil organic matter also play an important role in virus sorption (Armanious et al., 2016). Virions can therefore be viewed as small colloidal particles permeating the liquid phase of an interacting, porous soil medium (Sim and Chrysikopoulos, 2000). We speculate that phage virions will be passively distributed with water more easily than bacteria, since (1) they are much smaller; (2) interactions with soil surfaces are likely to be largely reversible; and (3) unlike bacteria, virions are not attached by exopolysaccharides to mineral and organic surfaces. Passive dispersion of phage virions in water implies their presence in all soil compartments larger than the smallest nano-pores (Fig. 2). The *biotic* propagation and dissemination, however, will be strongly concentrated in bacterial colonies, where rates of infection and subsequent lysis are maximal. Hence viruses are expected to be ubiquitous on micro- and nano-scales because of simple *abiotic* dissemination, but highly abundant in locations of bacterial colonies because of *biotic* distribution and propagation.

2.4. Viral infection of bacteria

In the past thirty years the importance of viruses in marine microbial ecology has been firmly established (Proctor and Fuhrman, 1990; Suttle, 2007). Lytic infection is widespread and affects up to 46% of the bacterial population in aquatic environments (Weinbauer, 2004), so that phage infection is not a rare misfortune, but instead a fact of prokaryotic life. The survival of aquatic bacteria relies on growth to compensate for infection, and participation in a perpetual evolutionary arms race between phage and host (Zhao et al., 2013).

The role of viruses in marine sediments is comparable to or greater than in the overlying water. Using dilution-based assays of viral production, Danavaro et al. (2008) attributed 16–89% of prokaryotic mortality in sediments to viral lysis, while Hewson and Fuhrman (2003) reported lytic turnover of 4–14% of the microbial population per hour! High phage abundance in wastewater treatment reactors (Brown et al., 2015) and in the human gastrointestinal tract (Łusiak-Szelachowska et al., 2017), despite rapid material replacement, indicates similarly rapid phage production. The collapse of cholera epidemics has been tied to bacterial population control by phages (Faruque et al., 2005). *Phage infection is therefore ubiquitous and constitutes a major cause of bacterial death in all ecosystems.*

Comparable infection data for soil bacteria are still scarce (Bowatte et al., 2010). Virus-to-bacterium ratios (VBR) reported for soil vary across six orders of magnitude (0.002–8200; Parikka et al., 2017; Emerson et al., 2018), including the entire range reported for marine ecosystems (Parikka et al., 2017; Weinbauer, 2004). Soil VBRs are, however, difficult to interpret ecologically, since (1) virion stability in soil could cause high viral abundance without proportional infection rates, and (2) VBRs are usually reported for the entire community, and therefore do not reflect species-specific virus-to-host ratios. Bowatte et al. (2010) used electron microscopy to observe phage infections in bacteria extracted from a loamy sand pasture soil and determined that from 12% to 48% of bacteria were visibly infected. Takahashi et al. (2011) found by the same approach visible infection in 8.9–12.1% of bacteria in a paddy soil. Values based on visualization certainly underestimate the true viral abundance and lytic infection percentage in soil due to (1) challenges in extracting bacteria and viruses from soil (Williamson et al., 2013), (2) because RNA and single-stranded DNA viruses are not readily detected by fluorescent direct count methods (Steward et al., 2013), and (3) because intracellular phage particles are only visible in the final stages of infection (Weinbauer, 2004) (Fig. 3). For instance, lytic infection of a marine *Vibrio* species was only visibly evident for 14–27% of the infection cycle's duration (Proctor and Fuhrman, 1990). At least for these three reasons, lytic phage infection could be even more common in soils than in aquatic systems. Indeed, the frequency of visible infection in paddy soil was higher than in the associated floodwater (Takahashi et al., 2013).

Microscopic observations of infection also fail to reflect the incidence of lysogeny, unless prophages are artificially induced to enter the lytic cycle. Lysogeny is widespread in aquatic ecosystems (Weinbauer, 2004) and 30–44% of bacteria in temperate soils have inducible prophages (Williamson et al., 2007, 2008), with another estimate in soil as high as 80–89% (Ghosh et al., 2008). Prophage sequences have been identified in soil (Ogunseitan et al., 1992), and there is also strong evidence of lysogenic infections in the genomes of soil microorganisms, including recognizable phage sequences (Brussow and Kutter, 2005).

We further speculate that infection rates in soil are even higher than in aquatic environments because (Table 3): (1) very strong dilution and mixing in aquatic ecosystems reduces the probability of physical encounters between phages and their hosts, relative to the limited liquid volume in soils; (2) the distribution of viruses in aquatic ecosystems is much more random over the water volume than in the soil, because viruses and hosts in soils are closely co-localized in microbial colonies in pores with high abundance of available C (Kravchenko et al., 2014); (3) ultraviolet light, which penetrates deeply into most aquatic ecosystems, damages and inactivates phages (Brussow and Kutter, 2005); and (4) adsorption of viral particles to solid surfaces can extend their survival (Kimura et al., 2008) and even enhance infectivity (Lipson and Stotzky, 1986).

Considering (1) these characteristics of the soil environment; (2) the large proportions of visibly infected bacteria in soil; (3) that lytic infections are only visually identifiable during a short period at the end of the lytic cycle; and (4) that lysogeny is widespread, *we conclude that the*

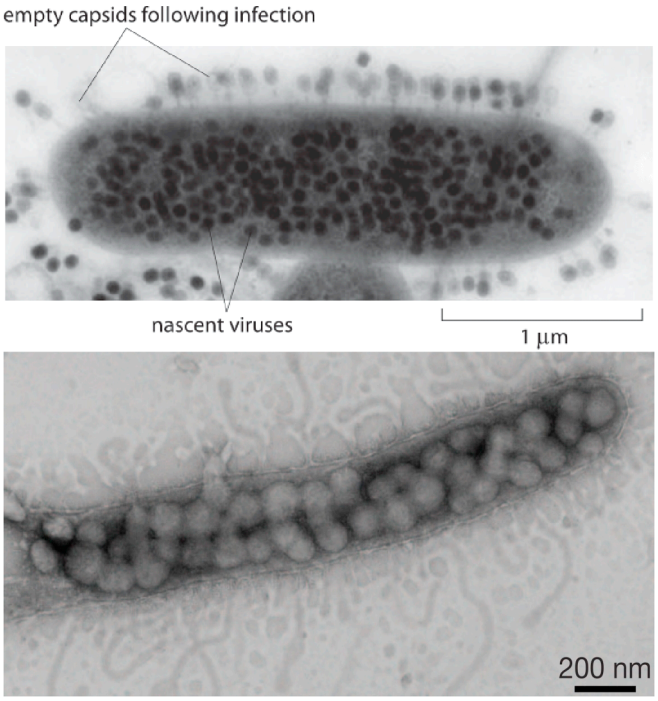

Fig. 3. Bacterial cells infected by phages. Top: Transmission electron microscopy (TEM) of *Escherichia coli* infected with phage T4. Dark viruses outside the cell have not yet injected their DNA into the bacterial host. (J.Wertz, http://book.bionumbers.org/how-many-virions-result-from-a-single-viral-infection). Bottom: SAR11 viruses in a marine bacterium (Zhao et al., 2013). Note that only this late stage of infection prior to lysis can be distinguished by TEM and other visualization methods. Earlier stages of phage development within the cells as well as lysogenic infections are not visible. The empty capsids (gray capsids in the top subfigure) remain outside the cell and can contribute to C sequestration in small micro- and nano-pores (see Necromass Stabilization and Fig. 6).

Table 3
Comparison of factors of virion distribution in aquatic and soil environments as related to the infection of bacteria.

Properties	Aquatic	Soil	[a]
Dilution	Very high	Low	+
Localization	Relatively even	Strongly localized in: Pores, water films, bacterial colonies	+
Host colonies	Small	Large	+
Bacterial encounters	Random	Specific + highly probable because of localization in colonies	+
Mixing	Fast	Extremely slow	–
Percent active cells[b]	50%–60%	Very low (0.1% … 10%)	–
Adsorption[c]	Minor	Important	+
UV incidence	Extensive	None	+

[a] The + and – show the higher or lower probability of infection in soil compared to aquatic ecosystems.
[b] The percentage of active microorganisms in the whole microbial community. The data for soil strongly depends on the method and activity parameter.
[c] Adsorption on surfaces of clay minerals, sesquioxides and organic residues.

majority of soil bacteria are infected by phages.

3. Functions of phages in soil and consequences for microbial life

Very high rates of infection have strong implications for microbial mortality and biomass turnover in soil. Here we suggest five functional implications of phage infections in soil: (1) "*Viral shunt*", (2) "*Forever Young*", (3) *Viral Regulatory Gate of EXOMET*, (4) *C stabilization as lysis necromass*, and (5) *Microscale divergence of C/N/P stoichiometry*.

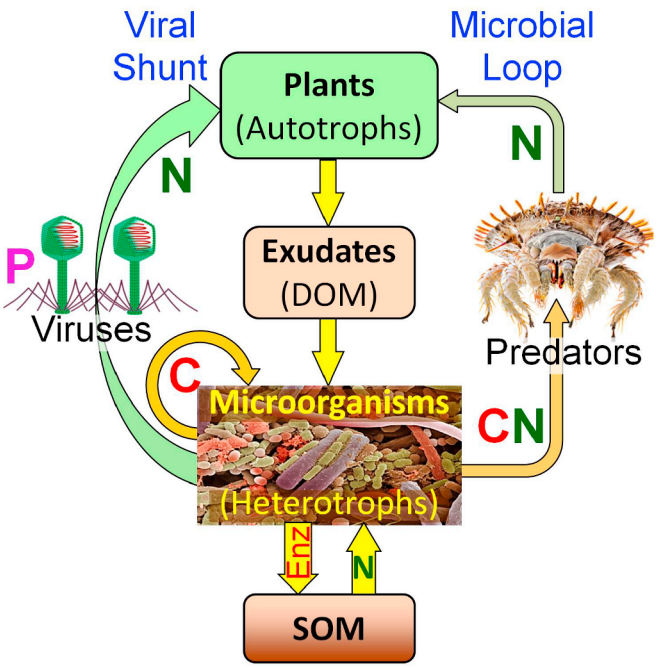

Fig. 4. Viral Shunt (left half) and Microbial Loop (right half). Plant roots release available C as exudates (exudates, dissolved organic matter - DOM) into the rhizosphere, which stimulates microbial growth and enzyme production (Enz) leading to N mineralization from soil organic matter (SOM) and N incorporation into microorganisms. *Microbial Loop*: Predatory animals feed on microorganisms, releasing mineral N. Plants take up the mineral N, which stimulates their growth and root development, creating a feedback to enhance release of organic C into the rhizosphere. *Viral Shunt*: Viruses infect and lyse microbial cells, releasing N into the rhizosphere. The N is directly available for plants and increases root growth and development with higher C release into the rhizosphere. Phosphorus (P) will be preferentially incorporated into the produced viral DNA or RNA (see Fig. 7). Note that the pool sizes on the Figure are not proportional to the real C or N stocks. (The pictures of microorganisms and of the mite were taken from https://www.riverfriendlyyard.com/science-behind-healthy-soil and https://www.nationalgeographic.com.es/naturaleza/grandes-reportajes/acaros-2_8862, respectively.)

3.1. The Viral Shunt: Microbial Loop reexamined

There is compelling evidence that bacterivores, particularly protozoa and nematodes, are important for microbial life and death in soil. Their roles in directing nutrients through the micro-food-web – termed the "*Microbial Loop*" – were initially suggested by Marianne Clarholm (1985) and further developed as follows (Zwart et al., 1994): Roots release C-rich substrates into the rhizosphere, which stimulate microbial growth, enzyme release and additional N mineralization from soil organic matter (SOM) (Fig. 4). Microorganisms use the root-released C and SOM-released N for growth. Protozoa and other fauna are attracted by the higher abundance of microorganisms and feed on the rhizosphere microbiota, releasing mineral N into the soil. Plants partly take up the mineral N, which stimulates their growth and root development, creating a feedback to enhance release of organic C into the rhizosphere.

The meta-analysis by Trap et al. (2016) concluded that, on average, grazing by bacterivores reduced microbial biomass by 16%, although some studies have reported increased bacterial biomass or abundance as a result of predation (Jílková et al., 2015; Xiao et al., 2014). More consistently, predators cause higher rates of respiration by enhancing bacterial turnover, and alter the microbial community structure (Trap et al., 2016). However, since viral populations are not controlled by

predation experimental design, it is currently unclear how bacterial activity and mortality is determined by phage infection. *Phage-induced mortality is expected to be a hidden universal factor of virtually all experiments*.

Despite the lack of rigorously controlled experimental data, several theoretical considerations indicate that phages could play a greater role in microbial turnover than predators (Emerson et al., 2018). Predatory protozoa and nematodes are too small to move soil particles themselves (Rønn et al., 2012), and are active only in aqueous environments (Neher, 2010). Active predators are therefore confined to water-filled pores or water films that are large enough to accommodate them. The physical environment therefore provides bacteria with extensive protection from predation. Rutherford and Juma (1992) used the principles of capillary forces to show that, in three soils, between 36% and 41% of water-filled pore volume was accessible to bacteria (diameter > 0.75 μm) but inaccessible even to the smallest protozoan predators (< 2.0 μm) at a matric potential of −30 kPa (Fig. 2). By the same approach, Postma and van Veen (1990) termed pores in the diameter range of 0.8–3.0 μm as "protective space" for *Rhizobium leguminosarum*, and found that it made up 19–23% of water-filled pore volume at −10 kPa (pF = 2.0, Fig. 2). Since most soil microorganisms live on surfaces rather than swimming freely (Rønn et al., 2012), it is valid to consider the distribution of solid surface area between pore sizes. Postma and van Veen (1990) showed that between 72% and 77% of the surface area of water-filled pores was in protective space. This protection will be further enhanced by pore volume exclusion: bottlenecks or isolated pores formed when larger pores are inaccessible to predators because they are only open through narrower access routes. The conclusion that soil pores provide considerable physical protection from bacterivores is borne out by the effects of soil texture and water potential on protozoa and nematode populations (Kravchenko and Guber, 2017). Gupta and Germida (1989) found that amoebae cause more dramatic reductions in bacterial populations in coarser-textured soil. Not only small pores or the absence of connections present physical barriers, but additionally the absence of water bridges at very negative soil water potential (pF > 3.5) obstructs the movement of protozoa and nematodes (Kravchenko and Guber, 2017). The importance of protective space is underlined by evidence that smaller pores are preferred for bacterial colonization (Ranjard and Richaume, 2001). We therefore conclude that *(1) only a small proportion of the pores available for and occupied by bacteria and fungi are accessible to predatory soil animals, but (2) all such locations are accessible to viruses* (Fig. 5).

A comparison of C pools and fluxes also indicates a large unobserved source of microbial mortality in soils. The C stock of all predators in soil (e.g., per m^2) and C transfer (based on ^{13}C labeling and tracing) from bacteria and fungi to respective predators is about 2-4 orders of magnitude less than the biomass of bacterial and fungal prey (Pausch et al., 2016). This is between 1-3 orders of magnitude less than the typical C and energy transfer between trophic levels in food webs known from general ecology (Odum and Barret, 2004). Protists make the most significant predator contribution to the turnover of bacterial C (Adl and Gupta, 2006), but their biomass in soil is typically 2-4 orders of magnitude lower than that of their bacterial or fungal prey (Fierer, 2017). Further, the decrease in animal population density with soil depth is much faster than that of bacteria and fungi (Ekschmitt et al., 2008; Potapov et al., 2017) and animal populations decline to near zero at a depth of 20–30 cm (Brady and Weil, 2000) or 50 cm (Potapov et al., 2017). The classical "*Microbial Loop*" can therefore explain only a small part of microbial death, and only in the topsoil. We suggest that the high rates of phage infection expected in soil (see previous chapter) drive a "*Viral Shunt*" (Jover et al., 2014, Table 4; Fig. 4). This is reflected in Antarctic desert soils, where mesofaunal predators are sparse and metagenomic evidence of widespread bacteriophages supports their role in microbial turnover (Wei et al., 2015). It is further supported by the rapid transfer of plant-derived C from bacteria to phages (Li et al., 2013). No single factor will account for all microbial death in soil, and it is expected that antibiotics and inter-bacterial predation (Findlay, 2016; Kumbhar et al., 2014), and environmental stress also contribute. However, *considering (1) a very high infection rate among soil bacteria; (2) a period of hours from infection to lysis (Weinbauer, 2004); and (3) burst sizes from 10 to over 200, we suggest that phages in particular induce rapid turnover of a large proportion of soil bacteria.*

The "*Viral Shunt*" concept (Fig. 4) could fully explain how roots are able to compete with microorganisms for N (Jover et al., 2014). Microorganisms are very strong competitors for N (Kuzyakov and Xu, 2013), and rapidly immobilize N around growing, C-releasing roots. However, it takes just a short time for viral infection to turn over the microbial population by cell lysis, releasing N for root uptake.

3.2. Concept: "forever young"

Unlike animals, which age and die, the lifetime of prokaryotes is not biologically programmed (but see discussion in: Engelberg-Kulka et al., 2006). Generally, bacterial death must be caused by external factors (Fig. 4). It is widely held that the main factor leading to microbial death is predation, particularly by protozoa, nematodes, and collembola. However, as shown above and visualized in Fig. 5, current evidence suggests that predation is not the main reason for the death of

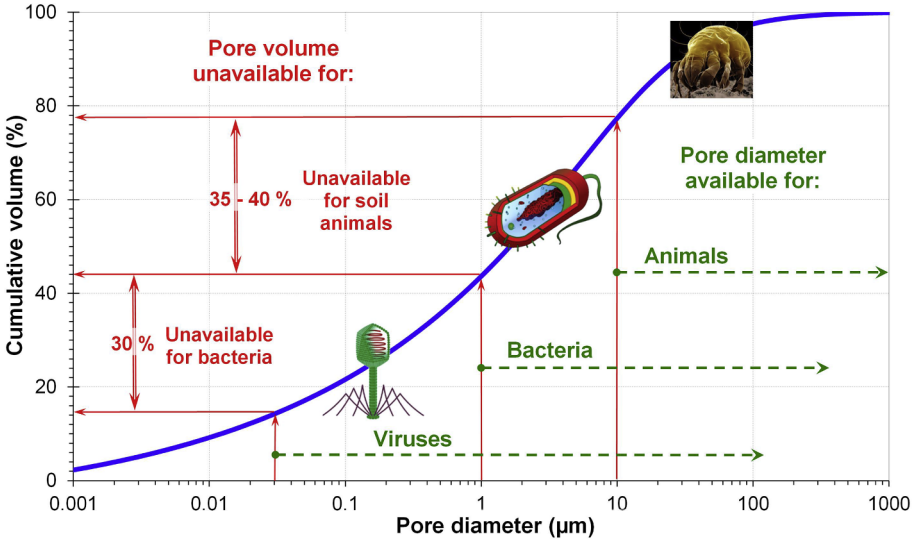

Fig. 5. Visualization of pore space available for viruses, bacteria and soil animals based on the distribution of pore sizes in soil. The pore size distribution is presented for an agricultural (Ap) horizon of a Eutric Luvisol (courtesy of A. Guber). The pore volume available for soil animals (assumed size > 10 μm) is just 1/3 of the volume available for bacteria. In contrast, viruses occupy all pores available for bacteria, fungi and soil animals. Note that the presentation of pore volume accessible for animals does not account for closed or isolated pores and bottleneck effects. Consequently, the pore volume accessible for animals is overestimated here.

Table 4
Evidence of a "*Viral Shunt*": Why predation cannot explain the majority of microbial death in soil.

Grouping	Reasons
Physical	• 35–40% of total pore space is not accessible to bacterial predators, corresponding to 50–70% of pore space available for bacteria (Fig. 3). • Decrease of soil animal abundance with depth is much faster than for microorganisms • Extremely difficult for predators to move through the soil (high density, sharp sand edges, large particles and aggregates)
Environmental	• Predator survival during bad conditions (e.g. drought, frost, high temperature) is expected to be much less than virions
Energetic	• C and energy fluxes between food web levels indicate that only a small proportion of bacteria are eaten • 10–1000 times smaller C and energy transfer between bacterial and fungal pool and the next tropic level
Biological	• Predators are lazy and efficient: they graze mainly on microbial colonies; individual cells have much higher chance to be left untouched • Plant N uptake despite strong competition from microorganisms implies rapid N cycling

microorganisms.

Viruses are major drivers of mortality among phytoplankton and heterotrophic bacteria in marine ecosystems, killing ~20% (and up to 50%) of living biomass per day (Suttle, 2007, 1994; Wommack et al., 2015). Here we propose that a major part of bacterial death in soil is caused by phage infection. Considering the very high infection rates for bacteria and the short time to lysis, we expect that the average lifetimes of bacteria in soil are weeks, at most. Of course, microbial lifetimes are not accurately described by one length of time and likely have a broad probabilistic distribution (e.g. log-normal). Nevertheless, the short average lifetime implies that bacteria in soil remain very young. Therefore, independent of the soil texture or the presence of soil animals (which are not always present in laboratory incubation studies), the populations of bacterial cells remain 'forever young' (Alphaville, 1984), even though the community structure may be stable for very long periods. Hence, phages maintain the young age of bacterial cells in soil.

Despite causing heavy mortality (Suttle, 2007), phage infections have not led to the extinction of all bacteria. This is because (1) a small number of individuals will develop resistance to the contemporary phage population (Gomez and Buckling, 2011; Zhao et al., 2013; Koskella and Brockhurst, 2014); (2) some cells will remain protected from infection by biofilms (Vidakovic et al., 2018); and (3) the growth and propagation rate of bacteria is able to counterbalance the rates of cell death. The surviving resistant bacteria benefit from the resources in the necromass (Haaber and Middelboe, 2009), building up a new population until the phage evolves to overcome their resistance.

We are aware that this concept "*Forever Young*", implying very fast microbial turnover, seemingly conflicts with the high energy and C costs expected for growing microorganisms (van Bodegom, 2007): If 50% of C taken up by microorganisms is lost as CO_2 in each cycle, the very fast bacterial turnover should deplete the whole soil C stocks within a few years. Even if very high estimates of carbon use efficiency (CUE) (0.85–0.90), suggested in some studies based on metabolic pathways of glucose and pyruvate (Dijkstra et al., 2011), are correct, the whole C in soil should be respired within 1–2 decades. This does not occur. The apparent contradiction can be explained by the following: (1) Recycling (Basler et al., 2015; Dippold and Kuzyakov, 2016): current measures of CUE are mostly based on the utilization of single substances, but viral lysis releases a spectrum of cellular components (Ankrah et al., 2014). Microbes growing on viral lysates therefore need not biosynthesize all biomass from a single C source but instead can draw on a diversity of metabolic building blocks to achieve much higher CUE than observed in laboratory physiological studies. (2) The very low proportion of active microbial cells in soil (Blagodatskaya and Kuzyakov, 2013; Lennon and Jones, 2011) and absence of the switch from lysogenic to lytic cycle in dormant cells means only the small active part has high C and energy costs. (3) Low CUE is apparent because a part of microbial necromass will be sequestered in nano-pores (< 0.1 μm, corresponding to cryptopores of Brewer, 1964) and will no longer be available for utilization by growing cells (see Section 3.4). Consequently, this entombed C (Liang et al., 2017) does not contribute to microbial biomass but will be counted as C uptake in the CUE calculation.

3.3. Viral Regulatory Gate of EXOMET

A few years ago, Sebastian Fontaine's group suggested an intriguing mechanism of extracellular oxidation of organic compounds after all microorganisms in soil had been killed – EXOMET (Maire et al., 2013). For at least three weeks after full soil sterilization by γ-irradiation, confirmed by microscopy and molecular techniques, CO_2 was still produced at 20–60% (depending on soil properties) of the intensity before sterilization. They proposed that this oxidation was mediated by extracellular enzymes and termed it 'exocellular metabolism' (EXOMET). The presence of extracellular enzymes leading to complete mineralization of low molecular weight organic substances (LMWOS), previously accepted to occur solely within living cells, was explained by microbial death (Maire et al., 2013). How such death might occur under natural conditions was not suggested. Predation cannot explain enzyme release, due to the degradation of proteins during digestion.

On the other hand, Kemmitt et al. (2008) found that microbial death by chloroform fumigation had no lasting effect on total soil CO_2 efflux. This was despite dramatic impacts on living microbial biomass, leading to the "*Regulatory Gate*" hypothesis – that an unidentified abiotic process is the rate-limited step in soil C mineralization (Brookes et al., 2017; Kemmitt et al., 2008). Despite intensive open discussions (Kuzyakov et al., 2009; Brookes et al., 2009; Paterson, 2009), a mechanistic explanation of the "*Regulatory Gate*" remains elusive.

We suggest that these two concepts can be joined and are related to the functioning of phages (Fig. 6). Exoenzymes remain active in soil for several weeks after non-predation microbial death (Schimel et al., 2017), hence cell death can increase EXOMET and will not necessarily be accompanied by reduced CO_2. The presence of metabolic enzymes outside of microbial cells, including enzymes responsible for mineralization of LMWOS, can be readily explained by viral lysis of microbial cells and the release of endoenzymes (Fig. 6). Hence the regulatory gate for soil CO_2 efflux by EXOMET is the release of intracellular enzymes into the extracellular soil solution, a process for which viruses are largely responsible. To the extent that EXOMET occurs under soil conditions, we expect viral lysis to be the 'Regulatory Gate' governing its activity.

3.4. C stabilization and sequestration in soil as microbial necromass

Lytic infection leads to the release of microbial cell constituents as dissolved organic matter and particulate organic matter (Jover et al., 2014). Various organic compounds will be released by lysis, including: (1) cytoplasmic contents, (2) colloidal fragments and globules, and (3) large cell wall fragments. Some of these materials (mainly from the first group, comprising proteins and nucleic acids) will be rapidly depolymerized and utilized by nearby surviving microorganisms, possibly within hours (Jones et al., 2005; Fischer et al., 2010; Gunina and Kuzyakov, 2015).

However, particles in the lower colloidal size range (< 0.05 μm, mainly the second group including small globules from membrane lipids and colloidal fragments of other cellular structures) may meet an alternative fate. Driven by diffusion and localized fluid flow, these

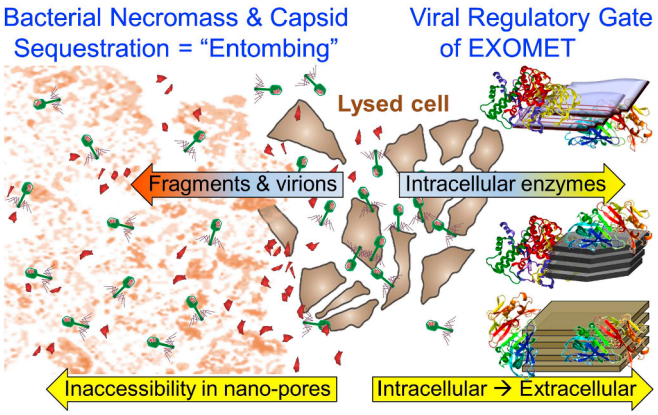

Fig. 6. Bacterial necromass and viral capsid sequestration in nanopores (left) and *Viral Regulatory Gate of EXOMET* (right). After viral lysis of bacterial cells, two pathways are possible. 1) *Bacterial necromass and capsid sequestration in nano-pores* ("*Entombing*", Liang et al., 2017): small particles remaining after cell lysis and empty viral capsids will be moved into nano-pores (< 0.1 μm) and will be not available for further microbial utilization. These particles remain inaccessible until they are eventually dislodged by water flow and removed from the pores, or the stable pores are themselves physically (or biologically e.g., by earthworms) disrupted. 2) Various endoenzymes will be released from the lysed cells, will be stabilized on clay minerals and may decompose low molecular weight organic substances. This leads to continued CO_2 production even after lysis of most microbial cells by chloroform fumigation (*Regulatory Gate*, Kemmitt et al., 2008) or by complete killing of microorganisms by γ-raditation (*EXOMET*, Maire et al., 2013). Cell residues after lysis and viral particles are presented in red-brown and green, respectively. The released endoenzymes stabilized on clays are presented on the right.

colloids could be readily transported into micro- and nano-pores (Fig. 6), where they will be protected from decomposition (Nam and Alexander, 1998). During virulent infection of a colony, such fragments could overwhelm the immediate degradative ability of the few survivors and considerable C amounts would become sequestered. New virions from lytic infections as well as empty capsids (Fig. 3, top) remaining outside the cell after infection also fall within the colloidal range and could become sequestered by similar mechanisms. To confirm explicitly the contribution of viral capsids to C sequestration in soil, biochemical (not genetic) biomarkers specific for viruses should be developed.

Necromass in the third group, consisting of large fragments of cell walls and tightly associated membranes, remains close to the site of the colony (Miltner et al., 2009). This will be available to the few survivors or future inhabitants of the microsite, will be utilized by them, and will not be stabilized over the long term. In the case of isolated bacterial cells (not located in a colony), there is a high chance that these components will persist for longer because of the absence of survivors (compare scanning electron micrographs of individual empty and dead cells in Fig. 3 of Miltner et al., 2012; and Fig. 3 in Schurig et al., 2012).

Consequently, microbial debris and viral particles after lysis may remain in inaccessible micro- and nano-pores and lead to soil C sequestration (Fig. 6) (Nam and Alexander, 1998). This is consistent with the large protein component of SOM (Kallenbach et al., 2016), and that SOM has a C/N ratio 2–4 times lower than plant litter input (Tipping et al., 2016). This concept of C and nutrient sequestration through microbial and viral necromass is analogous to the biological pump well known from ocean biology and recently suggested for soils as a microbial carbon pump and "entombing" effect (Liang et al., 2017). In the ocean, C and nutrients will be sequestered in deep sea sediments (Benitez-Nelson, 2000), whereas in soil they are "entombed" in micropores (Fig. 6). Nevertheless, the principle is similar: organic matter accumulates because, following microbial death (by viral lysis), necromass migrates to locations that are not supportive of rapid degradation.

Such C- and N-rich necromass products will be inaccessible as long as micro- and nano-pores remain stable, barring chance encounters with an appropriate series of enzymes in the same locations. To access these C- and N-rich organic sources – directly inaccessible for microorganisms and enzymes – some animals such as earthworms have developed strategies to brake and destroy the pores by passing soil particles through their gut and saturating the whole soil material with a suite of degradative enzymes (M. Kästner, personal communication).

3.5. Microscale divergence of C/N/P stoichiometry

The C/N ratio in soluble cell lysates is very close to that of the cells (Jover et al., 2014). In contrast, the C/P ratio is strongly decreased, reflecting that host P is preferentially incorporated into DNA (or RNA) of new virions. Nucleic acids have very high P content (C/N/P ≈ 10/4/1) compared to, e.g., the Redfield ratio of 106/16/1 for phytoplankton (Redfield, 1934) or 69/16/1 for heterotrophic bacteria (Suttle, 2007; Sterner and Elser, 2002). Because of the very high proportion of nucleic acids in viruses (Table 2), the P originally present in the host bacteria will become disproportionately incorporated into the new phages that are synthesized in the host (Fig. 7). Consequently, viral lysis causes a divergence in the C, N and P cycles at the microscale, with lysates from microbial cells depleted in P relative to living cells (Jover et al., 2014). Modeling various host-viral transformations of *Prochlorococcus* sp. MED4 phage under P limited conditions, Jover et al. (2014) showed that 33% of the original P content of the bacteria, but only 10% of C content, would be converted into virus particles, and the P-depleted residues would be released in the non-viral components (Fig. 7). The proportion of P allocated from host to virions increases with the size of capsid, P content in soil, and burst size (Jover et al., 2014). Because virions can be protected from microbial destruction and decomposition in small micro- and nano-pores (see Section 3.4), P will become temporarily inaccessible (Fig. 6). This could contribute to the P limitation for microorganisms and plants known in nearly all natural soils. The duration of this inaccessibility is probably longer than the mean inaccessibility of soil organic matter in general, and depends on the nanopore persistence. This divergence of C, N and P cycles at the microscale

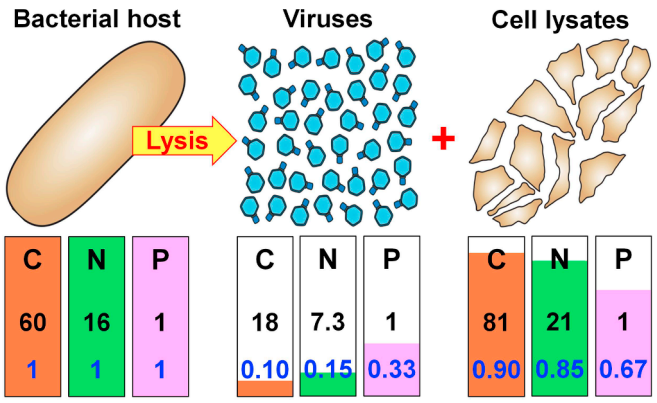

Fig. 7. Carbon (C), nitrogen (N) and phosphorus (P) stoichiometry in initial bacterial host, viruses, and remaining bacterial lysates after viral lysis (exemplary burst size = 40) relative to the initial content in bacterial host cells. The color bars and the lower numbers (in blue) are normalized to the total amount of each element in the bacterial host (C/N/P = 1/1/1), so that these values represent element partitioning after lysis. The upper numbers (in black) show the C/N/P ratio of each component. The C/N/P ratios (and color bars) show that P is enriched 3 times relative to C and 2 times relative to N in the produced viruses compared to the host, and P is correspondingly strongly depleted in the remaining lysates.The Figure is adapted from Jover et al. (2014) for marine ecosystems, based on a hypothetical infection of *Prochlorococcus* sp. MED4 by a podovirus with 70 nm diameter head and burst size of 40.

may have relevance for plant and microbial P acquisition strategies, and therefore influence ecosystems and nutrient cycling at the macro-scale. To our knowledge, this mechanism of P removal from biotic cycling and increased P limitation induced by stoichiometric divergence between microorganisms and viruses has not been investigated for soils.

These five concepts: (1) '*Viral shunt*', (2) '*Forever Young*', (3) *Viral Regulatory Gate of EXOMET*, (4) *C stabilization as lysis necromass*, and (5) *Microscale divergence of C/N/P stoichiometry* are closely linked together and build up the parts of the bacteriophage lytic system in soil (Fig. 8).

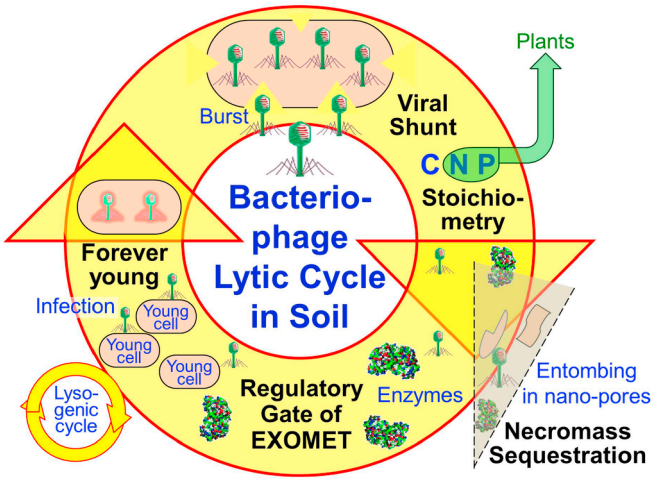

Fig. 8. Cycle of bacteriophages in soil: Linking five concepts directly related to viral infection and lysis of bacteria: *Viral Shunt*, *Microscale divergence of C/N/P Stoichiometry*, *Necromass Sequestration*, *Regulatory Gate of EXOMET*, and *Forever Young*. See detailed explanations in text.

3.6. Soil and environmental controls of viral abundance – the same as for bacteria?

The production of new phages in soil will be largely governed by the same factors that affect their bacterial hosts. In marine environments this is evident in the strong correlation between bacterial and phage abundance (Weinbauer, 2004). This correlation is also seen in soils (Williamson et al., 2017) and explains the positive relationship between organic matter content and viral abundance (Williamson et al., 2005). Viral abundance is also higher in wetter soils (Emerson et al., 2018), partly due to the positive effects on host species, but also because virions are damaged under dry conditions (Kimura et al., 2008). pH, soil depth and dissolved organic matter content are also important factors affecting the abundance and structure of viral communities (Emerson et al., 2018).

We speculate that the destruction of virus particles is accelerated by the activities of proteases and hydrolases in soil. While bacterial activity typically increases with temperature, the very low viral populations of hot deserts contrast with the moderately high counts from cold deserts (Williamson et al., 2017). This represents a dynamic balance between viral production and destruction, since low temperatures depress bacterial productivity but also promote viral persistence outside the host or inside low activity hosts (Breitbart, 2012). We conclude that viral abundance arises from a balance of forces: viral production is largely determined by host populations and activities, while the destruction of viral particles is determined by abiotic factors.

4. Conclusions and outlook

There are many exciting (but speculative) conclusions and a huge number of new research questions originating from this review and suggested concepts.

4.1. Conclusions

Viruses in soil, their functional importance and ecological consequences have been largely disregarded. Based on the data from aquatic ecosystems as well as specifics of the soil environment, we conclude that the majority of soil bacteria are infected by phages. Consequently, most bacterial cells contain a viral genome and are already on the pathway to lysis or carry a prophage that may be induced to enter the lytic pathway in the future. We discussed and adapted five new concepts based on this very high percentage of cells infected and the fast lysis of these cells. The abundance, the size, C and energy transfer and the localization of micro-, meso- and macrofauna indicate that the classical '*Microbial loop*' is insufficient to explain the C and nutrient fluxes of bacterial and fungal death. The '*Viral Shunt*' raises the importance of viruses for bacterial life cycles and shows that phages are important determinants of bacterial death rates, and therefore the release of easily available C and nutrients. Phages consequently accelerate element cycles in soil and provide nutrients for plants. A further implication of phage-induced mortality is that the active bacterial cells in soil should be very young. This is described in the concept '*Forever young*', showing that viral infection maintains a youthful bacterial population in soil. The controversial discussion of this concept involves many unresolved questions in soil science: the importance of dormancy, microbial maintenance and growth, and carbon use efficiency, among others. High P content in nucleic acids leads to preferential P incorporation (compared to C and N) into new virions. This may result in removal of available P, resulting in *microscale divergence of C/N/P stoichiometry* and so increasing the P limitation common in nearly all soils. This previously undescribed mechanism occurring at the microscale may have important consequences on the macroscale. The two previously proposed concepts of '*Regulatory Gate*' and *EXOMET* were conceptually united through the release of endoenzymes after viral cell lysis. Not only endoenzymes will be released, but also various cell fragments, with sizes similar to or smaller than virions. Just as soil animals cannot reach all the locations of bacteria in micro-pores, so bacteria and fungi cannot reach small micro- ($< 2\,\mu m$) and nano-pores ($< 0.1\,\mu m$), into which these cell fragments and capsids will be passively moved by water fluxes. These colloids of microbial necromass and capsids will remain undecomposed and contribute to medium- and long-term C sequestration in soil.

These concepts remain hypothetical and await experimental verification. Nevertheless, they demonstrate the need to revise our views on C, N, P cycles and energy fluxes in light of viruses in soil. Therefore, we call on the scientific community to reimagine the existence, life and death of microorganisms in soil from the microbial and viral viewpoints and not only from our macroscale.

4.2. Exciting outstanding research questions

Is bacterial life in soil really so strongly affected by viruses? Many of the points raised in this review indicate that bacterial life would be mundane without viruses, judging from the limited literature for soil, and the richer literature of marine, freshwater and sediment ecosystems. Direct experimental confirmation (or rejection) of many of the concepts is still necessary.

Is fungal and archaeal life in soil as strongly affected by viruses as bacteria are? Most studies of viruses have focused on bacterial, plant or animal hosts. This follows from the abundance of bacterial biomass in most environments and the macroscale importance of plant and animal pathogens. However, in soils, where fungal biomass is at least similar and frequently larger than that of bacteria, mycoviruses could play substantial roles in shaping the ecosystem. The same question arises for the enigmatic archaeal organisms.

Are all our experiments infected by phages? To what extent do native viruses alter the outcomes of, for example, incubation studies to assess microbial maintenance costs? Does this maintenance reflect fast

microbial death induced by ubiquitous phages and simultaneous intensive microbial growth? Do experimental treatments alter viral dynamics, e.g., lysogenic-to-lytic shifts? The potential influence of viruses on experimental outcomes has been largely overlooked, although they are active role-players in all non-sterile soil experiments.

The chicken or the egg? Who is driving whom? Do viruses determine the abundance, biodiversity and functions of bacteria, or do bacterial populations drive viruses? What governs coevolution, especially considering: (1) the absence of passive medium- and long-distance transport of viral communities in soil as is common in the ocean (Brum et al., 2015), (2) the extremely heterogeneous environment in soil from nano- to macro-scale, and (3) the impossibility of gene exchange in the absence of a pathway for physical transfer.

Are viruses active in or infective toward dormant cells? The calculations made here for phage-induced microbial turnover were focused mainly on *active* bacterial cells. These assessments were based largely on data from aquatic ecosystems. In these systems nearly all cells are active. In contrast, most microorganisms (90–99%) in soils are dormant (Lennon and Jones, 2011; Blagodatskaya and Kuzyakov, 2013). But dormancy does not imply that all physiological and biochemical processes are stopped (Joergensen and Wichern, 2018). It is completely unknown and is a wide field to understand the behavior of viruses in dormant cells, the consequences for hosts, and the relevance for various soil functions.

Can we trace the fluxes of C and other elements in viruses? To understand ecological functions, not only genetic information, but especially knowledge of the incorporation of elements into virions is necessary. This is very challenging, especially considering the very low 'biomass' of viruses and the necessity to combine isotopic with molecular biology approaches (Li et al., 2013; Lee et al., 2012). More detailed understanding of stoichiometric imbalances will also require simultaneous tracing of multiple elements, as might be achieved by ^{33}P and ^{13}C dual labelling, for example.

Is viral lysis involved in microaggregate genesis? Intense viral lysis of a bacterial colony produces a localized flush of intracellular compounds, such as proteins and polysaccharides, that have been implicated in soil particle aggregation (Rillig and Mummey, 2006). Might this represent an initial stage in microaggregate formation?

Can we develop non-genetic viral biomarkers? Genetic approaches require extraction and identification of DNA and RNA. These have great promise for the study of viruses, but can only reflect intact virions and do not allow empty viral capsids to be traced in soil (e.g. to evaluate their contribution to C sequestration, and C/N/P divergence). Therefore, the development of alternative biomarkers for viruses is necessary, perhaps based on the techniques of environmental proteomics.

What is the right scale? On which spatial and temporal scale is it necessary to design experiments, sample, and analyze the soil, in order to make accurate conclusions about viral abundance, dynamics, functions and consequences? This is surely not the scale of individual viral particles and probably not of individual bacterial cells, but perhaps communities. Yet even on this scale, it is challenging to draw conclusions about ecosystem functions.

Which element cycles are most affected by viruses? We have shown some important consequences of viral infections for microbial life and death in soil, and touched on the cycles of C, N and P. It remains unclear how relevant viruses are for element cycles at the soil profile and ecosystem scales. Various estimations of the roles of viruses in aquatic ecosystems lead to the expectation that most cycles of biogenic elements in terrestrial ecosystems are also driven to some extent by viruses.

How will virus-host relationships be shifted by climate change and what are the consequences for element cycles and ecosystem functions? Temperature optima for the survival of virions and bacteria differ. Consequently, increasing temperatures due to global warming may shift virus-host ratios. This may slow down bacterial turnover and decouple cycles of various elements.

4.3. Directions for future studies

There are three groups of general approaches for investigations: Observations, Experiments and Modeling. For Observations: Wider analysis of viruses in various soils and their relationship to the most important soil properties, including texture, organic matter, pH, climate (temperature and precipitation) and vegetation is needed, and should be related to microbial abundance. For soils, we have no idea about the viral abundance, genetics, functions, and association with bacteria, but surely the enormous bacterial diversity in soil and probably microbial evolution is controlled by phages. This group of studies is the simplest and can deliver many correlative datasets. Almost nothing is known about eukaryotic microbial viruses in soil, including, importantly, the role of mycoviruses in the development and dynamics of fungal communities (Williamson et al., 2017). This is an important research direction that will likely yield new revelations about the drivers of soil microbial life.

For Experiments: How can we design experiments with viruses in soil? Soil sterilization is unacceptable to study microbial processes, and the small size and great number of viruses make it impossible to maintain natural fungal and bacterial communities while excluding phages. However, phage bursts in soil might be stimulated by specific treatments, for example with food sources for bacteria, or environmental conditions (e.g. drought + rewetting, freezing + thawing, etc.). Addition of viral concentrates might also provide clues to viral ecology in soil, as it has in aquatic systems (Weinbauer, 2004). This group of studies requires the most imagination for experimental design and surely will make the most progress toward process and mechanic understanding.

For Modeling: Only very few models are focused on microbial life in soil. None of them considers viral infection. Usually, bacterial growth rates in soil models are (1) taken from pure cultures and reduced for non-optimal soil conditions, or (2) taken from maximal growth rates under non-limited food supply (e.g. after glucose addition) and slowed, or (3) based on death rates and maintenance (from CO_2 efflux) under *steady state*. Microorganisms in pure cultures or propagating at maximal growth rates (first and second approaches) are not infected or at least less affected by phages. But in soil, death rates depend greatly on viral abundance. Models can avoid some of the challenges of experimental studies to provide clues about the lives, survival, dispersion and functioning of microbial communities with and without viruses. Such models should consider not only infection rates and burst sizes, but especially movement and spatial localization or separation of viruses from their hosts. Can we apply the classical macro-ecological Lotka-Volterra predator-prey models to simulate the continuous micro-battle between bacteria and phages in the highly heterogeneous soil environment? Such research questions offer a nearly limitless playground for the modelers! Furthermore, there is an emerging need to incorporate virus-mediated processes into biogeochemical soil models and extrapolate the consequences for C, nutrient, energy and information fluxes to the ecosystem scale.

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