Trends in Microbiology

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Review

Adaptive strategies and ecological roles of phages in habitats under physicochemical stress

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Bacteriophages (phages) play a vital role in ecosystem functions by influencing the composition, genetic exchange, metabolism, and environmental adaptation of microbial communities. With recent advances in sequencing technologies and bioinformatics, our understanding of the ecology and evolution of phages in stressful environments has substantially expanded. Here, we review the impact of physicochemical environmental stress on the physiological state and community dynamics of phages, the adaptive strategies that phages employ to cope with environmental stress, and the ecological effects of phage–host interactions in stressful environments. Specifically, we highlight the contributions of phages to the adaptive evolution and functioning of microbiomes and suggest that phages and their hosts can maintain a mutualistic relationship in response to environmental stress. In addition, we discuss the ecological consequences caused by phages in stressful environments, encompassing biogeochemical cycling. Overall, this review advances an understanding of phage ecology in stressful environments, which could inform phage-based strategies to improve microbiome performance and ecosystem resilience and resistance in natural and engineering systems.

Highlights

Phages adapt to stressful environments by modulating reproductive strategies, infection efficiency, host ranges, and migration patterns.

Phages can enhance microbiome adaptability by encoding auxiliary metabolic genes (AMGs) related to stress resistance and promoting biofilm formation.

Phages drive a vital 'viral shunt', retaining organic matter and promoting nutrient cycling in resource-limited environments.

Through the expression of AMGs, phages modulate microbial nutrient metabolism, influencing biogeochemical cycles in stressful conditions.

Bacterial antiviral systems modulate the trade-off of detrimental cell lysis and beneficial horizontal gene transfer mediated by phages in stressful environments.

Phages in stressful environments

Stressful environments refer to natural habitats with extreme physicochemical conditions (such as deep sea, permafrost, arid deserts, and hot springs), as well as toxic polluted environments due to anthropogenic activities [1,2]. These habitats have diverse environmental stressors present, such as extreme temperature, drought, nutrient limitation, high osmotic pressure, and toxic pollutants (e.g., heavy metals, pesticides, and antibiotics), providing unique ecological niches for microorganisms [3–5]. Phages are viruses that infect bacteria [6]. With an estimated global population of 10^{31}, phages represent the most abundant and genetically diverse biological entities in the biosphere [6]. Phages can mediate bacterial mortality and metabolism through lytic and lysogenic infections, which can further impact microbiome structure and functioning [7,8]. With recent advances in bioinformatics and viromics, our understanding of the composition and ecological roles of viral communities has been rapidly expanded. Growing evidence shows that phages are intimately associated with resource conservation and biogeochemical cycles in both natural and engineered systems [9,10]. Also, phages in stressful habitats display genomic distinctions from those in mild environmental settings and exhibit significant ecological niche specificity likely triggered by environmental pressures [2,11,12]. They carry a large number of unique structural proteins and metabolic genes to adapt to their respective environmental pressures, profoundly impacting the evolution and environmental adaptation of their microbial hosts

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[13–15]. To this end, we holistically reviewed the interaction between phages and bacteria in stressful environments from perspectives of viral physiology, ecology, and adaptation, which will lead to an improved understanding of the key roles that phages play in ecosystem stability under physicochemical stress.

Impact of physicochemical stress on phages

Exposure to environmental stress (e.g., extreme temperature, radiation, oxidative stress, high hydrostatic pressure, drought, and antibiotics) can cause detrimental effects on the individual structure and physiological processes of phages (Figure 1). For instance, high temperatures from 65°C to 80°C and UV irradiation between 210 nm and 290 nm can inactivate phages through capsid structure damage and genetic disruption [16,17]. External osmotic pressure (20 atmospheres) can diminish phage infection efficiency [18]. Nutrient starvation and cryogenic stress (–20°C for 72 h) could reduce phage infectivity by decreasing the available phage receptors on the cell wall of their bacterial hosts [19]. Antibiotics could interfere with phage replication, transcription, and translation within host cells. For example, aminoglycoside at sublethal concentrations (0.6 μg/ml) can inhibit phage replication [20]. Furthermore, the oxidative stress-caused SOS response may induce prophages into the lytic life cycle [21]. Table 1 presents detailed information, including stressors, damage processes, phages, and host bacteria [17–20,22–34].

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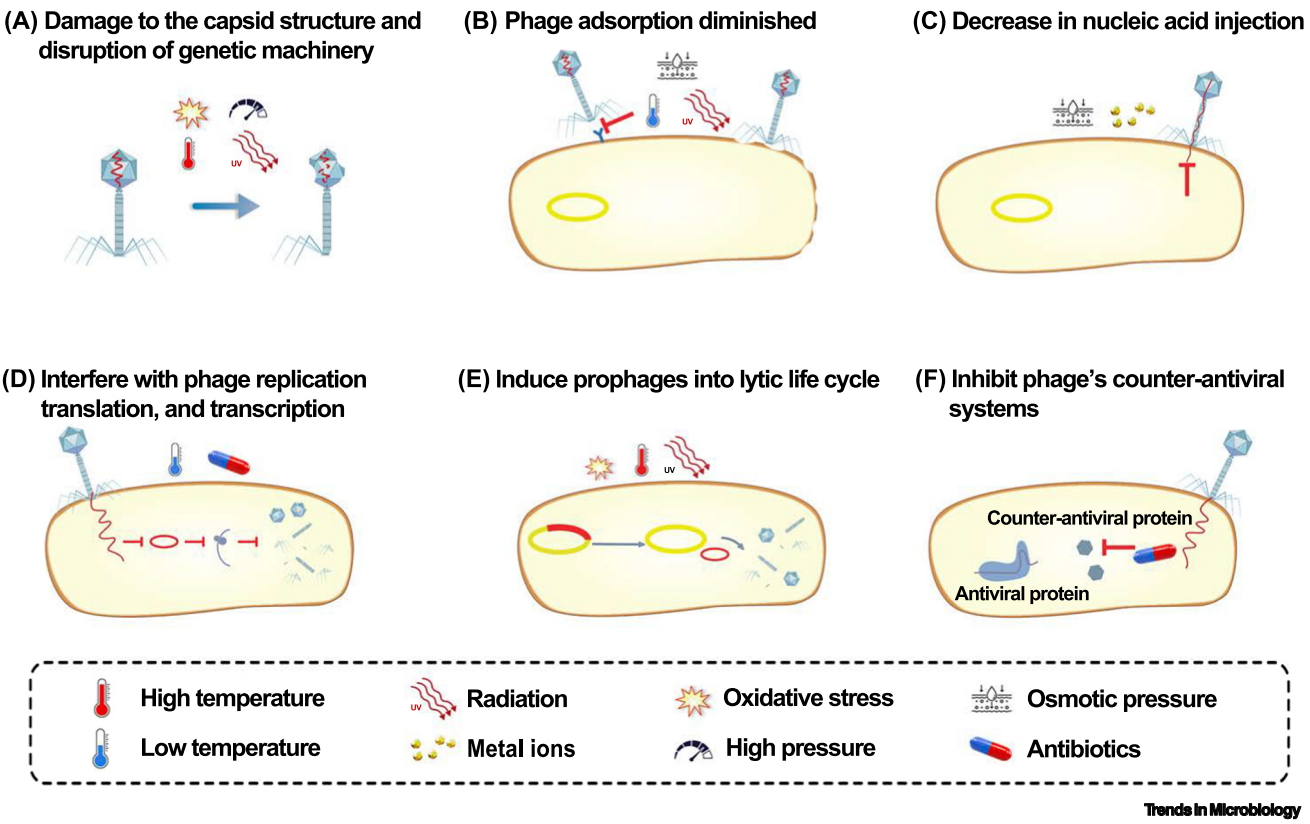

Figure 1. Impact of physiochemical stress on phage structure and life cycles. (A) Environmental stresses can damage capsid structure and disrupt genetic machinery. (B) Phage adsorption diminishes due to the damage or loss of phage receptors on the host surface. (C) External osmotic pressure and multivalent cations can impact phage infection by impeding genome injection. (D) Antibiotics interfere with the replication, transcription, and translation of phages. (E) Oxidative stress-caused SOS response and heat induces prophages into the lytic life cycle. (F) Antibiotics inhibit viral counter-antiviral systems.

Table 1. Impact of physiochemical stress on phage structure and life cycles[a]

Effects of stress	Stress	Phage	Bacteria	Refs
Damages capsid structure and disrupts genetic machinery	Urea (3–4 M)	OMKO1	*Pseudomonas aeruginosa*	[17]
	Heat (65°C to 80°C)	OMKO1	*P. aeruginosa*	[17]
	Microwave irradiation (2450 MHz for 20 s)	T4	*Escherichia coli*	[22]
	Microwave irradiation (2450 MHz for 20 s)	T7	*E. coli*	[22]
	Silver ions (1.8×10^{-4}–1.8×10^{-2} M)	MS2	*E. coli*	[23]
	Ozone (0.1 $mg\cdot l^{-1}$)	MS2	*E. coli*	[23]
	UV (65.2 $mW.cm^{-2}$)	MS2	*E. coli*	[23]
	Pressure (300 MPa)	*Lactobacillus paracasei* phages	*L.s paracasei*	[23]
	Pressure (100 MPa)	*Lactic acid bacteria* phages	*Lactic acid bacteria*	[23]
	Femtosecond laser (50 mW cm^{-2})	M13	*E. coli*	[23]
	Femtosecond laser (APNTPs for 10 min)	λ	l*E. coli*	[23]
	Femtosecond laser (APNTPs for 10 min)	Rambo	*E. coli*	[23]
	Plasma	MS2	*E. coli*	[23]
	Plasma	Φ174	*E. coli*	[23]
	Plasma	λ	*E. coli*	[23]
	Plasma	T4	*E. coli*	[23]
	Quaternary ammonium (>848 ppm)	ΦX-174	*E. coli*	[24]
	Quaternary ammonium (>848 ppm)	MS2	*E. coli*	[24]
Prevents phage adsorption and genome injection	Nutrient starvation	Coliphages	*E. coli*	[19]
	Cryogenic stress (–20°C for 72 h)	Coliphages	*E. coli*	[19]
	Osmotic pressure (20 atm)	λ	*E. coli*	[18]
	Magnesium ions (>64 mM)	λ	*E. coli*	[25]
	X-rays	λ	*E. coli*	[26]
	Streptomycin, kanamycin, hygromycin	phAE159	*Mycobacterium tuberculosis*	[27]
	Streptomycin, kanamycin, hygromycin	D29	*M. tuberculosis*	[27]
	Hygromycin (100 ppm), Apramycin (100 ppm)	Alderaan	*Streptomyces*	[20]
	Chloramphenicol (0.8× MIC), erythromycin (1× MIC), tetracycline (0.25× MIC)	DMS3vir	*P. aeruginosa*	[28]
Induces prophages into the lytic lifecycle	Plasma (0.12 kJ cm^{-2} for 10 min)	λ	*E. coli*	[29]
	Benzo[a]pyrene (200 ppm)	Enteroviruses	Intestinal bacteria	[30]
	Hydrogen peroxide (0.5 mM)	*Streptococcus* phage	*Streptococcus*	[31]

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Glossary

Auxiliary metabolic genes (AMGs): unique and nonessential functional genes found in the genomes of phages. AMGs encode proteins that modify the metabolic processes of host bacteria, enhancing the adaptability and survival of both the phages and their hosts in various environments.

Constant diversity: an ecological hypothesis that describes how viruses prevent any prokaryotic population from over-expansion, thus promoting microbial community diversity maintenance.

Generalized transduction: during phage assembly, bacterial DNA is erroneously packaged into phage capsids due to a misrecognition of the phage's packaging signals. These phages can then insert the bacterial DNA into any recipient cell they infect, leading to genetic transfer.

Hi-C sequencing: Hi-C technology, derived from Chromosome Conformation Capture (3C), is a sequencing method used to analyze the 3D structure of chromatin, providing insights into the 3D genome.

K-strategy: an ecological theory that refers to a reproductive strategy characterized by slow growth rate, large body size, production of fewer progeny, and long life expectancy. K-strategists are more easily selected in unstable and unpredictable environments.

Kill the winner: an ecological hypothesis in which virulent phages predominately prey on fast-growing bacteria and thereby provide necessary living space and nutrients for slower-growing bacteria. As a result, bacterial diversity and ecosystem stability are maintained.

Lateral transduction: some prophages bypass the typical excision step and, instead, directly replicate their genome within the bacterial chromosome. This replication also includes large contiguous sections of the host DNA, which are subsequently packaged into viral particles. These particles can effect high-frequency gene transfer, encompassing extensive portions of the host genome.

Piggyback the persistent: an ecological hypothesis in which phages become more dominated by those exhibiting temperate rather than lytic lifestyles driven by persistent physical and chemical stress, which influence microbial metabolism, thereby altering microbial communities.

Table 1. (continued)

Effects of stress	Stress	Phage	Bacteria	Refs
	Copper ions (3.1×10^{-6} M)	*Cyanophage* AS-1	*Anacystis nidulans*	[32]
	Polychlorinated biphenyls (1.0 ppm)	Marine viruses	Marine bacteria	[33]
	Heat (42°C for 10 min)	λ	*E. coli*	[34]
	UV (>5 $J.m^{-2}$)	λ	*E. coli*	[34]

[a]Abbreviations: APNTPs, atmospheric pressure non-thermal plasmas; MIC, minimum inhibitory concentration; ppm, parts per million.

Physicochemical stress that influences the physiology of phages and their host can also lead to the alteration of the composition and succession of viral communities. For example, viral diversity increased and abundance decreased in pesticide-contaminated and phosphorus-deficient soils [14,35]. Additionally, the composition and diversity of phages in composting systems continuously evolved with fluctuations in nutrients and temperature [36,37]. Increases in organochlorine contamination could transfer the assembly of soil viral communities from deterministic to stochastic assembly processes for both viral taxa and **auxiliary metabolic genes** (**AMGs**) (see Glossary) [38].

Adaptive strategies of phage communities under physicochemical stress

K-strategy

For phages, smaller burst sizes and longer latent periods during infections could reduce host lysis, which is beneficial for the preservation of the limited host resource for sustainable exploitation in stressful habitats [39]. Such survival strategies can avoid the collapse of host populations, termed the 'tragedy of the commons' (the irreversible depletion of a shared resource due to the lack of regulations and individual incentives for its sustainable use and conservation) [40]. Meanwhile, such survival strategies facilitate the generation of higher-quality progeny phages with larger individual sizes and stronger resistance [39]. For instance, a study on four lytic *Vibrio* phages revealed that the slowest-replicating phage exhibited the highest thermal tolerance and chloroform resistance [41]. Moreover, larger phages have larger genomes and tend to have more stable capsid structures and a capacity to encode a package of protective enzymes such as photolyases associated with slower decay rates [42]. In addition, larger phages have greater autonomy during infection as they encode various multifunctional tail fibers and enzymes that can modify DNA and RNA, allowing for controlled replication, transcription, translation, regulation of host metabolism, or evasion of host defenses [43]. Therefore, while both types of phage exist in viral communities under resource-limited and potentially structurally damaging stressful conditions [44], **K-strategy** rather than **r-strategy** phages are more likely to increase their relative abundance in such conditions (Figure 2A) [45].

Lysogenic cycles

The lysogenic cycle refers to the integration of the temperate phage genome into the host chromosome after infecting the host cell [21]. The lysogenic mode may represent an important adaptive strategy for phages in response to adverse environments (Figure 2B). Specifically, residing within the host cell can reduce the phage's direct exposure to physicochemical stress and the demand for host resources compared with free phages (e.g., virulent phages and temperate phages in lysis cycles) [21]. A higher proportion of lysogeny was normally observed in stressful environments, such as severe Cr-contaminated soils [11], arid deserts [46], glaciers [47], and deep sea (about 3000 m) [48]. Notably, most phages of acidophilic hyperthermophiles are non-lytic and persist in host cells in a stable state [49]. In hot springs, almost all bacterial cells contain at least one prophage [50]. Moreover, temperate phages can offer various potential advantages

r-strategy: an ecological theory that refers to a competitive strategy characterized by rapid growth rate, small body size, early reproduction, production of more progeny, and short life expectancy. r-strategists are more easily selected in stable environments.
Specialized transduction: this mechanism involves the excision of a prophage from the bacterial chromosome – but including adjacent bacterial genes by mistake. These genes are then packaged with the phage DNA into new viral particles that can integrate them into the genome of another bacterium upon infection.

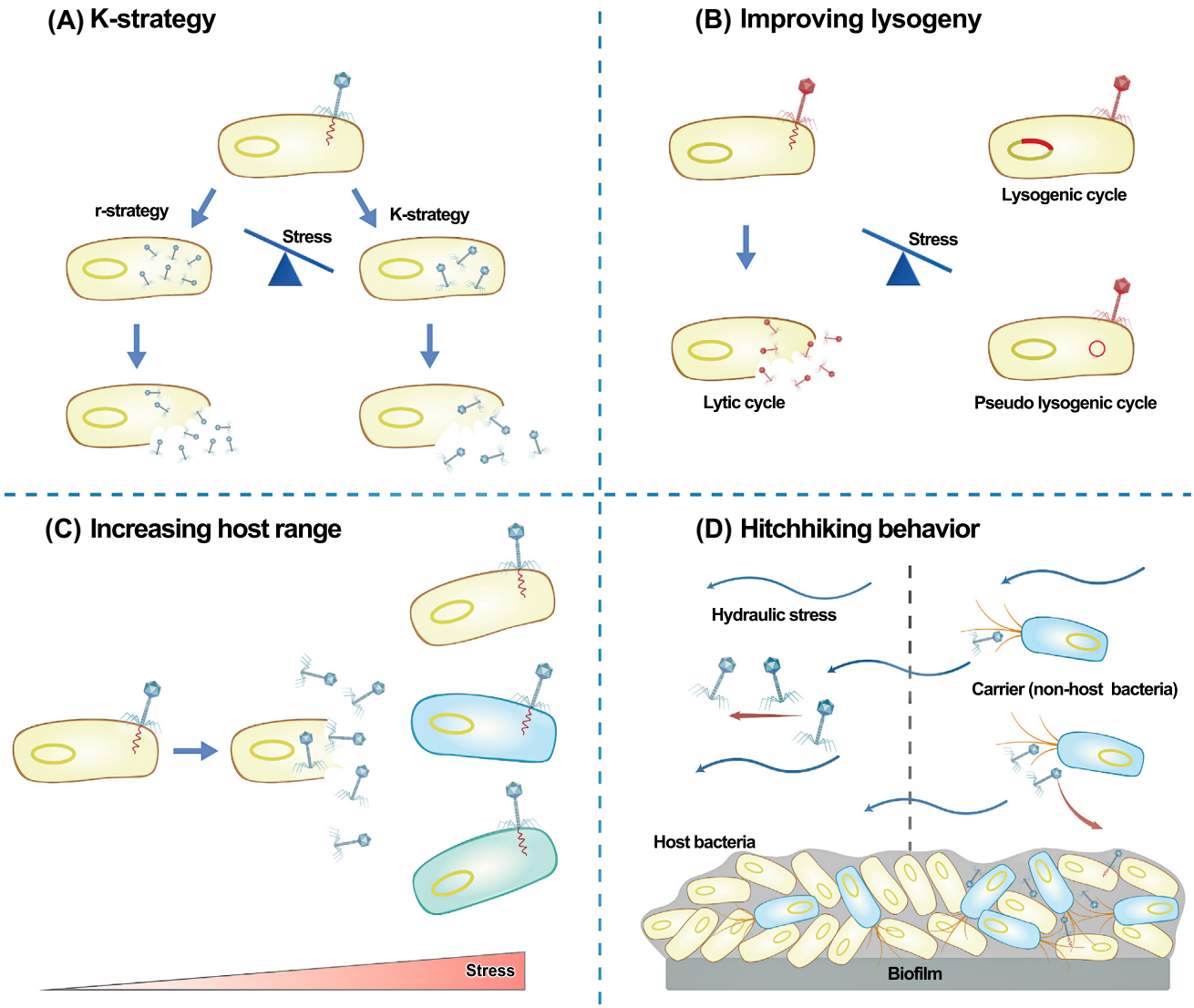


Figure 2. Adaptive strategies of phages under environmental stress. (A) Phages within K-strategy are more likely to be selected under stressful environments. The selected phages are characterized by low propagation rates but enhanced resistance to stress resistance. (B) Phages in the lysogenic cycle are more likely to escape extracellular environmental stress and may establish mutualistic symbiosis with the host. (C) Polyvalence is advantageous for phages to survive in stressful habitats with low host density. (D) Hitchhiking on motile bacteria enables phages to escape stressful habitats and colonize new ecological niches. Figure adapted with permission from Yu, Z. *et al.* [59] copyright 2021 American Chemical Society.

to the host (e.g., broaden their hosts' metabolic repertoire, confer or enhance virulence, and promote biofilm formation), and thus contribute to their environmental adaptability to high temperatures, extreme pH, and high concentration of heavy metals, further ensuring a stable living condition for intracellular viruses [21,51]. However, the strategy of lysogeny may not be universally applicable across all stressful environments as the maintenance of the lysogenic state is closely tied to host physiological conditions and environmental conditions. For instance, it has been observed that an increase in radiation intensity or acute contamination by benzo[a]pyrene could cause DNA damage via the SOS response, thereby triggering the phage lytic cycle [29,30].

Polyvalence

Polyvalent phages can bind to multiple receptors, which may come from multiple hosts (broad-host-range phages) or the same host (narrow-host-range polyvalent phages), whereas monovalent phages exhibit specificity toward a single receptor [52]. Polyvalent phages are often accompanied by a decrease in infectivity efficiency and burst size, which represents a trade-off between precision and breadth of recognition sites [52]. In stressful environments characterized by low host biomass, polyvalence is advantageous for phages to utilize alternative receptors to infect the host or rapidly find new hosts [11]. In addition, low infection efficiency and burst rate of polyvalent phage also contribute to maintaining the stability of microbial communities under stressful conditions, which is a trade-off between replication rate and host range [21]. With the advances in bioinformatics techniques – for example, clustered regularly interspaced short palindromic repeats (CRISPR) spacer sequence matching [53] and Kernelized logistic matrix factorization and similarity network fusion [54] – a widespread occurrence of broad-host-range phages has been reported in recent decades. Metagenomic studies of various settings (e.g., Cr and pesticide-contaminated soils [11,14]) have shown that the proportion of broad-host-range phages significantly increases with increasing pollutant concentrations (Figure 2C). Given the possible false-positive results produced by bioinformatics-based host prediction, high-throughput chromosome conformation capture (**Hi-C sequencing**) serves as a complementary approach to enhance confidence in host assignments [55]. Specifically, phage–host interactions revealed by Hi-C sequencing showed a large amount of potentially lysogenic phages with a broad host range in dry soil and oligotrophic groundwater [56,57].

Hitchhiking on other motile organisms

Motile bacteria can adapt to environmental changes through chemotactic movement [58]. While non-motile bacteria, which do not possess these mechanisms, have evolved alternative strategies for collective transport – hitchhiking on motile microorganisms [58]. Phages themselves lack autonomous motility and primarily rely on random diffusion to encounter their hosts [42], but they can enhance their migration capabilities by attaching to the surfaces and mucus of motile bacteria, or even their surrounding sheaths, thus enabling them to evade unfavorable habitats or colonize new ecological niches (Figure 2D) [59–61]. For instance, phage T4 can co-transport with hyphal-riding bacteria (*Pseudomonas putida*) by utilizing fungal hyphae (*Pythium ultimum*) to cope with heterogeneous and drought conditions [62]. Additionally, a hitchhiking interaction has been observed between phage (PHH01) and their non-host mobile bacteria, *Bacillus cereus*. This interaction appears to simultaneously augment the phage's resilience to hydraulic stress and promote its adaptability within biofilms [59]. The widespread occurrence of this hitchhiking phenomenon within wastewater treatment systems has been confirmed through detailed electron microscopy and viromic analysis [59]. In these interactions, phages also enhance the colonization ability of non-host bacteria by cleaving host bacteria in the new ecological niche during this process, resulting in a mutually beneficial relationship between hitchhikers and carriers [59].

The ecological effect of phages on microbial adaptability

In stressful environments, phages play a pivotal role in boosting the resilience and adaptability of microbial communities by reducing microbial intergenerational consumption, enhancing stress response, and fostering the formation of biofilms. The enhancement of the host's survival ability, in turn, ensures that phages can benefit from occupying relatively stable conditions to maintain their continued existence and proliferation.

Regulating host metabolic activity

One important strategy employed by microorganisms is to enter a state of low metabolic activity when faced with adverse environmental conditions [63]. Studies on deep-sea microorganisms

have revealed that filamentous phages (f327 and SW1) involved in chronic infections can reduce growth metabolism to slow down intergenerational consumption [63,64]. Simultaneously, they boost the motility and chemotaxis of their hosts by encoding genes related to the transcription of viral shock (*Psps*), bacterial flagellar assembly and chemotaxis, and energy production [63,64]. Moreover, the maintenance of the phage lysogenic state and the chronic infection process have also been shown to inhibit the growth rate of hosts to cope with nutrient and energy resource scarcity [65]. Furthermore, studies on virome in the desert, have uncovered that phages can manipulate host sporulation through various AMGs involved in sporulation regulatory factors (e.g., *spo0A*), transcriptional regulators (e.g., *LexA, LuxR*), and other genes associated with processes including chromosome segregation, DNA damage repair, and cell wall-related functions [66]. Moreover, viral infection may also directly trigger dormancy defenses in archaeal and bacterial hosts [67], which may bring bacteria additional resistance to adverse environmental conditions, such as drought and extreme temperatures.

Transferring or directly expressing AMGs related to stress resistance

Horizontal gene transfer (HGT) plays a crucial role in microbial genetic diversity and environmental adaptation [68], where phages serve as significant mediators of microbial HGT through transduction [69]. Specifically, phage-mediated transduction includes **specialized transduction** [70], **generalized transduction** [71], as well as **lateral transduction** [72,73]. Notably, specialized transduction and lateral transduction are closely related to the integration and induction of lysogenic phage genomes. Therefore, the increase in the relative abundance of lysogenic phages under stressful conditions, and the elevated rates of lysogenic conversion induced by environmental changes could increase the frequency of HGT in microbial communities via specialized transduction and lateral transduction [51]. For example, the proportion of lysogenic phages is higher than that of virulent phages in hydrothermal vents, and lysogenic phages increase HGT among thermophilic vent bacteria [74]. The study on paddy soil revealed that phage-mediated HGT of *arsM* [encoding an As(III) S-adenosylmethionine methyltransferase enzyme] is critical for the restoration of microbial adaptability [75]. Moreover, phages with a broad host range have a greater chance to infect more diverse native prokaryotes (even across genera) and enlarge the gene pool for HGT [76]. For example, some *Erysipelothrix* phages have the ability to acquire ARGs (*mel* and *tetM*) from their immediate host species and then, transmit it to even other bacterial genera (e.g., *Streptococcus, Streptococcus pneumoniae,* and *Bacillus coagulans*) [77]. Therefore, an expanded host range of phages in stressful environments can further facilitate the rapid spread of resistance genes within microbial communities.

Moreover, the genomes of phages in stressful conditions have been found to harbor numerous AMGs that confer microbial resistance to stress (Figure 3). These include genes such as *arsM* and efflux pump genes, which are associated with the detoxification of heavy metals [11,75] and antibiotics [78]. It also includes dehalogenase genes like *L*-DEX and demethylmenaquinone methyltransferase (*ubiE*), instrumental in microbial degradation of organic pollutants [14,30]. Additionally, genes such as *dfrB* (dihydrofolate reductase), *NAMPT* (nicotinamide phosphoribosyltransferase), and *nadM* (nicotinamide-nucleotide adenylyltransferase) play a crucial role in bolstering the antioxidant capabilities of microbial communities [79]. Genes that impact cell membrane fluidity (e.g., *lpxD)*, are also part of this repertoire, contributing to fatty acid metabolism and enabling microbes to adapt to extreme temperatures and saline environments [80]. Furthermore, the gene encoding the thiouridine synthase subunit E (*tusE*, a homolog of *dsrC* related to the sulfur relay system) was harbored by phages, exhibiting a high abundance in environments such as deep-sea hydrothermal vents [81,82]. This gene encodes a sulfur transfer protein used for tRNA thiol modifications which can enhance the structural stability of tRNA, improving the tolerance of microorganisms to high temperature, acid stress, and heavy metals [81].

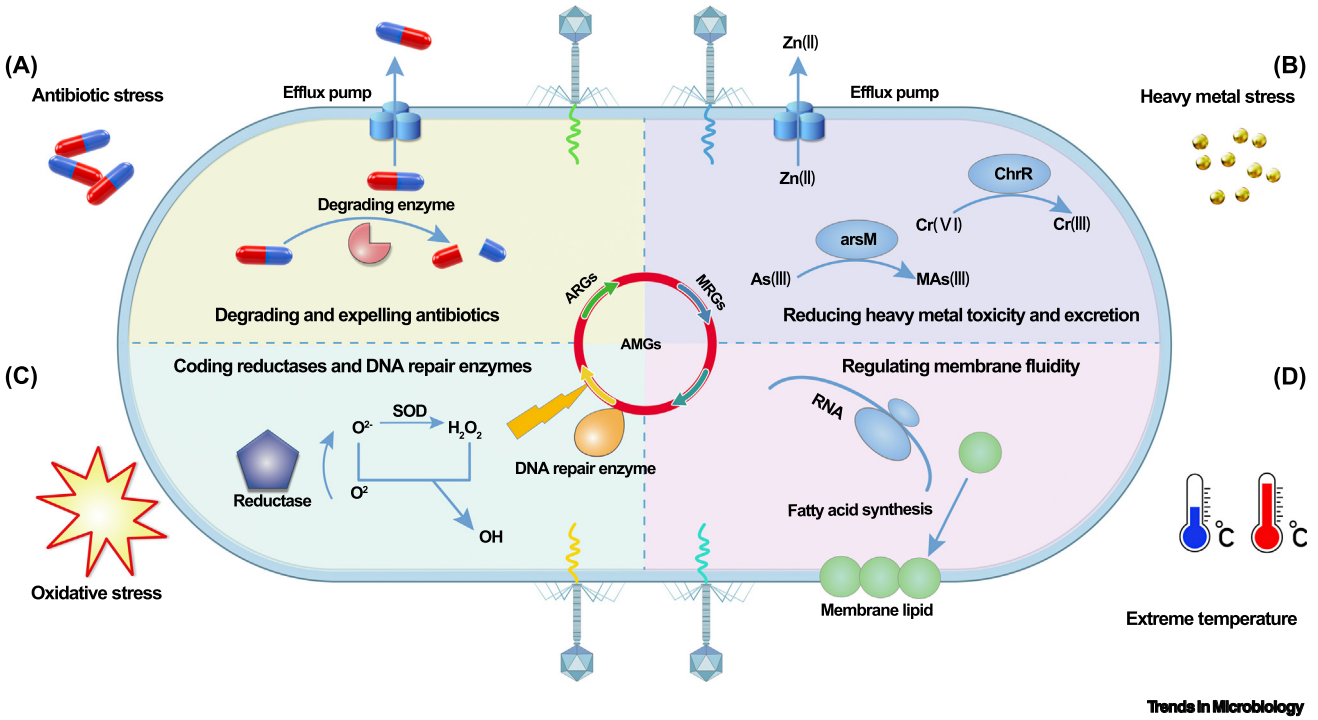


Figure 3. Phages can enhance host adaptability and resistance to stress by encoding auxiliary metabolic genes (AMGs). (A) Phages improve bacterial degradation and excretion of antibiotics through the carriage of antibiotic-resistance genes (ARGs). (B) Phage-carried AMGs related to metal detoxification enable the host to survive in habitats polluted with heavy metals. (C) AMGs encode oxidoreductases and DNA-repair enzymes, facilitating the host to cope with oxidative stress caused by disinfectants and UV radiation. (D) Phage-carried AMGs are involved in fatty acid synthesis and metabolism and participate in regulating host cell membrane fluidity, thereby enhancing the physiological stability of cells under extreme temperatures. Abbreviations: MRGs, metal resistance genes; SOD, superoxide dismutase.

Promoting microbial biofilm formation

Biofilms, ubiquitous in nature, greatly enhance the ability of microorganisms to cope with stressful environments (such as drought, heavy metals, hydraulics, and antibiotics), benefiting their survival, molecular exchange, communications, and proliferation [83]. Phages are important constituents of biofilm communities, and increasing evidence suggests that phages can induce, regulate, and even enhance biofilm formation [84–86]; this would enhance the overall resilience and adaptability of microbial communities to stressful environments. First, selective pressure induced by low-density phage infection can accelerate the development of biofilms (e.g., *Pseudomonas aeruginosa*, *Salmonella enterica*, and *Staphylococcus aureus*) (Figure 4A) [87]. For instance, predation from low concentrations of virulent phages can upregulate the expression of quorum sensing and polysaccharide secretion genes, thereby promoting the colonization of the filamentous bacterium *Piscinibacter* (host) onto the *Thauera* (non-host) aggregate, which enhances the hydraulic stability of the two-species aggregate [86]. Moreover, cell lysis caused by prophages and virulent phages results in the release of biofilm-promoting molecules, including eDNA, polysaccharides, and intracellular proteins, which can enhance the adhesiveness of the biofilm and maintain its structural integrity (Figure 4A,B) [88]. For example, lysis of *Shewanella oneidensis* MR-1 triggered by prophage induction promoted biofilm formation, while the mutant strains lacking prophage presence struggled to form biofilms [88]. In addition, phages can facilitate the formation and stability of microbial aggregates by enriching and expressing AMGs associated with the synthesis of extracellular polymeric substances (EPS) under external forces like hydraulic stress (Figure 4B) [89]. Moreover, filamentous phages (like M13 and Pf) are known to induce prokaryotic aggregation through horizontal bridging and serve as biofilm

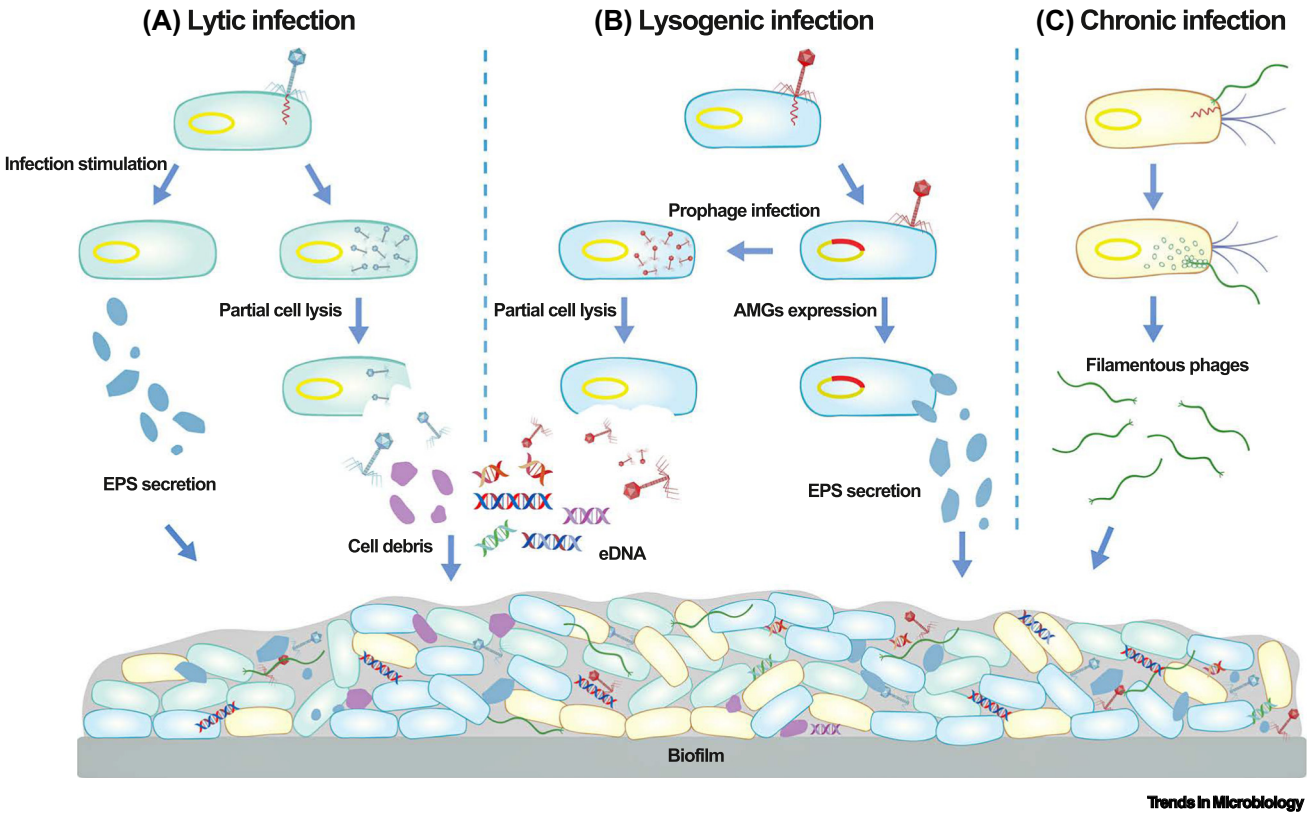
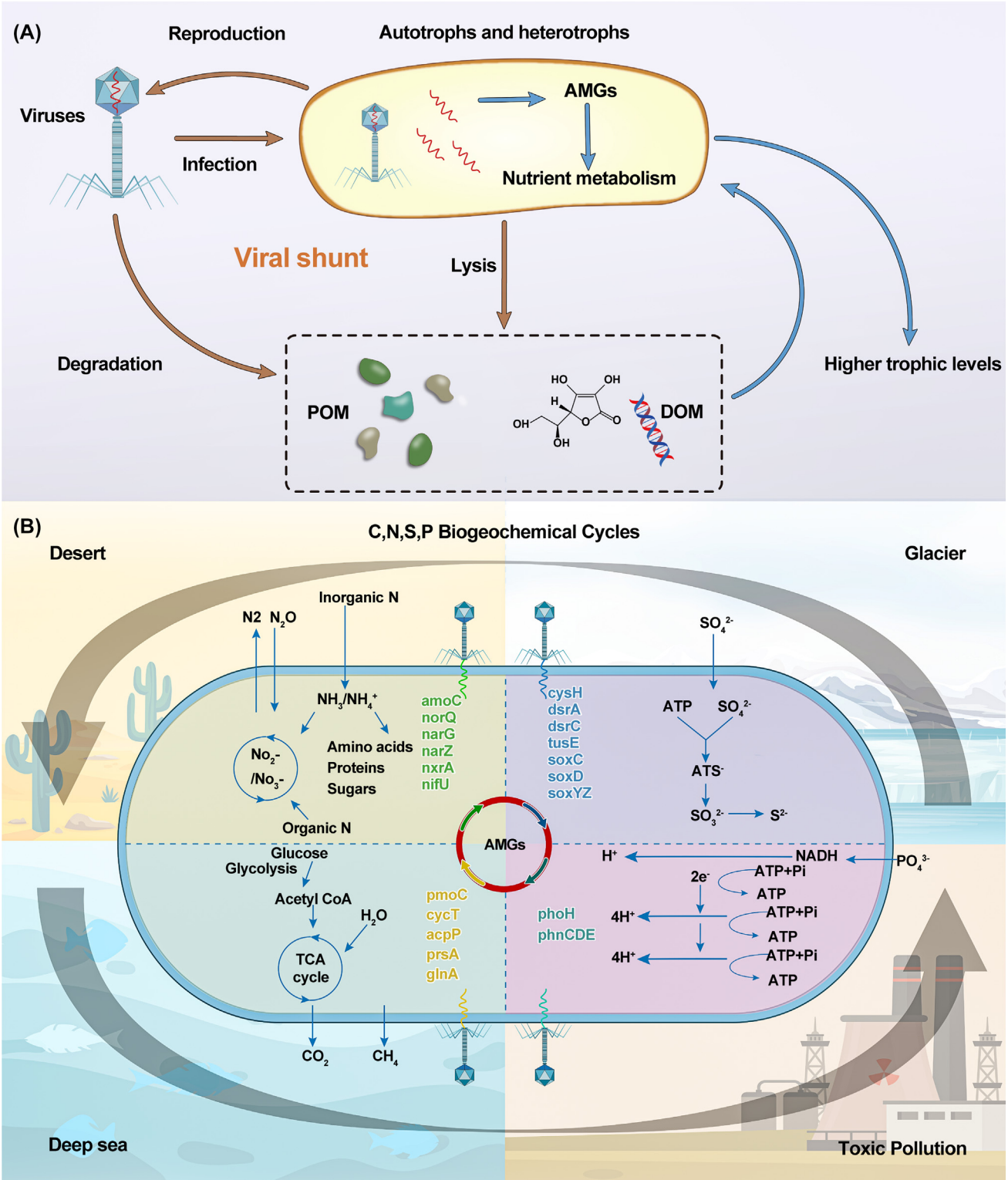


Figure 4. Phages facilitate biofilm formation and thus enhance host adaptability and resistance to stress. (A) Predation pressure and partial cell lysis by lytic phages enhance the secretion of extracellular polymeric substances (EPS), which is conducive to biofilm formation. (B) Lysogenic phages carry auxiliary metabolic genes (AMGs) related to biosynthesis and secretion of EPS, whose expression can facilitate biofilm formation; prophage induction results in partial cell lysis, contributing to the biofilm matrix. (C) Filamentous phages can enhance biofilm structural stability through their horizontal bridging and serve as biofilm matrix components. Abbreviation: eDNA, environmental DNA.

matrix components, thus enhancing biofilm structural stability and resistance to desiccation and antibiotics (Figure 4C) [90].

The role of phages in bacterial metabolism and biogeochemical cycling in stressful habitats

The ecological functions of phages encompass top-down regulation of microbial community structure, lysis of host cells, and acceleration of elemental cycling [91]. Phages are estimated to be responsible for 20–40% of bacterial death globally [8]. Due to the low abundance or absence of advanced predators, this contribution could be more pronounced in stressful environments. For instance, in the deep sea (below 1000 m), nearly 80–100% of cell mortality has been attributed to viral lysis [91]. The microbial mortality induced by phages has become a crucial factor influencing nutrient cycling within microbial food webs and shaping microbial community structures [92]. Specifically, phages facilitate the direct return of organic carbon to the food chain by lysing host cells, thereby shortening the organic carbon cycling pathway and creating a unique 'viral shunt' within the micro-food web cycle (Figure 5A) [93]. This allows for the maintenance of microbial biomass in resource-limited environments, such as deep seas, glaciers, and deserts [94–96]. At a macroscopic level, the 'viral shunt' significantly contributes to the biogeochemical cycles of the biosphere [9].



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In addition, some phages can participate in the bacterial nutritional metabolism process by encoding and expressing AMGs. For instance, some phages in deep sea, polar, and desert ecosystems have been found to carry AMGs associated with carbon metabolism, such as *pmoC* and *glnA* [91,97]. These genes are closely linked to carbon utilization and stocks, enabling the regulation of carbon loss in resource-limited conditions. This can also provide their hosts with much-needed carbon sources and an adaptive advantage in acquiring essential nutrients [98]. Furthermore, phages can enhance microbial utilization of phosphorus in phosphorus-deficient environments, such as acidic mine drainage sediments and deep-sea habitats, by encoding genes associated with phosphorus metabolism, such as *phoH* and *pstS* [99]. In deep-sea hydrothermal vents, phages infecting sulfur-oxidizing bacteria carry a plethora of AMGs related to sulfur metabolism, including *cysH*, *soxC, soxD, soxYZ,* and a gene-encoding phosphoadenylyl sulfate (PAPS) reductase [81,82]. These genes bolster deep-sea sulfur metabolism, thereby impacting the biogeochemical sulfur cycle [82]. Moreover, phage-encoded AMGs related to nitrogen metabolism (e.g., *nxrA* and *nifU*), also provide substantial support for the inclusion of phages in geochemical models of stressed environments [81,100]. Consequently, phage communities can serve as important libraries of genes related to microbial metabolic regulation, capable of modulating microbial ecology and biogeochemistry in stressed environments (Figure 5B). The current understanding of AMGs is predominantly based on computational approaches, and more comprehensive investigation and validation are required to explore the diversity, prevalence, distribution patterns, and functional activities of AMGs.

Antiviral systems and counter-antiviral systems in stressful environments

Bacteria have evolved various antiviral systems to prevent phage infection and activation [101], while phages have also developed efficient counter-antiviral systems, such as DNA modification and anti-CRISPR proteins [102]. These antiviral and counter-antiviral systems act as important mediators of bacterium–phage interactions, balancing beneficial HGT incidents and metabolic burden on bacteria in harsh environments [103]. The increased understanding of antiviral systems and counter-antiviral systems provides new insights into the strategies for microbial adaptation to adverse conditions.

The bacterial antiviral systems are influenced by various factors such as phage abundance and diversity in the environment, resource availability, environmental stress, and fitness costs [104]. In stressful environments with limited resources and low biomass, the overall microbial community may decrease the carriage or expression of antiviral systems. For instance, the enrichment and expression of microbial antiviral systems decrease under drought conditions, which is consistent with the reduced bacterium–phage collision and downregulation of microbial metabolism in drought [105]. Furthermore, in stressful environments with high virus-to-bacteria ratios, a key strategy involves broadening the defense spectrum of antiviral systems while simultaneously reducing the metabolic burden, as observed in drinking water microbiomes with disinfectants [79]. In addition, 'selective immunity' may become an essential antiviral strategy for bacterial communities in stressful environments, helping to obtain beneficial HGT mediated by lysogenic phages. For example, the type III CRISPR-Cas system exclusively targets phage transcription, which is profitable to increase compatibility with beneficial temperate phages, and ensure defense against virulent phages [106]. Moreover, at the population level, defense systems

Figure 5. Schematic of viral participation in bacterial nutrient metabolism and the biogeochemical cycle. (A) Virus-mediated lysis of microbial cells (viral shunt) and the degradation of viruses can release POM and DOM in environments. Figure adapted with permission from Wang, S. *et al*. [93]. (B) By encoding auxiliary metabolic genes (AMGs), phages participate in their host's nutritional processes, involving essential elements such as carbon, nitrogen, phosphorus, and sulfur. Abbreviations: DOM, dissolved organic matter; POM, particulate organic matter; TCA, tricarboxylic acid.

(e.g., CRISPR-Cas) could be depleted when they endow immunity to laterally transferred beneficial genes (antibiotic resistance genes), while regained and enriched through HGT in environments where phages are a major source of mortality [107]. However, numerous unresolved questions persist regarding the bacterial antiviral strategies in stressful environments, including their energy allocation in the face of biological (phage) and abiotic stress, the triggers for changes in antiviral strategies, and the elucidation of novel antiviral systems or mechanisms.

Furthermore, an intriguing phenomenon warrants discussion: antiviral systems are normally considered critical weapons for bacteria to prevent phage infection and destruction [101]. However, it has been observed that these antiviral systems [such as the CRISPR-Cas system and restriction–modification (RM) system] are widely distributed in the genome of phages (especially lysogenic phages), and they are enriched along with AMGs beneficial to the host under disinfection or heavy metal stress [79]. For phages, this can help hosts defend against competitive phages and improve the survival rates of themselves and hosts. For bacteria, prophages protect their hosts from secondary infections by excluding superinfection, while acting as mediators for the HGT of antiviral system components, thus enhancing the overall viral defense capacity of the community [108]. Therefore, temperate phages carrying antiviral genes are more likely to establish mutualistic symbiosis with their hosts in stressful environments [79].

In the arms race with their bacterial hosts, phages have also evolved diverse counter-antiviral systems [109]. Among these, evading host RM systems by encoding methyltransferases is the most prevalent anti-defense strategy in hostile environments [79]. A recent study has revealed that viruses in deep-sea cold seep sediments exhibit a rich repertoire of adaptive strategies to counter bacterial antiviral systems, including the encoding of anti-CRISPR (Acr) proteins and methyltransferases, along with the presence of reverse transcriptase (RT) enzymes that contribute to viral adaptability [110]. The counter-antiviral system carried by phages is also influenced by various factors such as environmental conditions and their hosts. For instance, some antibiotics that target protein translation can enhance bacterial defense to phages. For example, chloramphenicol, erythromycin, and tetracycline at minimum inhibitory concentration can interfere with the translation of anti-CRISPR protein (DMS3mvir-AcrIF1) to block bacterial CRISPR-Cas immunity [28]. However, our current understanding regarding the diversity of counter-antiviral systems and how environmental pressures directly impact their evolution remains limited.

Concluding remarks and future perspectives

On top of an understanding of the composition and diversity of phages in stressful environments, this review offers an ecological and evolutionary perspective on the adaptation of phages and their interactions with bacterial hosts in stressful environments. In low-biomass stressful environments, the prevalence of the lysogenic lifestyle in phages aligns with the '**piggyback the persistent**' hypothesis. This hypothesis posits that temperate phages assist their hosts in adaptation to stressful environments by modulating metabolism, resistance, and antiviral systems while ensuring the stable survival of their own genome – a mutualistic symbiosis with the host. Conversely, virulent phages under stressful conditions, as described by the '**kill the winner**' and '**constant diversity**' hypotheses, play a crucial role in promoting nutrient cycling, facilitating community evolution, and maintaining microbial diversity. More research is needed to gain a predictive understanding of how adverse environmental conditions (including physicochemical factors highlighted in this review) influence the ecological and evolutionary dynamics of phage–bacterium interactions at the community level. Moreover, it is important to develop breakthrough biological technologies that facilitate research on phage–host interactions and to utilize bioinformatics and artificial intelligence tools (holism) and laboratory experiments (reductionism) to better elucidate the

Outstanding questions

How can ecological network models and microbial interactions help to assess the ecological impact of phages in habitats under physiochemical stress?

How substantial is the influence of viral AMGs on bacterial responses to environmental stress and its role in facilitating nutrient cycling within ecosystems?

How do prokaryotic antiviral systems and counter-antiviral systems of phages evolve in response to environmental changes, and what are the ecological consequences?

What is the distribution and composition of RNA viruses in stressful environments, and how do they affect the microbial community ecology?

How do the genome features (e.g., genome size, GC content) of phages and their hosts change in response to environmental disturbance?

ecological mechanisms of phages in stressful environments. Addressing these questions (see Outstanding questions) will greatly contribute to our understanding of microbial stress adaptation mechanisms and ecological impacts under phage involvement, which is crucial for expanding basic knowledge and developing phage-based applications in natural and engineering systems.

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Declaration of interests

The authors have no interests to declare.

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