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Review

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# Bacteriophage-driven microbial phenotypic heterogeneity: ecological and biogeochemical importance



Xiaolong Liang[1] ✉, Shuo Yang[2], Mark Radosevich[3], Yongfeng Wang[1], Ning Duan[3] & Yongfeng Jia[1] ✉

Bacteriophages (phages) reprogram host metabolism and generate phenotypic heterogeneity, yet the mechanisms and ecological implications remain poorly understood representing a major knowledge gap in microbial ecology. This review explores how phage infection alters microbial physiology, contributes to single-cell variation, and influences population dynamics. We highlight the potential consequences of phage-driven heterogeneity for microbial community structure and biogeochemical cycling, underscoring the importance of integrating phage-host interactions into ecological and ecosystem models.

## Background

Microorganisms, with prominent abundance and diversity on Earth, play essential roles in biogeochemical cycles and ecosystem function. Microbial cells exist and function mostly in groups of varying complexity ranging from populations of genetically identical cells to complex communities consisting of diverse taxonomic groups[1,2]. Microbial community properties, such as productivity and resilience to environmental perturbations, are dependent on their genetic diversity[3]; however, the connection between microbial community properties and diversity remains a fundamental question to be discovered and explored. Recent research has revealed diverse metabolic phenotypes between genetically identical cells under homogeneous environmental conditions[4,5]. To achieve a better understanding of microbial ecology, advances in single-cell technology and phenotypic heterogeneity that occur within isogenic microbial populations have developed into a primary research focus in microbiology and quantitative biology.

Phages are infectious biological agents that only replicate within prokaryote cells using host cellular machinery. Phages are highly abundant in Earth's ecosystems with an estimated total abundance of $10^6$ globally[7,8]. Phage infection can lead to horizontal gene transfer, alteration of microbial metabolism, and host cell mortality[9,10]. The affected cellular-level functions add up to ecosystem impacts, as phage infections typically account for 20–40% of microbial mortality in oceans and are important in controlling microbial diversity and function[7,11]. Such a high prevalence of phage-infected microbial populations is a defining ecosystem feature, underscoring the pivotal role of phages in modulating microbial phenotypic heterogeneity through epigenetic modifications and regulatory shifts in cellular processes. Phage infections can contribute to phenotypic heterogeneity within microbial populations in ways that are not well understood, while also driving broader microbial diversification through integration of genetic material. Phage infections drive phenotypic heterogeneity in microbial populations via multiple mechanisms, including differences in infection status, phage life cycle strategies, and variability in infection progression. In natural microbial populations, not all cells become infected simultaneously[12]. This heterogeneous infection landscape creates metabolic and physiological diversity between infected and uninfected subpopulations, affecting cellular function, stress resilience, and population dynamics[13,14]. The outcome of phage infection in host phenotypes is largely determined by the infection mode, whether lytic or lysogenic. Lytic infections result in rapid phage production, driving immediate host population turnover. In contrast, lysogeny, where the phage genome integrates into the host chromosome, can contribute to long-term phenotypic heterogeneity[15,16]. Moreover, individual cells within a population may exist at different stages of infection, ranging from early-stage viral genome replication to imminent lysis or stable lysogeny. These dynamic infection processes create a spectrum of phenotypic states, shaping microbial interactions, evolutionary trajectories, and ecosystem functions.

While previous research demonstrated phage infection exposes top-down control over microbial communities and regulates host processes[15,16], the precise mechanisms through which phages reprogram host metabolism, induce phenotypic heterogeneity, and shape ecosystem functions remain poorly understood. This represents a major knowledge gap in microbial ecology. In this review, we summarize the key mechanisms by which phages influence microbial phenotypic heterogeneity and discuss their ecological and biogeochemical implications.

[1]CAS Key Laboratory of Forest Ecology and Silviculture, Key Laboratory of Pollution Ecology and Environmental Engineering, Institute of Applied Ecology, Chinese Academy of Sciences, 110016 Shenyang, China. [2]School of Life Sciences, Fudan University, 200433 Shanghai, China. [3]Department of Biosystems Engineering and Soil Science, The University of Tennessee, Knoxville, TN, 37996, USA. ✉e-mail: liangxlucas@outlook.com; yongfeng.jia@iae.ac.cn

## Mechanisms regulating phenotypic heterogeneity

Phenotypic differences between individual cells in clonal microbial groups were initially described in 1970s[17], and the concept of phenotypic heterogeneity shows that the individuals within an isogenic microbial population express dissimilar metabolic functions. Diverse molecular mechanisms might give rise to microbial phenotypic heterogeneity, and stochasticity in gene expression has been shown as a frequent cause of phenotypic diversity in metabolic functions[18]. The cell-cell heterogeneity known as an evolutionary strategy of bet hedging enables a population to be resilient to external perturbation[4]. Bet-hedging strategies in microbial populations arise as a response to unpredictable and fluctuating environmental conditions, where a single phenotypic state may not maximize long-term fitness. In bet-hedging, genetically identical cells stochastically express distinct phenotypes, ensuring that some subpopulations are pre-adapted to future stressors, even at the cost of reduced short-term growth or competitiveness[19,20]. Bet-hedging is particularly favored under rapid and unpredictable environmental shifts[21], preemptive stress preparedness[22], and constraints on regulatory adaptation[23].

With rapid advances in technology for single-cell analysis, research on microbial phenotypic heterogeneity has gained increasing attention with a particular emphasis on the prevalence, molecular mechanisms, consequences, and magnitude of such effects[24]. Studying individual host-phage systems is critical for understanding the molecular basis of microbial phenotypic heterogeneity. The molecular causes and ecological functions of microbial phenotypic heterogeneity were explored in some studies[25,26]. For example, Kiviet et al.[25] reported that stochastic gene (i.e., coding metabolic enzymes) expression can produce variations in the catalyzed metabolic reactions and propagate to cause fluctuations of instantaneous single-cell growth. In exploring the gene network architectures behind oxygen-dependent high variance of the trimethylamine oxide (TMAO) respiratory system in *Escherichia coli*, Carey et al.[26] discovered that IscR, an oxygen-sensitive transcription factor of two signaling genes (i.e., *torT* and *torS*), is the key regulator of this system. In general, IscR can bind to the region between *torT* and *torS* repressing the expression of these two genes in an oxygen-dependent manner. Transcription of TMAO reductase-encoding *torCAD* is regulated by TorT/TorS signaling system (TorT: TMAO receptor; TorS: a phosphatase when alone or the sensor kinase when combined with TorT-TMAO), and the repressed expression of *torT* and *torS* would increase the variance in *torCAD* expression which ultimately stimulate the phenotypic diversity among the population. Furthermore, the remarkable microenvironments that individual microbial cells of a population experience and respond to are complex giving rise to additional phenotypic variations[24]. Collectively, these findings underscore that phenotypic heterogeneity arises from the interplay between stochastic intracellular processes and microenvironmental variability, emphasizing the need to dissect both molecular regulatory networks and spatial ecological contexts to fully understand single-cell level diversity in microbial populations.

Microbes are most likely co-occurrent with more abundant infectious phages (dependent on their inhabiting environments), and viral infections should be critical factors affecting microbial activity in the microenvironments. Recent research has developed new approaches, e.g., nanometer-scale secondary ion mass spectrometry (NanoSIMS), single cell Raman spectroscopy, and single-cell microfluidic technology, for assessing phenotypic heterogeneity within isogenic microbial populations[5,27,28]. NanoSIMS coupled with stable isotope probing (nanoSIP) allows precise quantification of the isotope content in individual microbial cells and enables direct measurement of single-cell phenotype expression. While previous efforts in microbial phenotypic heterogeneity research have focused on the environmental drivers and molecular stochasticity[4,24], the role of phage infections that are widespread and associated with large fractions of microbial populations, have been overlooked.

## Critical roles of phages driving heterogeneous phenotypes

Phage-host interactions are typically addressed through community level analyses including -omics approaches (e.g., genomics, transcriptomics, proteomics, or metabolomics), stable isotope probing, and bulk characterization of phage-host abundance ratio and phage reproduction strategies[29–31]. These techniques provide an indispensable picture of the major processes occurring at the community scale; however, they are not capable of interpreting phage-host dynamics at single-cell level where phage-mediated metabolic transformation actually happens.

Phage-imposed regulation of host metabolism has consequences for carbon degradation and biogeochemical reaction rates[6,32–34]. While extensive research has explored impacts on microbial population dynamics and community-level processes, contrastingly little attention has been given to the precise mechanisms by which phages reprogram host metabolism at single-cell level. This knowledge gap is critical, as phage-induced metabolic reprogramming can create substantial phenotypic heterogeneity among infected cells while also driving genetic and functional diversification within clonal bacterial populations, ultimately shaping ecological interactions and evolutionary trajectories. Investigating how phage infections drive single-cell phenotypic variability provides a novel framework for exploring microbial ecology, with implications for predicting microbial functional responses in dynamic environments[35,36]. Here, we propose that phage infection serves as a fundamental driver of cell-to-cell metabolic heterogeneity, necessitating a reevaluation of phage-host interactions beyond traditional population and community-level perspectives.

To incorporate phages into ecological and biogeochemical models, the following key questions remain to be answered. How do phages reprogram host metabolism and impact cellular outputs? How does phage infection within the community affect the interaction network and productivity in ecosystems? To what extent are phages driving microbial phenotypic heterogeneity and affecting biogeochemical cycles? How does variability in host metabolism change susceptibility to phage infection?

We can better understand phage-induced phenotypic heterogeneity and its ecological implications by using single-cell analytical tools that resolve viral infections at subpopulation resolution (Fig. 1). Techniques such as nanoscale secondary ion mass spectrometry (NanoSIMS), stable isotope probing (SIP), bioorthogonal non-canonical amino acid tagging (BONCAT), and fluorescence or halogen in situ hybridization (FISH/HISH) have emerged as powerful approaches to map host metabolism and viral activity in complex microbial communities. For example, BONCAT-FISH has been used to identify translationally active bacterial subpopulations during phage infection, revealing how infection alters protein synthesis and nutrient assimilation[37,38]. Similarly, NanoSIMS-SIP studies have shown that phage-infected cells exhibit altered carbon and nitrogen uptake relative to uninfected neighbors, highlighting infection-driven shifts in elemental cycling[35,39,40]. Microfluidics-based single-cell tracking has also revealed how infection heterogeneity affects bacterial fate decisions under nutrient limitation, providing dynamic insights into survival strategies and metabolic trade-offs[41].

Despite their promise, these approaches remain underutilized in phage ecology, especially in structured habitats such as soils and biofilms where physical microenvironments and diffusion barriers shape infection dynamics. Applying these single-cell technologies in tandem with metagenomics and viral tagging could link phage-host interaction variability to ecosystem-scale processes such as nutrient flux, community resilience, and biogeochemical feedback. Integrating high-resolution single-cell phenotyping with ecological modeling frameworks is essential for advancing our ability to predict microbial community responses to environmental change in virus-rich ecosystems. Such integration will not only fill critical knowledge gaps but also enable quantitative assessments of how phage infections shape microbial functional traits, influence community stability, and regulate ecosystem-level processes. Together, these efforts define a promising frontier for elucidating the ecological significance of phage-driven phenotypic heterogeneity across diverse environmental contexts.

Phages are known to preferentially infect actively growing cells, as successful replication often depends on access to abundant nucleotides, ATP, and intact translation machinery[34]. Consequently, cells with slower growth or reduced metabolic activity may evade infection altogether or

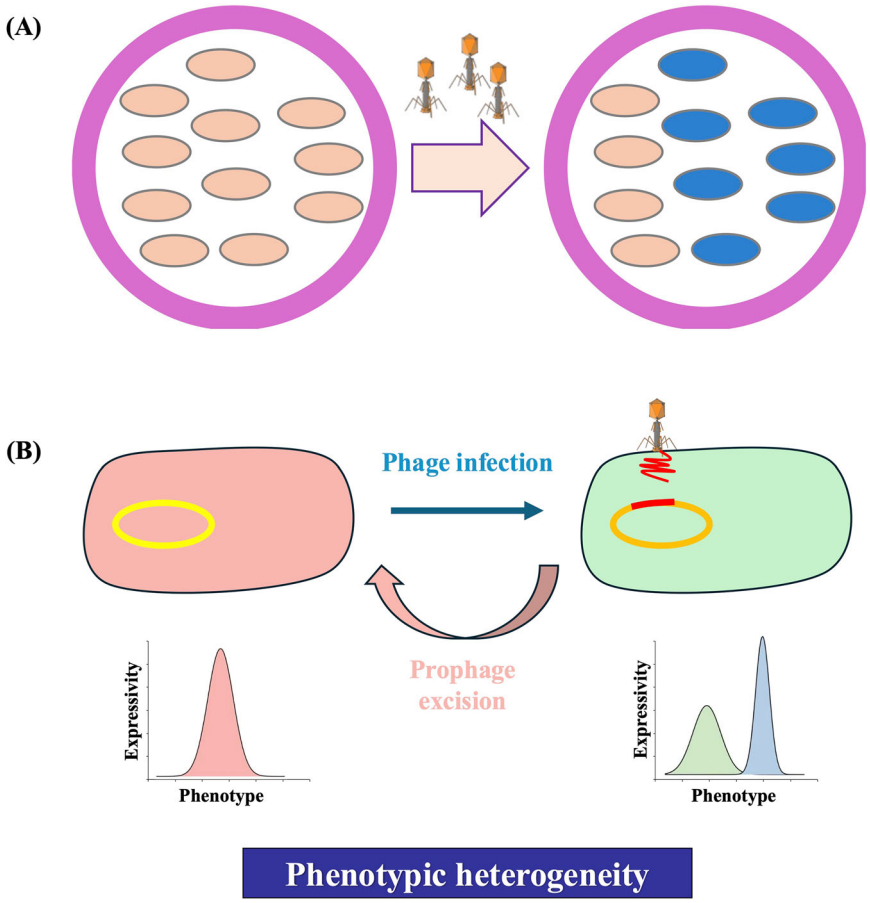


**Fig. 1 | Phage-induced microbial phenotypic heterogeneity. A** In the absence of phage infection, bacterial cells exhibit phenotypic variation that arises primarily from intrinsic stochasticity in gene expression and regulatory noise. **B** Phage infection —whether through lysogeny (prophage integration, indicated by the red genomic region) or active lytic replication (illustrated by the cartoon phage)— modifies host gene regulation, leading to a bimodal phenotypic distribution. This heterogeneity arises due to differences between lysogens and non-lysogens, variability in prophage induction, or phage-mediated metabolic reprogramming. The resulting bimodal pattern exemplifies a bet-hedging strategy, where subpopulations adopt distinct phenotypic states. However, the impact of phage infection on host metabolic regulation is context-dependent; for instance, Carey et al.[42] showed that phage infection suppressed bet-hedging, illustrating that the regulatory outcomes of phage-host interactions can vary widely across systems, with some phages enhancing and others constraining phenotypic heterogeneity.

delay phage replication, contributing to phenotypic heterogeneity in infection outcomes. For instance, recent single-cell studies using time-lapse microscopy and microfluidics have shown that cells in a low metabolic state exhibit delayed lysis or enter pseudolysogenic states, allowing them to survive transient phage exposure under nutrient-limited conditions[42]. Moreover, metabolic traits influence phage adsorption and post-entry fate. Specific surface receptors required for phage binding are often linked to nutrient transport systems, which are regulated according to metabolic status[43]. This creates feedback loops where host physiology governs not only entry but also the trajectory of infection, i.e., lytic, lysogenic, or abortive. In structured environments such as biofilms, metabolic gradients generate spatial heterogeneity in phage susceptibility, resulting in infection hotspots among metabolically active cells and refugia for slower-growing ones. These insights emphasize that metabolic variability is not merely a byproduct of environmental fluctuation but a critical regulator of phage-host dynamics with direct implications for infection efficiency, viral propagation, and population stability.

## Metabolic regulation via prophage interruption of host gene expression

Temperate phages integrate their genomes into host chromosome as prophages in lysogenic cycles and potentially regulate host gene expression. Prophages are prevalent in bacterial genomes and can constitute 10 to 20% of the host genome[44,45]. Prophage integration may occur directly within a gene and cause destruction of the host gene function or take part in host gene regulation. During lysogeny of λ phage in *E. coli*, the λ-coded lytic-repressing cI protein specifically represses the expression of the *pckA* gene encoding phosphoenolpyruvate carboxykinase, an essential enzyme for gluconeogenesis[46]. The cI-repressing activity lowers bacterial growth rate and may benefit the host under nutrient poor conditions (Fig. 2A).

Microbial cells exploit the central metabolic pathways for carbon catabolism and energy output, but the regulation process may be heterogeneous between cells as alternative strategies for a population to overcome fluctuating conditions. Following the investigation of heterogeneity in *E. coli* TMAO respiratory strategy mentioned above[26], Carey et al.[47] showed that a temperate bacteriophage HK022 disrupts the genes *torT* and *torS* by integrating between them and shuts off aerobic transcription of *torCAD* eliminating the above-mentioned bet hedging strategy of TMAO respiration in *E. coli* (Fig. 2B). The mechanism of phage HK022 altering host respiration strategy is that the integration of prophage HK022 in host genome substantially elevates *torS* expression without changing the expression of *torT*. The excessive TorS not binding to TorT would specifically dephosphorylate TorR leading to aerobic repression of *torCAD*. The authors also demonstrated that the phage-exerted effect is upregulating as the transcription of *torS* in HK022 lysogen originates from within the prophage. This study illustrates how prophages can modulate host gene expression leading to alterations in metabolic pathways and physiological traits that influence host fitness under specific environmental conditions. In some cases, these phage-mediated effects may confer advantages, such as increased survival or metabolic flexibility, particularly in fluctuating environments where bet-hedging strategies allow populations to endure unpredictable stresses[19,20]. For example, prophage integration can enhance resistance to environmental stressors by regulating host metabolic functions, as seen in *E. coli* where phage HK022 represses *torCAD*, altering respiratory strategies[47]. However, such phenotypic heterogeneity does not universally translate to a fitness benefit. In some instances, prophage-encoded regulatory modifications may impose metabolic costs or constrain adaptive potential, leading to trade-offs in population dynamics. Therefore,

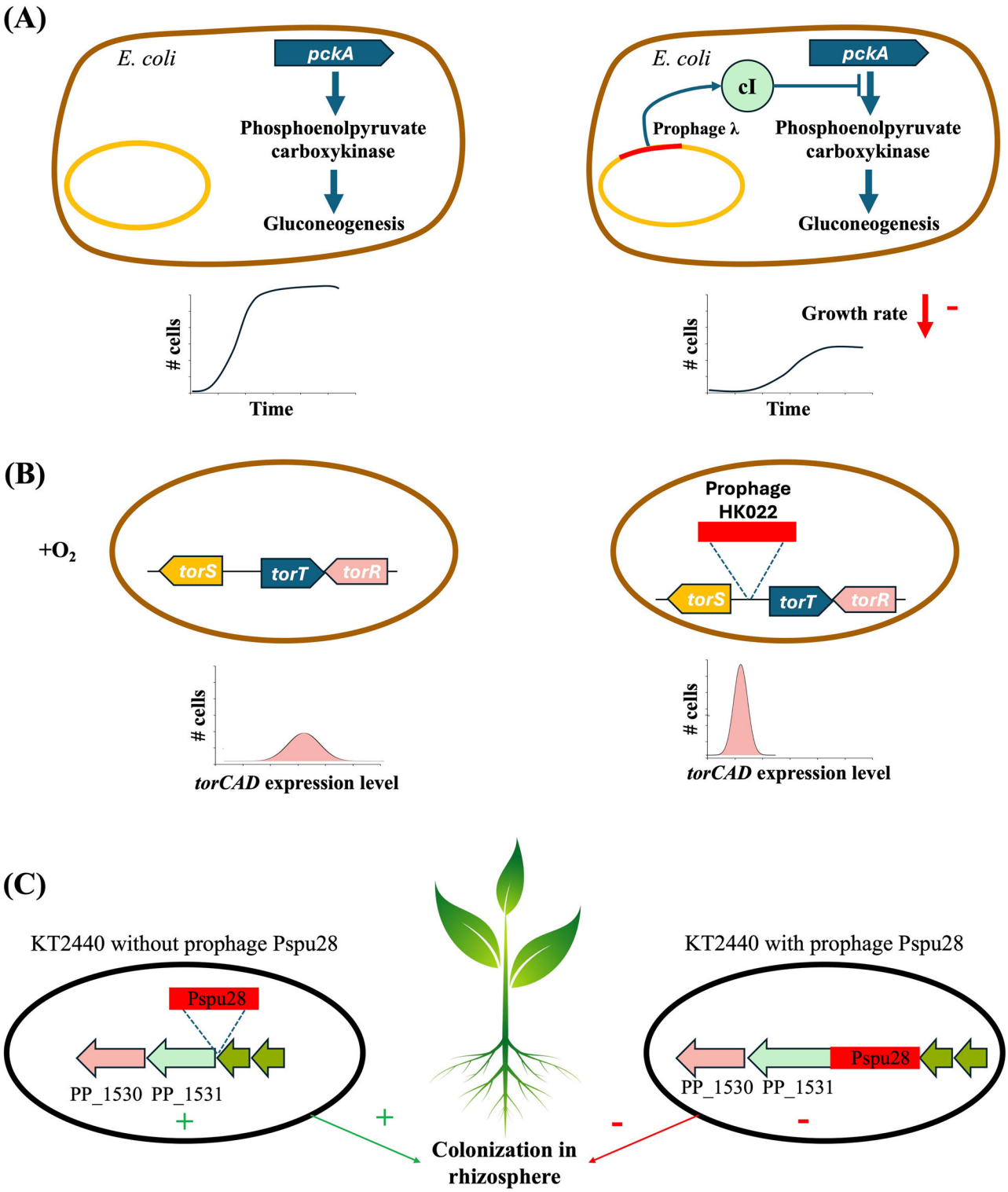


**Fig. 2 | Schematic of phages causing host phenotype variations. A** Lysogeny of λ phage represses the expression of the *pckA* gene in *E. coli* leading to lower cellular growth rate. **B** The integration of prophage HK022 in host genome causes aerobic repression of *torCAD*. **C** The excision of prophage Pspu28 increases the expression level of the neighboring PP_1531 gene and benefits host colonization in rhizosphere.

while prophages can contribute to microbial diversification and ecological resilience, their role in host fitness is context-dependent, requiring further empirical studies to delineate the selective advantages and constraints imposed by phage-host interactions.

Excision of prophages from lysogenic bacterial populations can occur stochastically and may dramatically alter the host cell performance and their interactions with the environment. KT2440 resides in the rhizosphere and has versatile metabolism. Multiple genomic islands and prophages are present in a rhizobacterial strain, *Pseudomonas putida* KT2440[48], and a prophage of 39.4 kb containing 53 open reading frames named Pspu28 was postulated to be associated with host population fitness[49]. Pspu28 can be excised from host genome induced by an excisionase, and the excised phages

coexist with the integrated prophages in KT2440 populations. The excision of prophage Pspu28 significantly increases the expression level of the neighboring gene, the promoter of PP_1531, encoding a predicted arsenate reductase (Fig. 2C). Though the regulatory mechanism is not resolved, integration/excision of Pspu28 may represent a living strategy for KT2440 in the rhizosphere. The loss of Pspu28 from host genome increased cellular fitness of KT2440 under intraspecific competition in rhizosphere.

In a comprehensive study of *Staphylococcus pseudintermedius* genomic analysis, Brooks et al.[50] showed that prophage SpST71A disrupted a competence operon *comG*, essential for natural genetic competence, in ST71 clone, and the strains carrying a prophage-interrupted *comG* or horizontal gene transfer restricting CRISPR/Cas had less nucleotide diversity and exhibited lower recombination rates than strains not containing these systems.

## Metabolic regulation by prophage excision

Prophages may function as a symbiont and benefit their host cells in providing innovative phenotypes. By precise deletion of all prophages in *E. coli*, Wang et al.[51] revealed the significant functions of prophages enhancing cell growth, increasing biofilm formation, strengthening resistance to quinolone and β-lactam antibiotics, and overcoming osmotic, oxidative and acid stresses. Recent studies have shown reversible lysogeny in diverse bacterial populations via integration and excision of phage genomes from host chromosomes[52]. Bacterial strains utilize the prophage excision system as a typical survival strategy under certain challenging conditions.

Wang et al.[53] investigated the associations between prophages and host physiology in *E. coli*, especially how CP4-57 prophage excision in *E. coli* biofilms is regulated and impacts host phenotypes. The results showed that Hha, a DNA-binding protein, can induce expression of excision genes and reduce tmRNA Ssra transcription which altogether induce prophage CP4-57 excision in early biofilm development. The elimination of CP4-57 induces mobility and represses carbohydrate metabolism leading to reduced cell growth. A whole-transcriptome analysis revealed that prophage CP4-57 excision activated flagella-related genes and repressed the expression of key enzymes involved in the tricarboxylic acid cycle and required for lactate utilization. This study showed a diversified population with a low frequency, around $10^{-4}$, of prophage excision in biofilm formation leading to specialized functions of mobility and carbon metabolism.

The competence (Com) system in the intracellular pathogen *Listeria monocytogenes* is deactivated, as a temperate phage is integrated into the Com master activator gene, *comK*, which disrupts the function of Com system. However, the study by Robinovich et al.[54] found that prophage excision is induced during intracellular (primarily in phagosomes) growth of *L. monocytogenes*, without producing progeny phages, allowing the restoration of a functional *comK* gene. The prophage excision event activated Com system, which facilitated intracellular growth and enhanced bacterial escape from phagosomes. Notably, the authors observed that approximately 80% of intracellular *L. monocytogenes* expressed *com* genes, generating population-level phenotypic heterogeneity.

In many cases of lysogeny, phage genomes integrate into bacterial chromosome regions within or near host genes, and the subsequent excision of prophages can activate or disrupt host gene expression, thereby contributing to phenotypic variability. In probing the DNA-level mechanism for cold tolerance of *Shewanella oneidensis* by whole-genome deep-sequencing, Zeng et al.[55] discovered that the cold adaptation is regulated by the excision of a novel P4-like cryptic prophage named CP4So. The authors observed a 10000-fold increase of the cryptic prophage excision in a small fraction (0.1–3%) of the bacterial population induced by temperature downshift, and interestingly, the small group of generated prophage-free cells were able to enhance biofilm formation and promote survival of the entire population. This study also revealed the molecular regulatory system in which the histone-like nucleoid-structuring protein (H-NS) acts as repressor of CP4So excision by binding to the promoter of the putative excisionase gene, and the H-NS level at cold temperatures was reduced resulting from de-repression of CP4So excision. All these cases demonstrate prophages capable of functioning as host to a DNA regulatory element.

## Defining phage-mediated phenotypic heterogeneity in an ecological context

Phage-mediated phenotypic heterogeneity arises when genetically identical microbial cells exhibit divergent infection outcomes and physiological responses following phage exposure. Phage-driven phenotypic heterogeneity manifests not only within individual hosts but also scales across microbial populations and ecosystems. In natural environments, genetically identical bacterial populations often exhibit divergent infection outcomes due to differences in metabolic activity, stress responses, and local microenvironments. These differences, derived from various molecular mechanisms, including stochastic gene expression[56], epigenetic modifications[19], and phage-induced metabolic reprogramming[47], can lead to variable fates of phage infection. This heterogeneity, although stochastic at the single-cell level, creates structured variability in population function and resilience. For example, de Groot et al.[56] demonstrated that bacterial populations employ growth rate-dependent stability as a bet-hedging strategy, enabling subpopulations to maintain distinct phenotypic states that enhance survival under fluctuating environmental conditions. In *E. coli*, metabolic pathway differentiation under variable oxygen conditions enables subsets of the population to thrive in distinct environmental states[26]. Similarly, in *Vibrio cholerae*, phage-mediated lysogeny contributes to population stability by maintaining a balance between susceptible and resistant subpopulations, mitigating the impacts of phage predation[57]. These outcomes can co-occur within the same population, driven by variation in cellular metabolic state, spatial context, phage genotype, and environmental stressors[11,58].

In structured environments like biofilms and soil aggregates, the spatial heterogeneity of resources and redox conditions gives rise to diverse metabolic states, which in turn modulate phage susceptibility. For instance, nutrient-limited or slow-growing cells often evade lytic infection, frequently escape lytic phage infection, serving as reservoirs for phage-tolerant phenotypes or carriers of inducible prophages that contribute to latent viral persistence within microbial communities[52]. Concurrently, metabolically active microsites, characterized by high microbial turnover, can become hot spots for intensified viral replication and accelerated host cell lysis. These spatially resolved infection patterns introduce functional stratification within microbial communities, which influences carbon turnover, nutrient release, and the timing of microbial succession.

Recent research has revealed that phage infection not only eliminates specific microbial taxa but also drives subpopulation-level differentiation through expression of auxiliary metabolic genes (AMGs). These genes carried by temperate or lytic phages encode functions related to carbon metabolism, phosphorus acquisition, nitrogen cycling, and oxidative stress responses, and can temporarily reprogram host metabolism during infection[9,36]. Importantly, AMGs are not uniformly expressed across all infected cells; instead, their activity contributes to phenotypic mosaics, where different infection outcomes within the same genotype lead to variable ecological roles.

Experimental evidence is beginning to show that this heterogeneity has measurable effects on ecosystem processes. For example, in marine mesocosms, viral activity has been shown to influence net primary production by modulating the turnover of phototrophic microbial populations[36]. In soil systems, viral predation and lysogeny alter nitrogen availability and microbial carbon use efficiency[59,60], suggesting a broader ecological role for phage-induced heterogeneity in terrestrial biogeochemical cycling.

The ecological implications of phage-mediated phenotypic heterogeneity are profound. By modulating host gene expression and metabolism at the single-cell level, phages influence microbial population structure, species interactions, and biogeochemical processes. This heterogeneity may provide microbial communities with greater resilience to environmental fluctuations and selective pressures, contributing to ecosystem stability.

Methodologically, the field is beginning to bridge single-cell resolution with ecological scale using integrated tools. As discussed in the section on the critical roles of phages in driving phenotypic heterogeneity, advanced single-cell approaches such as NanoSIMS-SIP and BONCAT-FISH have enabled in situ resolution of phage-infected subpopulations, revealing altered nutrient assimilation patterns at the microscale[38]. Complementary tools, including fluorescence-labeled viral probes (e.g., viralFISH) and single-cell genomics, are further uncovering the diverse infection trajectories that remain obscured in bulk community-level analyses. Additionally, time-resolved microfluidics and high-throughput live-cell imaging platforms now facilitate real-time monitoring of infection dynamics, uncovering stochastic switching between phage susceptibility and resistance[41].

Despite these advances, the functional implications of phage-induced phenotypic heterogeneity remain only partially resolved. The extent to which such heterogeneity influences microbial interactions, resource partitioning, and emergent community dynamics is still poorly characterized. In particular, the role of infection-driven functional diversity in shaping cooperation, competition, and microbial network stability is largely unexplored. Similarly, the impact of subpopulation-level variability on ecosystem resilience—especially under environmental stressors such as nutrient pulses, drought, or climate-driven shifts—remains an open question. To address these gaps, future efforts must integrate high-resolution molecular and imaging techniques with quantitative biogeochemical assays and trait-based or systems-level ecological models. Such interdisciplinary approaches are essential for uncovering the ecological and evolutionary consequences of phage-driven heterogeneity across spatial and temporal scales.

Phage-mediated phenotypic heterogeneity thus represents more than a mechanistic curiosity; it is an organizing principle in microbial ecology. It drives within-species diversity, enables population persistence under phage pressure, and modulates the flow of energy and matter through microbial food webs. Continued efforts to resolve this heterogeneity at scale will be critical for understanding and forecasting the behavior of microbiomes under environmental change.

## Metabolic regulation by phage-encoded auxiliary metabolic genes (AMGs)

Phages may obtain host genes during infection, and the functional host-originated genes, called AMGs, can modulate host cell metabolism in subsequent infections. AMGs have been widely detected in viromes[61,62], and the expression of AMGs in host cells boosts a variety of activities such as carbohydrate metabolism[63], sulfur oxidation[62], nitrification[64], and the metabolism of methane[65].

Phage-introduced AMGs are important for host functions especially in adverse environments, and the expression level of AMGs is usually much higher than the host-encoded gene copies. A whole transcriptome sequencing (RNA-seq) study of responses of P limitation-stressed marine cyanobacterium, *Prochlorococcus* NATL2A, to phage (cyanomyovirus P-SSM2) infection showed that phage infection elevated the transcripts of adenosine triphosphate (ATP) synthase and ribosomal protein genes which were exhausted in uninfected P-starved cells[66]. As Cyanophage P-SSM2 contains a P-acquiring gene, *pstS* encoding high-affinity phosphate-binding protein, the phage *pstS* transcript was 20-fold more abundant than the host copy during phage infection. Besides augmentation of host functions and introduction of new functionality, the phage-derived metabolites may function as regulatory elements in the host and shift metabolic flux. Thompson et al.[67] discovered that cyanophages carry a gene for the Calvin cycle inhibitor, CP12, which also exists in cyanobacteria and can direct carbon flux from glucose synthesis to the pentose phosphate pathway (PPP) by inhibiting two key enzymes in the Calvin cycle. Expression of phage CP12 together with other phage genes involved in photosynthetic light reactions and PPP facilitated light and PPP reaction activity and increased NADPH production which likely supported phage reproduction in the host cells.

In lytic replications, the expression of phage AMGs is also dominant compared with host genes of similar functions, and metabolic activities in host cells are transformed for productions of progeny phages. Waldbauer et al.[68] explored the biosynthetic processes in phage-infected cells using an isotope-labeling proteomics approach (i.e., supply of $^{15}$N-labeled extracellular N post-infection) and the authors found that up to 41% of nitrogen (N) in progeny phage proteins was derived from extracellular N while nearly none of the host protein showed isotope incorporation. The authors also discovered some phage-derived AMGs, and AMGs-related proteins were produced de novo. Together, these results suggest selectively active metabolism in host cells, such as phage component synthesis and AMG-related processes, during infection.

## Metabolic regulation by phage-host antagonistic coexistence

Infection by bacterial phages may induce substantial changes of metabolism in phage-infected microbial cells (also termed virocells) and affect the ecosystem in a drastically different way compared with uninfected cells[69,70]. Considering the extremely high phage infection rates among microbial populations, virocells are predominant in a wide range of ecosystems on earth and substantially influence global biogeochemistry[31,71]. However, research concerning the mechanisms of phage-directed metabolic reprograming in virocells and their ecological consequences is still rare.

Phage-host experiments using model systems have proven to be an effective way for filling the knowledge gaps[36,70]. An examination of the impacts of lytic phage infection on the intracellular metabolism in *Pseudomonas aeruginosa* cells through high coverage metabolomics analysis showed that phage infection actively transformed the host physiology (i.e., an increase in pyrimidine and nucleotide sugar metabolism) through phage-specific depletion of host resources, reprogramming the host metabolism and phage-encoded auxiliary metabolic genes[32]. A recent work by Howard-Varona et al.[34] investigated the function of unrelated phages, siphovirus PSA-HS2 and podovirus PSA-HP1, in reprograming host metabolism in contrasting virocells by infecting a marine *Pseudoalteromonas* bacterium independently. Temporal-scale multi-omics revealed clear evidence of HS2 and PP1 in reprogramming metabolism and resource acquisition in their respective corresponding virocells. HS2 infection repressed high energy-consuming metabolism, including motility and translation, while HP1 infection restrained host transcription and reprogrammed the central carbon and energy metabolism (i.e., synthesis of sulfur-rich amino acids thereafter degraded via the glyoxylate-TCA cycle) for enhancing translation in virocells. Together these studies consistently demonstrate that the gene expression in virocells is remarkably different, and most host genes are down-regulated compared to cells without phage infection in the same population throughout different stages of infection.

While phage infection has non-negligible roles in transforming host transcription and metabolism, the specific responses of the hosts vary among phage-host pairs[34,72]. Host cells can acquire phage resistance and modulate phage infection[73]. The developed host resistance to infecting phages is always concomitant with modified phenotypes. A chemostat experiment incubating *Cellulophaga baltica* strain MM#3 with two lytic phages, $FS_M$ and $FS_T$, was performed to investigate the strain development in response to the phage activity[6]. A large population witnessed an increased phage resistance during 3 weeks of incubation. The BIOLOG assay revealed a diversification of the metabolic properties among the population, as the phage resistance was accompanied with a reduction in the strains' capacity of metabolizing various carbon sources. Thus, phage infections in microbes, even within a clonally derived population, can induce the emergence of phage-resistant variants through genetic mutations or other heritable changes. This process occurs alongside modifications in physiological properties that contribute to phenotypic heterogeneity among infected and uninfected cells. However, phage-induced phenotypic heterogeneity reflects transient, non-heritable variations in cellular states that can influence population dynamics.

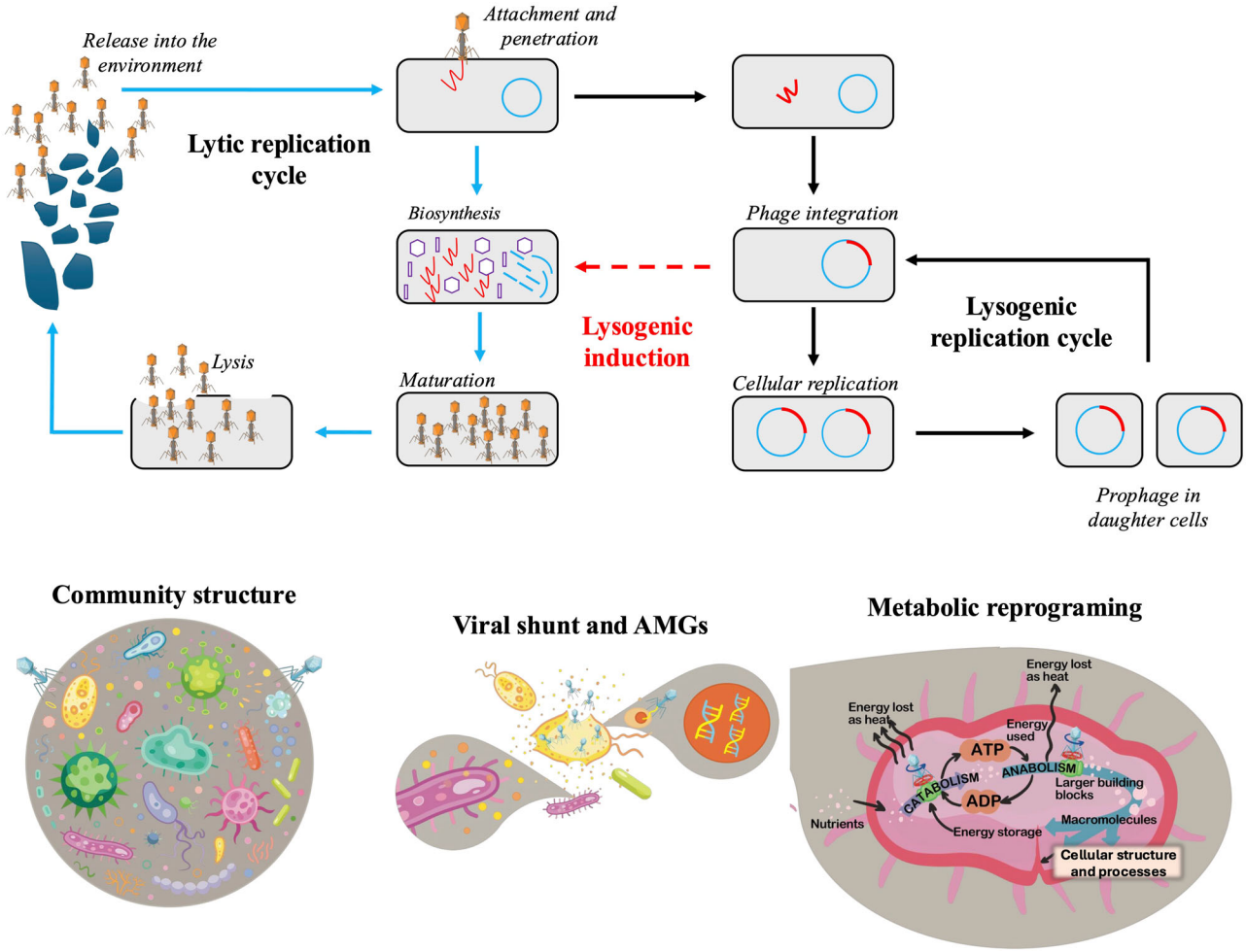


**Fig. 3 | Schematic of phage replication cycles and impacts on microbial phenotypes.** Phages are obligate parasites of host cells, they mainly have two types of reproduction, lytic and lysogenic. In lytic cycles, the phage binds to the cell surface; injects its genome into the cytoplasm; the viral genome replicates and produces other component parts; assembly new phages and lysis of the host. In lysogenic replication, the viral genome integrates into host genome after attachment and injection and is stably maintained as a prophage. The prophage replicates as the host cell grows and divides. The lysogenic state can be converted to the lytic pathway which is induced by viral/host regulatory system under various environmental signals.

## Phage-host interactions and phenotypic heterogeneity in structured microbial communities

Phages and hosts battle consistently for survival displaying and array of defense and counter-defense mechanisms. Lytic phages such as T4 phage invade their host leading to acute replication of themselves and ultimate lysis of host. Temperate phages such as lambda (λ) phage either enter lytic cycles to lyse the host or integrate into host genome as prophages (Fig. 3), and the integration and excision is regulated by both phage- and host-encoded genes[55,74]. Prophages can be triggered into lytic cycles when the host cell is under physiological stress. The association between phages and hosts can be very complicated and is regulated by a variety of factors such as environmental stresses, host physiological state, genetic resistance, and the nature of phages[75–77]. For example, high bacterial growth rates are usually accompanied with high phage abundance and burst size, possibly due to the sensitivity of phage replication to host translation machinery and intracellular resources for phage biosynthesis and assembly. The bacterial growth rate also plays a critical role in survival against phage infection, and phage-host interactions have important consequences in the phenotypic composition of a bacterial population and should be considered when studying bacterial population dynamics[76]. Bacterial adaptive and innate immune systems (e.g., CRISPR-Cas and restriction-modification systems) and phage anti-defense strategies for overcoming host immunity evolve coincidently in natural environments and under environmental settings, and the antagonistic phage-host coevolution can lead to extensive variations in bacterial phenotypes[78,79]. Microbial communities frequently inhabit spatially structured environments, such as biofilms, where gradients in nutrients, signaling molecules, and cell density create heterogeneous microenvironments that modulate phage-host interactions and amplify phenotypic variability at the single-cell level.

Biofilms are spatially structured microbial communities embedded in a self-produced extracellular matrix, and biofilm formation is a common strategy used by microbial communities to adapt to hostile conditions, influences local environmental conditions, phage mobility, and infection dynamics[80]. The biofilms possess distinct environmental properties than the outer space, and the mobility of phages and the susceptibility of bacterial populations to phage infection are markedly influenced within biofilms. The phage-host interactions also play an immense role in biofilm formation or removal. For example, phage genomes encode lysis-causing endolysins and polysaccharides-, exopolysaccharides-, or lipopolysaccharides-degrading depolymerases that disrupt the biofilm matrix[81]. Though mostly functioning as predators of microbial populations, phages engage in diverse symbiotic relationships driving biofilm formation or strengthening biofilms via

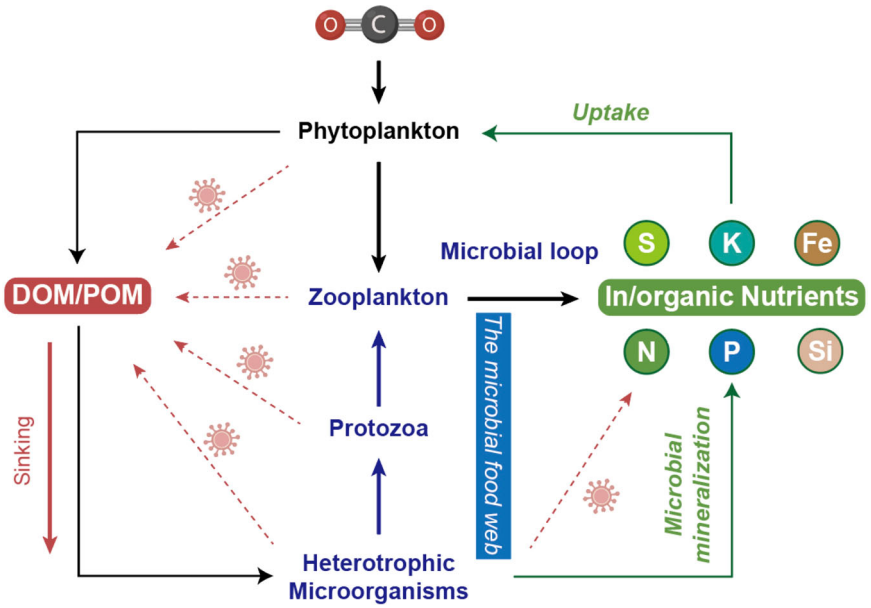


**Fig. 4 | Viral shunt and microbial loop in aquatic ecosystem.** Phytoplankton release available C as exudates in form of dissolved organic matter (DOM) into the environment which stimulates microbial growth and enzyme production. Heterotrophic microorganisms utilize and transform organic matter into in/organic nutrients for phytoplankton. Predatory animals feed on microorganisms and release mineralized products contributing to the pool. In the meantime, almost all organisms of primary producers and primary consumers can be infected and lysed by phages. The impact of the viral lysis of high percentages of microbial production is that it shunts large fractions of carbon and nutrients back to soluble pool instead of going through the food web and stimulates nutrient uptake by heterotrophic microorganisms and phytoplankton. The cell debris generated from viral lysis also contribute to the particulate organic matter (POM) pool for microbial mineralization and potential carbon deposition.

lysogenic activities[51,55] and by contributing to cell-lysis sensing[82]. These represent only a subset of the ways in which phages and bacterial hosts can establish symbiotic relationships, as emerging research continues to reveal additional mechanisms by which phages shape microbial functions, ecological dynamics, and evolutionary trajectories. The formation of biofilms is closely related to microbial population density-based cell-to-cell communications, such as quorum sensing (QS), as QS autoinducers can modulate the expression of biofilm-producing genes. The linkage between phage reproduction strategy and bacterial QS was recently revealed which has brought immense interest and efforts in this exciting research direction. Ghosh et al.[83] demonstrated the first experimental evidence of cell-density dependent prophage induction linked to QS systems via a study performed using soil and groundwater bacteria and a model *E. coli* system. Silpe and Bassler[57] elucidated the first regulatory mechanism controlling QS-mediated lysis-lysogeny decision of the vibriophage, VP882, in *Vibrio cholerae*. Recent research by Tan et al.[84] discovered that *Vibrio anguillarum* QS can regulate the lysogeny-lysis transition of the H20-like prophage in host cells thus modulating host physiology. The authors demonstrated that the host QS activity under high cell density repressed induction of the H20-like prophage. The abundance ratio of free phage particles to host cells increased eightfold in cultures of the QS-deficient *ΔvanT* mutant relative to wild-type cultures at high cell density which was caused by the repressed prophage induction by host QS. Host QS repressed biofilm formation, while the H20-like prophage stimulated host biofilm formation. Finally, both biofilm formation and prophage induction were repressed by QS at high cell density, while proteolytic activity of the host strain was positively regulated by QS system[84]. These intricate interactions among phages, QS, and biofilm formation can generate phenotypic heterogeneity across spatial and temporal scales within microbial communities. This variability can significantly impact biogeochemical processes by modulating microbial functional traits, shifting community structure, and altering the dynamics of nutrient cycling in structured environments.

## Biogeochemical implications of phage-driven processes

Previous research has demonstrated the significance of phages in regulating microbial community composition, ecosystem processes, and biogeochemical cycling[36,84–86]. Phages exert top-down control over microbial abundance and diversity via selective mortality of microbial hosts. The phage functionality extends beyond host mortality, fundamentally altering the flow of organic matter and nutrients within ecosystems. Phage lysis of microbial cells releases intracellular components, such as amino acids, nucleotides, and phosphorylated compounds, that can be readily assimilated by other living microorganisms in the system; a process known as the viral shunt[87,88] (Fig. 4). Phage-mediated cell lysis shunts large fractions of carbon and nutrients back to the soluble nutrient pool instead of going through the food web, stimulating nutrient uptake by heterotrophic microorganisms and primary producers[86].

Recent high-resolution biogeochemical modeling and isotopic labeling studies have demonstrated that phage-mediated lysis can regenerate up to 40% of microbial biomass daily in productive ocean regions and soils, significantly accelerating turnover of labile organic matter and enhancing microbial nutrient acquisition[1,89,90]. Beyond DOM, lysed cells contribute necromass, macromolecular components including lipids, peptidoglycans, and nucleic acids, that enrich the particulate organic matter (POM) pool. The necromass not only fuels microbial decomposition and secondary production but also interacts with soil and sediment matrices to promote long-term carbon stabilization, especially under mineral-associated and low-oxygen conditions[91–93]. Furthermore, viral lysis can release iron, sulfur, and phosphorus-containing molecules that influence redox-sensitive cycles in both marine and terrestrial systems, thereby linking phage activity to broader biogeochemical feedbacks[1,94]. The very high percentages of microbial production neutralized by phage lysis each day suggest substantial function of phage lysis in biogeochemical cycles[88]. These findings underscore that phage-mediated processes operate at scales that are ecologically and geochemically consequential. However, quantifying the spatial and temporal variability of these effects remains an important frontier for both experimental and modeling studies.

Unlike lytic life cycles, temperate phages can enter lysogeny as a prophage for an indefinite period[95]. Prophages as large DNA insertions in the host chromosome may disrupt host gene expression, introduce new functions through lysogenic conversion, and serve as regulatory switches of host genes via genome excision[52,96], yet little is known how phage infection changes host phenotypes during the lysogenic cycle. Metagenomics has revealed exceedingly high biological diversity of phage communities and a wide range of phage-coding potential functionalities[97,98]. Phages can transform their infected host cell metabolism partly through phage-encoded metabolic genes[36,99]. In search for phage-encoding carbon metabolism genes from the Pacific Ocean Virome (POV) dataset, Hurwitz et al.[61] recovered 35 phage gene families with potential for reprogramming central metabolic pathways and modulating carbon flux within and between cells. By studying the growth dynamics and metabolism of roseophage-infected *Sulfitobacter* sp. 2047, Ankrah et al.[100] revealed that phage infection significantly elevated

the metabolic activity and most metabolites in the host cells in which ~75% of total nutrients were redirected into virions. Considering the immediate and substantial phage-imposed interference on host metabolism, along with the stochastic nature of infection among genetically identical populations, phages contribute to microbial phenotypic heterogeneity both within infected subpopulations and between infected and uninfected cells. This heterogeneity arises from variations in metabolic reprogramming among infected cells, as well as broader population-level diversification resulting from differences in infection status.

During lytic infection, phages exert substantial and immediate control over host metabolism by hijacking transcriptional and translational processes to prioritize viral replication. Specifically, they can repress central metabolic pathways, alter carbon and nitrogen fluxes, or redirect energy toward phage biosynthesis[32,34]. This metabolic reprogramming is further modulated by host physiological state and environmental context, contributing to phenotypic heterogeneity both among infected cells and between infected and uninfected subpopulations[101,102]. The inherently stochastic nature of infection dynamics, combined with differences in host metabolic readiness, amplifies this variation, leading to functionally diverse microbial subgroups that differ in nutrient uptake, growth efficiency, and stress responses. Despite growing evidence of these effects, most biogeochemical models still lack parameters that reflect phage-induced metabolic variability. Incorporating this heterogeneity, especially through trait-based or population-structured modeling approaches, could improve predictions of nutrient cycling and ecosystem resilience[103,104]. While some ecosystem models have begun to include viral lysis and mortality effects, future efforts should integrate the metabolic and phenotypic consequences of infection at the single-cell level to better capture the ecological roles of phages.

### Outlook

Recent studies underscore the ecological importance of microbial phenotypic heterogeneity, yet its underlying drivers remain insufficiently understood. This review highlights the diverse mechanisms by which phages contribute to such heterogeneity, including lysogeny, metabolic reprogramming, and variability in infection dynamics. These processes generate functional diversity both between infected and uninfected cells and among cells undergoing different infection states. Phage-driven heterogeneity at the single-cell level has far-reaching implications for microbial population structure, ecosystem interactions, and biogeochemical cycling, positioning phages as key modulators of microbial function beyond their role in host lysis.

Current understanding of phage-host interactions at the ecosystem scale is primarily derived from community-level -omics approaches (e.g., metagenomics, metatranscriptomics, proteomics, and metabolomics), stable isotope probing, and bulk measurements of phage-host abundance and activity. While these methods offer critical insights into community-wide processes, they often obscure the heterogeneity and regulatory shifts occurring within individual cells where phage-mediated metabolic reprogramming originates.

To resolve this gap, advanced single-cell techniques such as NanoSIMS, nanoSIP, FISH, and HISH offer powerful avenues to link phage gene expression with cell-specific activity. These tools can identify infection states, quantify nutrient assimilation, and track metabolic shifts at subpopulation scales. For example, nanoSIP has been used for investigating the activity and biogeochemical consequence of marine phages[44,45]. NanoSIP complemented with techniques like FISH or HISH can link single-cell identity to function. Recent advances in single-molecule DNA-FISH and multiplexed rRNA-FISH also enable direct visualization of mobile genetic elements, providing complementary resolution to metagenomic data, particularly in structured habitats like biofilms[105].

However, few studies have yet applied these approaches explicitly to quantify phage-induced phenotypic heterogeneity or to link it with nutrient fluxes. Future efforts should integrate single-cell measurements with population-level infection dynamics to inform predictive models of microbial function. Embedding phage-driven phenotypic variation into trait-based or functional group modeling frameworks will help bridge mechanistic understanding of infection with ecosystem-level processes. Doing so is critical for advancing our ability to predict how microbial communities and the ecosystem services they regulate respond to environmental change.

## Data availability

No datasets were generated or analyzed during the current study.

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## Author contributions

X.L. conceived this review and wrote the main manuscript. M.R. and Y.J. contributed to the conception and revised the manuscript. S.Y., Y.W. and N.D. prepared the four figures in the manuscript. All authors have read and approved the manuscript.

## Competing interests

The authors declare no competing interests.

## Additional information

**Correspondence** and requests for materials should be addressed to Xiaolong Liang or Yongfeng Jia.