Community context matters for bacteria-phage ecology and evolution

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Abstract

Bacteria-phage symbioses are ubiquitous in nature and serve as valuable biological models. Historically, the ecology and evolution of bacteria-phage systems have been studied in either very simple or very complex communities. Although both approaches provide insight, their shortcomings limit our understanding of bacteria and phages in multispecies contexts. To address this gap, here we synthesize the emerging body of bacteria-phage experiments in medium-complexity communities, specifically those that manipulate bacterial community presence. Generally, community presence suppresses both focal bacterial (phage host) and phage densities, while sometimes altering bacteria-phage ecological interactions in diverse ways. Simultaneously, community presence can have an array of evolutionary effects. Sometimes community presence has no effect on the coevolutionary dynamics of bacteria and their associated phages, whereas other times the presence of additional bacterial species constrains bacteria-phage coevolution. At the same time, community context can alter mechanisms of adaptation and interact with the pleiotropic consequences of (co)evolution. Ultimately, these experiments show that community context can have important ecological and evolutionary effects on bacteria-phage systems, but many questions still remain unanswered and ripe for additional investigation.

Introduction

Bacteria and their viral symbionts, bacteriophages (phages), have long been important study systems in biology [1–3]. While early work focused on molecular and genetic details, the last few decades have seen growing interest in examining their ecological and evolutionary dynamics. The study of bacteria-phage ecology and evolution has largely been driven by two motivations: 1) to understand the microbial world *per se*, since bacteria and phages are ubiquitous, abundant, and diverse, with widespread importance, including for human health [4, 5]; and 2) as tractable models of generalized host-symbiont or predator-prey dynamics [6, 7].

However, studies on the ecology and evolution of bacteria and phages have predominately utilized one of two approaches: experiments with very simple communities (reductionist) or observations of very complex communities (holistic) [8]. For instance, reductionist work has described in-depth the ecology and coevolution of many bacteria-phage pairs (reviewed in [6, 9–11]). In parallel, holistic studies have examined semi-natural and natural systems to infer bacteria-phage ecology and (co)evolution [11–20]. Nonetheless, while both reductionist and holistic approaches have provided useful insights, they are limited in what we can learn about bacteria-phage ecology and evolution in multispecies contexts.

Here, we address this intellectual gap via synthesis and review of the recent and emerging body of phage-bacteria experiments in medium-complexity communities, something that has long been highlighted as a needed focus [11, 21–23]. Specifically, we discuss experiments that directly test how the ecology and evolution of "focal" bacteria and phages are altered by the presence versus absence of other bacterial species ("community context", Fig 1). We focus our discussion on experiments with three or more bacterial species (for two-species communities see Table S1, partially reviewed in [24]), and omit those lacking experimental manipulation of community context (Table S1, reviewed in [11–20]) or with eukaryotes (Table S1, communities with microbial eukaryotes reviewed in [25, 26]). The experiments we highlight bridge the gap between the historical holistic and reductionist approaches.

These experiments comprise part of a larger trend to better incorporate complexity in our ecological and evolutionary understanding of biological systems. Broadly, there is growing interest in how species richness alters the ecology and evolution of community members [27–31]. On the microbial side, for instance, there is an emerging focus on how the evolution of bacteria is altered by the presence of other bacterial species (Table S1, reviewed in [22, 31, 32]). In parallel, recent efforts have sought to incorporate more realism into laboratory bacteria-phage experiments, including the effects of abiotic environment (e.g. [33–35]) and spatial structure (e.g. [21, 24, 36]). Finally, studies are increasingly showing that biological communities can be affected *by* bacteria-phage ecology and evolution (reviewed in [11, 37]).

While we do not discuss these topics in detail, they similarly constitute efforts to more deeply understand multispecies microbial communities.

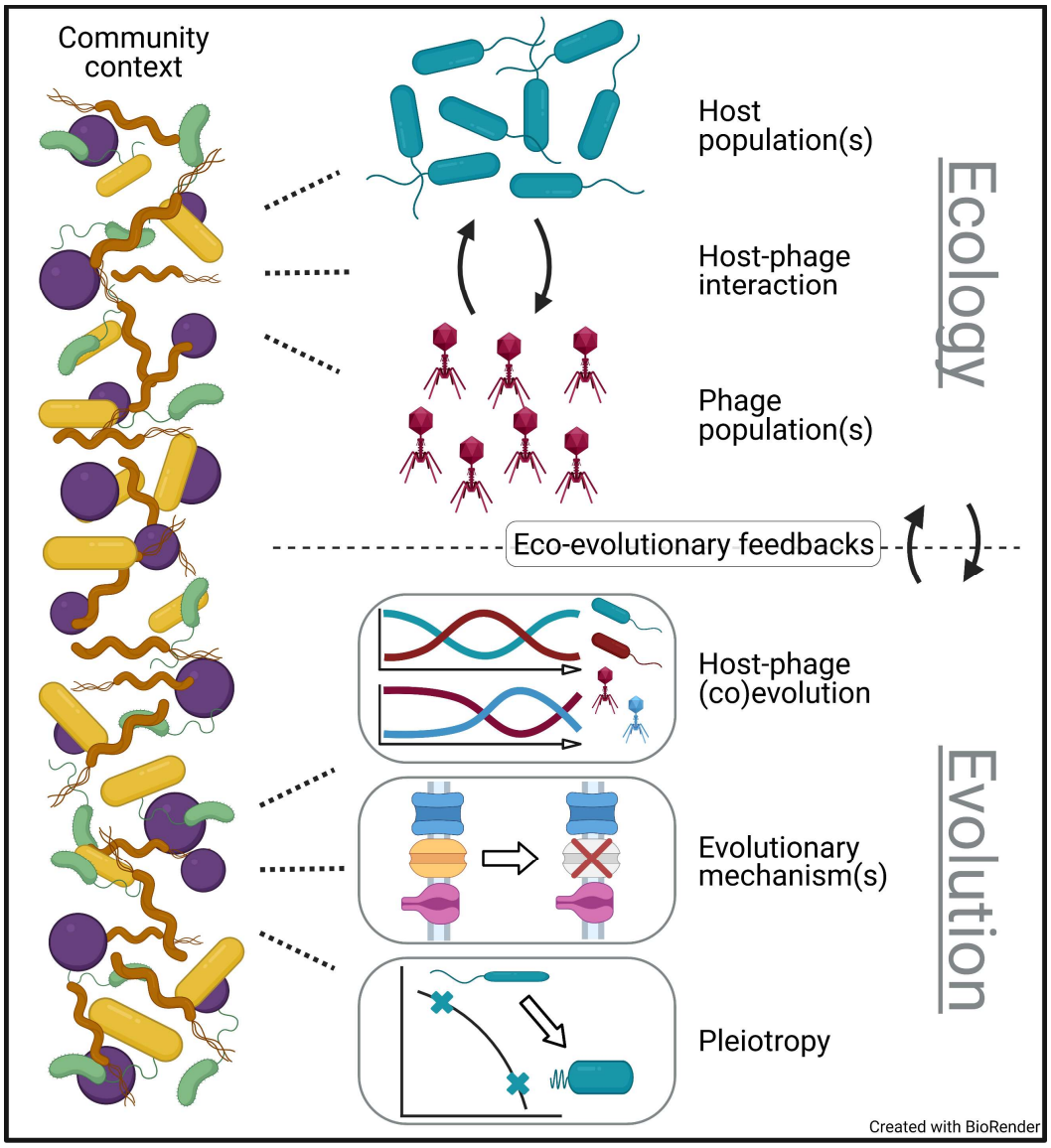

Figure 1. Effects of community context on focal bacterial and phage ecology and evolution. Bacterial community context (left) may affect the ecology and evolution of interacting phage and bacteria populations in various ways (dotted lines). Ecological effects include direct effects on the density of the focal bacterial and phage populations, as well as higher-order effects that alter the interaction between the focal bacteria and phage populations. Evolutionary effects can include: changes to (co)evolutionary dynamics, like fluctuating selection dynamics (depicted); mechanisms of evolution, like which receptor mutation bacteria acquire to evolve phage resistance (depicted); and pleiotropic consequences, like those involved in a trade-off between two traits (depicted). Moreover, ecological and evolutionary effects of community context may affect each other through eco-evolutionary feedbacks. Filled arrows denote abstract interactions, while unfilled arrows denote changes through time.

Effects of community context on focal populations' ecology

The presence of other bacterial species can directly alter the density of the focal bacterial population or the focal phage population as well as modifying the interaction between the focal bacteria and phage populations.

Ecological effects on focal host density

Much of the existing empirical work has shown that community presence suppresses the density of the focal host bacterial species (Table 1). For instance, Gómez and Buckling carried out experiments with *Pseudomonas fluorescens* strain SBW25, its associated phage phi2 (Φ2), and natural soil microbial communities with undetermined species content. They found that community presence depressed the density of *P. fluorescens* in the presence of phage, relative to no-community treatments [36]. Similarly, Johnke et al. used a wastewater model community to investigate the dynamics of multitrophic predator-prey systems, including one bacteria-phage pair [38]. Their data show that community presence had a significant negative effect on the density of the focal host *Klebsiella* sp. relative to community-absent treatments (linear model community presence-absence two-tailed contrast, intercepts $p = 0.049$ in absence of phage, $p = 0.36$ in presence of phage, slopes $p = 0.83$ in absence of phage, $p < 0.01$ in presence of phage, Fig S1, [39]). In a final example, Mumford and Friman investigated the dynamics of *Pseudomonas aeruginosa*, its associated phage PT7, and a community modeling burn or cystic fibrosis infections that includes *Staphylococcus aureus* and *Stenotrophomonas maltophilia* [40]. Although they measured microbial densities only at the end of experimental evolution, they found that the presence of any or all community members suppressed the focal host density relative to community-absent treatments (Fig S2). Interestingly, they found that a quorum sensing-deficient mutant of the focal host was less sensitive to community suppression than the wild-type, suggesting that this suppression by community presence may be partially mediated by density-dependent behavioral changes of the focal bacteria. Across all of these studies, findings that community presence suppresses focal host density are consistent with longstanding ecological theory on competitive release, where removal of competitors can enable a focal species to increase in density [41]. Moreover, because community presence drives smaller population sizes, it may have secondary effects, including increased susceptibility to ecological drift and stochastic extinction [42] as well as weakened natural selection, stronger genetic drift, and decreased mutation supply.

Ecological effects on focal phage density

Similar to the shared pattern of suppression of focal bacterial density, community presence generally suppresses the density of the focal phage population (Table 1). For instance, Gómez and Buckling's work with soil communities showed that community presence decreased phage

densities, relative to no-community treatments [36]. Similarly, Alseth et al. investigated the evolution of resistance to phage DMS3vir by *P. aeruginosa*, using a model infection community where *S. aureus*, *Burkholderia cenocepacia*, and *Acinetobacter baumannii* were also present [43]. Although no effect of community presence on phage density was observed during experimental evolution (linear model two-tailed contrasts against community-absent treatment, intercepts $p > 0.42$ for all four treatments, Fig S3, [39]), experimental manipulation of the initial frequency of the non-focal community showed a clear suppressive effect of community presence on phage density. Finally, in Mumford and Friman's experiments with a model infection community, community presence also decreased focal phage density ([40], Fig S2). However, this suppression of phage density was eliminated when the focal host was quorum sensing-deficient, possibly because this mutant host strain was itself less strongly suppressed by community presence. In contrast to those three studies, when Johnke et al. carried out similar experiments with a wastewater community they found that community presence actually increases phage density (linear model community present-absent two-tailed contrast, $p = 0.012$, Fig S1, [38, 39]). Excepting the findings of Johnke et al, these studies are generally consistent with phage density being bottom-up limited by the density of their host bacteria. However, to our knowledge none of the studies have verified this ecological mechanism, leaving open the possibility that community presence suppresses phage density through other processes. Regardless of the mechanism, smaller population sizes of both phages and their hosts are likely to make phages more prone to extinction events, potentially favoring alternative reproduction strategies like lysogeny or environmental durability [44, 45].

Effects on the ecological interaction between focal populations

Community presence can also alter the interaction between the focal bacterial and phage populations (Table 1). For instance, in their wastewater system, Johnke et al. observed that the effects of community presence and phage presence synergize: while individually each suppresses focal host density, when both are present together they drive the focal host population extinct (Fig S1,[38]). In other cases, community presence can reverse the effect of phage presence. For example, Gómez and Buckling found that phage suppressed the density of their host *P. fluorescens* in the presence of other soil bacterial species, but in the absence of the community phage-presence actually increased the density of the focal host [36, 46]. The exact mechanism driving this effect remains unexplained. Similarly, Mumford and Friman found that community presence altered the effect of phage, with the direction of change dependent on the community membership (Fig S2, [40]). In their infection community they found that phage addition in monocultures depressed host density, while phage addition in the presence of other bacterial species suppressed, had no effect, or even elevated focal host density, depending on competitor identity and whether the focal host was wild type or quorum sensing-deficient. The conflicting findings of these three studies suggest that much further work is warranted before

we can understand how community presence and phage presence interact with each other in a generalizable way.

Box 1. Alternative tests of the ecological effects of community presence

All the studies which have tested for the ecological effect of community context have done so by directly contrasting focal densities between community-added and community-absent treatments. This is essentially a test for the presence of interspecies bacterial competition. However, other statistical comparisons may provide additional insights into the degree of effect of community presence.

For instance, one simple hypothesis is that all bacterial species undergo equal pairwise competition, and thus community presence should reduce the focal population density proportional to the total number of species in co-culture. To assess the utility of this comparison, we used data from two previous studies [38, 40] and a Markov chain Monte Carlo (MCMC) approach to generate Bayesian posterior likelihood distributions of the mean densities of focal bacterial populations in the presence or absence of other community members [39, 47–51] (see Supplemental Methods). In both studies, the suppressive effects of community presence were larger than would be expected from equal competition. In Johnke et al.'s experiments, community presence suppressed focal host density only in the absence of phage (Table S2). In Mumford & Friman's experiments, community presence suppressed focal host density more strongly when the focal host was wildtype strain PAO1 than when it was a quorum sensing-deficient mutant strain (Table S2).

We can apply a similar approach to phage density measures. If phage density is bottom-up limited, we would expect equal pairwise competition among bacterial species to reduce phage density proportional to the total number of bacterial species in co-culture. Using the same methods, we find that all of the likely suppressive effects from [38, 40] were larger than would be expected from equal competition (Table S3). In contrast, in Alseth et al.'s experiment, phage density in the presence of *B. cenocepacia* was not likely different from the community-absent treatment, but was suppressed less than we would have predicted from equal competition (Table S3, [43]).

Overall, our analyses suggest that testing experimental data against additional hypotheses can augment the insights we gain. Indeed, the approach used here can be employed to implement a variety of hypothesis tests, including the absence of competition, equal competition, species identity-dependent competition, and others. Widespread adoption of such analysis approaches will accelerate our understanding of the ecological effects of community presence.

Table 1. Ecological effects of community presence on focal bacteria-phage populations.

Reference	Focal Host – Phage	Community	Effect on Focal Host Density[a]	Effect on Focal Phage Density[a]	Effect on Focal Host-Phage Interaction[a]
[43]	*P. aeruginosa* – DMS3vir	*S. aureus*, *B. cenocepacia*, *A. baumannii*	-	↓ as initial focal frequency was manipulated; ↔ in experimental evolution conditions	-
[52, 53]	*E. coli* – P10	Murine gut community	-	-	-
[36, 46, 54]	*P. fluorescens* – phi2 (Φ2)	Soil communities	↓	↓	Effect of phage on focal host was: ↑ in absence of community; ↓ in presence of community
[38]	*Klebsiella* sp. – *Klebsiella*-phage	*P. putida, Staphylococcus* sp.	↓ in the absence of phage ↔ in the presence of phage	↑	Synergistic suppression by community and focal phage led to focal host extinction
[55]	*Pseudoalteromonas* sp. #1 – *Pseudoalteromonas* sp. #1 phage; *Pseudoalteromonas* sp. #2 – *Pseudoalteromonas* sp. #2 phage; *Photobacterium* sp. – *Photobacterium* sp. phage; *Vibrio sp.* – Vibrio sp. phage	All four species pairs were co-cultured	-	-	-
[40]	*P. aeruginosa* – PT7	*S. aureus, S. maltophilia*	↓ (at end of experimental evolution)	↓ or ↔ (depending on focal host genotype, at end of experimental evolution)	Effect of phage on focal host at end of experimental evolution was ↓/↔/↑ depending on community membership and focal host genotype

[a] The "↓", "↔", and "↑" symbols denote when community presence suppressed, had no effect, or elevated the density of the focal population, respectively. The "-" symbol denotes when the study did not measure the effect of community context.

Effects of community context on focal species' evolution

The presence of other bacterial species can also alter the evolution of one or both of the focal bacteria and phage populations by: altering the progression and dynamics of (co)evolutionary change, changing the mechanisms by which evolution proceeds, or modifying the pleiotropic consequences of adaptation.

Bacteria-phage (co)evolution

In response to one another, bacteria and phages can (co)evolve adaptations conferring changes in resistance and infectivity, respectively [56]. However, existing evidence is conflicted over how community context affects the nature and rate of such antagonistic (co)evolution (Table 2).

For instance, some studies have found that community presence constrains the (co)evolution of one or both of the focal species (Table 2). In Mumford and Friman's experiments with an infection community, although community presence did not alter the qualitative coevolutionary dynamics, it did reduce the frequency of evolved resistance to both contemporary and ancestral phages [40]. However, this effect only occurred when the host was wild type and not quorum sensing-deficient, a difference that may be, in part, driven by genotype-dependent ecological effects (see ecology section). Similarly, Johnke et al. observe that community presence prevents coevolution: in the absence of the community, bacteria and phages evolve in a fluctuating selection dynamics pattern; in the presence of the community, the host bacteria go extinct [38]. These two experiments are consistent with the idea that community presence constrains the evolution of focal species, possibly by limiting available niche space, imposing new selective pressures, or driving otherwise-unexperienced trade-offs between biotic and abiotic pressures [31].

In contrast, others have found that community presence has little effect on (co)evolutionary dynamics (Table 2). For instance, with four marine bacteria-phage pairs, Middelboe et al. observed the evolution of complete resistance to phage infection regardless of community context [55]. Similarly, Gómez and Buckling tested the effect of soil community presence on bacteria-phage (co)evolution. Although their resistance data were suggestive of community presence accelerating coevolution, they measured neither an effect of community presence on the rate of genomic evolution, the type of coevolutionary dynamics, the degree of local adaptation by phages, nor any interaction with phage presence in affecting focal host adaptive radiation [36, 46, 54]. These studies show that community presence can sometimes have little effect on focal bacteria-phage coevolution.

In one final example, community presence actually enabled greater evolution in the focal species. De Sordi et al. investigated the evolution of *Escherichia coli* phage P10 along with two strains of *E. coli* —one host and one nonhost [52, 53]. After inoculating all three *in vitro*, in germ-free mice guts, and in conventional mice guts (with an intact microbiome), they found that only the conventional mouse gut environment enabled the phage to evolve infectivity for the nonhost *E. coli* strain. They went on to show that a third strain of *E. coli*, present only in the conventional mouse gut, acted as an eco-evolutionary bridge enabling the phage to adapt to infect the nonhost *E. coli* strain. Thus, in these experiments, community presence accelerated the evolution of phage host-range shifts and expansion by enabling new coevolutionary trajectories.

Overall, community presence can retard, have no effect, or in rare cases accelerate bacteria-phage (co)evolution. Many factors could drive these differences, although experimental conditions like bottleneck size and evolutionary timescale appear not to drive the patterns observed here (Table 3). Future work is necessary to identify the determinants of community context-driven changes in (co)evolution.

Evolutionary mechanisms

Community presence can also affect the mechanisms by which focal bacteria and phage populations tend to evolve. For instance, Alseth et al. showed that community presence can alter the ways that bacteria evolve resistance to phage infection [43]. In their model infection community, they found that the relative fitness of two resistance mechanisms was altered by community context: in the presence of other species, *P. aeruginosa* primarily evolved CRISPR-based resistance; in the absence of other species, *P. aeruginosa* primarily evolved surface mutation-based resistance. While the exact mechanism remains unknown, it may be related to prior work in the system finding cooperative phage anti-CRISPR systems [57] and nutrient-dependent evolution of resistance [58]. Regardless, to our knowledge, this is the first study to show how community context can alter the fitness landscape of bacterial resistance to phage, so more experiments are necessary to test how community presence can affect the evolution of other resistance mechanisms [59, 60].

Pleiotropy

In bacteria-phage communities, (co)evolution can drive changes beyond the resistance or infectivity phenotype. Such examples of pleiotropy may exist as an evolved trade-up, when the secondary effect is beneficial, or as an evolved trade-off, when the secondary effect is deleterious (a “cost” to evolution). Both trade-offs and trade-ups can have important effects, from competitive ability to virulence [61].

Some existing work has clearly documented community context interacting with the trade-offs and trade-ups faced by focal bacteria. For instance, Alseth et al. showed that community presence drove focal bacteria to escape a trade-off between phage resistance and virulence because community presence favored resistance mechanisms that did not trade-off with virulence (see evolutionary mechanisms section) [43]. On the other hand, Johnke et al. documented trade-ups associated with community and phage presence [38]. In their experiments, focal host *Klebsiella* sp. evolved a higher growth rate in treatments with phage or community presence alone, but was driven extinct in the treatment with both phage and community presence.

In contrast to these studies, others have failed to find evidence that community context interacts with the trade-offs and trade-ups faced by focal species. For instance, Gómez and Buckling found that the presence of a microbial soil community had no effect on the cost of evolved resistance to phage [36]. Similarly, Mumford and Friman found that focal *P. aeruginosa* paid a genotype-dependent cost to adapt to phages or competitors, but that these costs were independent and did not interact [40]. More work is needed to determine how the pleiotropic effects of adaptation to antagonists and community presence generally interact, especially across diverse systems whose molecular details differ.

Table 2. Evolutionary effects of community presence on focal bacteria-phage species.

Reference	Focal Host-Phage	Community	(Co)evolution[a]	Evolutionary Mechanisms[a]	Pleiotropy[a]
[43]	*P. aeruginosa* – DMS3vir	*S. aureus*, *B. cenocepacia*, *A. baumannii*	-	Favored CRISPR-based resistance over surface mutation-based resistance	Resistance was associated with loss of virulence, whose cost was elevated by community presence
[52, 53]	*E. coli* – P10	Murine gut community	Enabled phage host range expansion mutants	-	Community presence enabled phage to traverse trade-off front
[36, 46, 54]	*P. fluorescens* – phi2 (Φ2)	Soil communities	No effect on coevolutionary dynamics (FSD vs ARD)[c], local adaptation, rate of genomic evolution, host adaptive radiation; resistance to contemporary phages was elevated	-	No effect on cost of phage resistance
[38]	*Klebsiella* sp. – *Klebsiella*-phage	*P. putida, Staphylococcus sp.*	Constrained host evolution: host went extinct	-	Adaptation to community or phage presence separately resulted in higher growth rates
[55]	*Pseudoalteromonas sp. #1* – *Pseudoalteromonas sp. #1 phage*; *Pseudoalteromonas sp. #2* – *Pseudoalteromonas sp. #2 phage*; *Photobacterium sp.* – *Photobacterium sp. phage*; *Vibrio sp.* – *Vibrio sp. phage*	All four species pairs were co-cultured	No effect[b]	-	-
[40]	*P. aeruginosa* – PT7	*S. aureus, S. maltophilia*	No effect on coevolutionary dynamics (FSD vs ARD)[c]; constrained evolution of resistance to phage	-	Focal host paid genotype-dependent cost to adapt to community and/or phage presence, with no interaction between the two costs

[a] The "-" symbol denotes when the study did not measure the effect of community context.

[b] Culture differences between treatments limit the strength of conclusions that can be drawn

[c] Coevolutionary dynamics are often characterized qualitatively as Arms Race Dynamics (ARD) or Fluctuating Selection Dynamics (FSD) [62]

Table 3. Evolutionary timescale and bottleneck size in experimental studies. Details on reviewed experiments are listed here, including the type of experiment (batch or continuous), size of the initial inoculum population (N_0), the size of the population before the first transfer (for batch culture) or at peak (for continuous culture) (N_1), the dilution per transfer (for batch culture) or per day (for continuous culture) (D), the number of transfers (for batch culture only) (n), and the total duration of the experiment (t). N_1 was estimated from data (Figs S1, S3) or visually from published figures. The number of bacterial generations in each experiment was estimated as: batch culture generations = $\log_2(N_1/N_0) + n \times \log_2(D)$, or continuous culture generations = $\log_2(N_1/N_0) + t \times D$. When N_1 was unavailable for batch culture experiments, it was assumed growth from inoculum to first transfer was equivalent to subsequent dilutions, i.e., number of generations = $(n+1) \times \log_2(D)$.

Reference	Focal Host-Phage	Design	N_0 (cfu)	N_1 (cfu)	D	n (number of transfers)	t (duration)	Number of Generations
[43]	*P. aeruginosa* – DMS3vir	Batch	3.3×10^6	$\sim2\times10^7$	100	2	3 days	~15.9
[52, 53]	*E. coli* – P10 (*in vitro* only)	Batch	10^8	-	10	3	24 days	~13.3
[36, 46, 54]	*P. fluorescens* – phi2 (Φ2)	Batch	$10^{6.75}$	-	-	0	48 days	-
[40]	*P. aeruginosa* – PT7	Batch	3.8×10^5	-	7	4	16 days	~11.2
[38]	*Klebsiella* sp. – *Klebsiella*-phage	Cont.	8.7×10^6	3.0×10^8	0.1	-	3 days	~5.4
[55]	*Pseudoalteromonas* sp. #1 – *Pseudoalteromonas* sp. #1 phage;	Cont.	$\sim10^{4.9}$	$\sim10^6$	1	-	9.375 days	~13.0
			$\sim10^{4.5}$	$\sim10^{5.9}$				~14.0
	Pseudoalteromonas sp. #2 – *Pseudoalteromonas* sp. #2 phage;		$\sim10^{4.25}$	$\sim10^4$				~9.4
	Photobacterium sp. – *Photobacterium* sp. phage;		$\sim10^{4.5}$	$\sim10^{5.8}$				~13.7
	Vibrio sp. – *Vibrio* sp. phage							

Concluding Remarks

There is a long history of studying the ecology and evolution of bacteria-phage communities, and the studies reviewed here have highlighted the importance of biotic community context as a key factor. Generally, these experiments have found community presence to have consistent ecological effects but divergent evolutionary effects. In part, this could arise from differences in how ecology and evolution have been measured: while ecological dynamics have often been measured similarly, evolutionary dynamics have been assessed with a diversity of metrics. At the same time, the molecular details specific to each experiment may be more important for evolution than ecology. Further work, especially using similar approaches across systems, is necessary to resolve this difference.

To our knowledge, the nine studies reviewed here are the only published experiments to manipulate bacterial community context while measuring the ecological or evolutionary dynamics of bacteria-phage populations. Given the dearth of studies, many questions remain unanswered (Box 2), but two limitations of the existing experiments particularly stand out. First, many of these studies have been conducted in communities with 3 or 4 bacterial species. More speciose communities are likely to more strongly suppress focal species densities and constrain focal coevolution, more closely resembling natural communities [31]. Thus, observing the ecological and evolutionary effects of species-rich communities is an important avenue for future work. Second, nearly all of these studies have observed dynamics over short timescales, typically 10 – 15 generations (Table 3). Given this, it's likely that much of the observed evolutionary change is the result of selection for phage resistance in the focal host. It remains to be seen what ecological and evolutionary dynamics emerge over longer timescales in multispecies bacteria-phage communities.

Just as future experiments must expand the scope of approaches and ask novel questions, there is also a need for research to coalesce conceptually and methodologically (Fig S4). Ultimately, only by determining the effects of community context on bacteria-phage communities can we fully understand these important systems, both as laboratory models and for their central roles in the natural world and human health.

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Box 2. Outstanding Questions

- **How does community presence interact with the ecological effects of phage presence?** We might predict independent effects of community and phage presence, but existing findings have observed a variety of interactions.
- **How does community presence affect the evolution of bacterial resistance to phage?** Prior work has shown that community presence can favor CRISPR-based resistance, but how community presence affects numerous other resistance mechanisms remains untested.
- **How does community presence affect phage host-range evolution?** Phages can evolve to overcome host resistance or to shift their host range. Some experiments have found that community presence may facilitate host-range expansion.
- **Does community presence accelerate or decelerate (co)evolution of focal species?** Community presence could accelerate evolution by creating ecological opportunities or decelerate evolution by imposing constraints. Existing evidence has often found no effect or a decelerating effect.
- **How does community presence affect coevolutionary dynamics?** Coevolutionary dynamics are often qualitatively categorized (e.g., arms race; fluctuating selection). Existing work has found no effect of community presence on these dynamics.
- **What are the null expectations for how community presence should alter bacteria-phage evolution?** Conceptual and theoretical advances are needed to establish null expectations for how community presence should affect the evolution of focal bacteria-phage species.
- **How do findings scale to communities with multiple phage hosts?** Communities with multiple phage hosts, like multiple host-phage pairs or single phages with multiple hosts, could act as models of macro-parasite ecology.
- **How do existing findings scale to more species-rich communities?** The results reviewed here are largely in the context of communities with 3 – 5 bacterial species, which may or may not follow the same patterns and rules as more (or less) diverse communities.
- **How do findings change over longer timescales?** The results reviewed here are largely drawn from experiments lasting just 10-15 evolutionary generations. Over such short timescales ecological dynamics may still be stabilizing, and some evolutionary dynamics may have yet to emerge.
- **How do ecology and evolution interact in multispecies bacteria-phage communities?** Ecological and evolutionary processes can occur on similar timescales. Future experiments should pursue approaches that enable characterization of eco-evolutionary feedbacks.

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Supplemental Information

Community context matters for bacteria-phage ecology and evolution

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Methods

While many studies carried out similar analyses of the ecological effects of community presence, we sought to expand these findings by carrying out additional analyses for three recent papers. We acquired the data [1–3], used dplyr [4] and tidyr [5] for cleaning and manipulation, and used ggplot2 for plotting [6]. We then carried out standard frequentist statistical analyses, as well as evaluating the utility of alternative comparisons in a Bayesian framework.

For the frequentist analyses, we were fundamentally interested in how community context alters the density of focal bacterial and phage populations. Specifically, we carried out a linear model in R using lm for each study [7]. In each linear model, the response of log-transformed density data was assessed for each focal host genotype-community presence-phage presence combination. When time series data were available, the fit involved both an intercept and a slope term for all density data after T0. All data and analyses are available at https://github.com/mikeblazanin/phage-community-review

For the alternative comparisons (Box 1), we were interested in how changes in density following the addition of other community members compared to predictions for how density might have changed. To assess this idea, we fit Bayesian models to log-transformed density data as the response variable. Such models assumed that all data points from each focal host genotype-community presence-phage presence combination arose from a Normal distribution

with a unique mean, but with equal variance across all treatments. The priors were chosen to be uninformative: the shared standard deviation was a uniform distribution between 0 and 100, and the means were a normal distribution with mean 0 and precision (τ) 0.001. Using the rjags interface [8] for JAGS [9], after 1,000 adaptation steps and 1,000 burn-in steps, 50,000 samples were collected using default settings. Then, the mean values for different treatments were contrasted in a paired manner (i.e., the first sampled mean of community absent vs the first sampled mean of community present, and so forth for each of the 50,000 samples). For the plain community-absent contrast no modification was done, but for the alternative prediction (of equal competition among bacterial species) the community-absent mean was divided by the number of species in co-culture then subtracted from the mean of co-culture. When time-series data were available, all density data after T0 were used and an intercept and slope were fitted, both with priors of a normal distribution with mean 0 and precision (τ) 0.001. Reported in Box 1 and Tables S2 and S3 are contrasts between intercept values. All data and analyses are available at https://github.com/mikeblazanin/phage-community-review

Figures

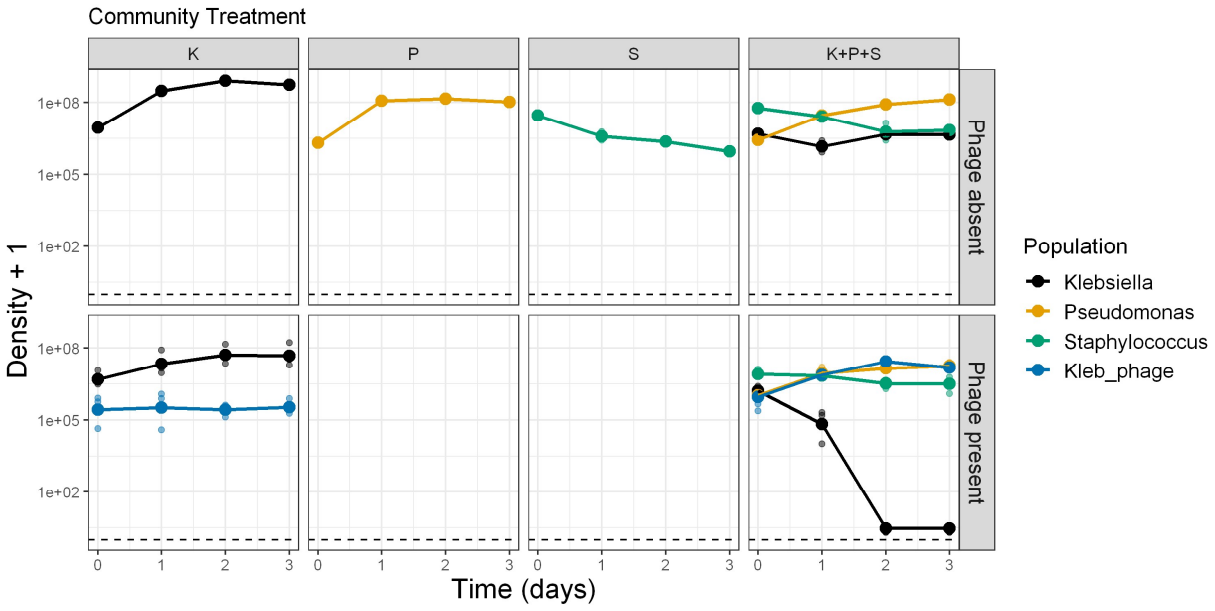

Figure S1. Density dynamics of wastewater community, related to Table 1 and Box 1. Data taken from [1]. Community treatments along the top denote which species were included in treatment (K is *Klebsiella*, P is *Pseudomonas*, and S is *Staphylococcus*). Presence versus absence of the *Klebsiella*-specific phage in treatments is indicated on the right-hand side. Small filled circles denote the density of individual populations, while large filled circles and lines denote mean densities (in log-space).

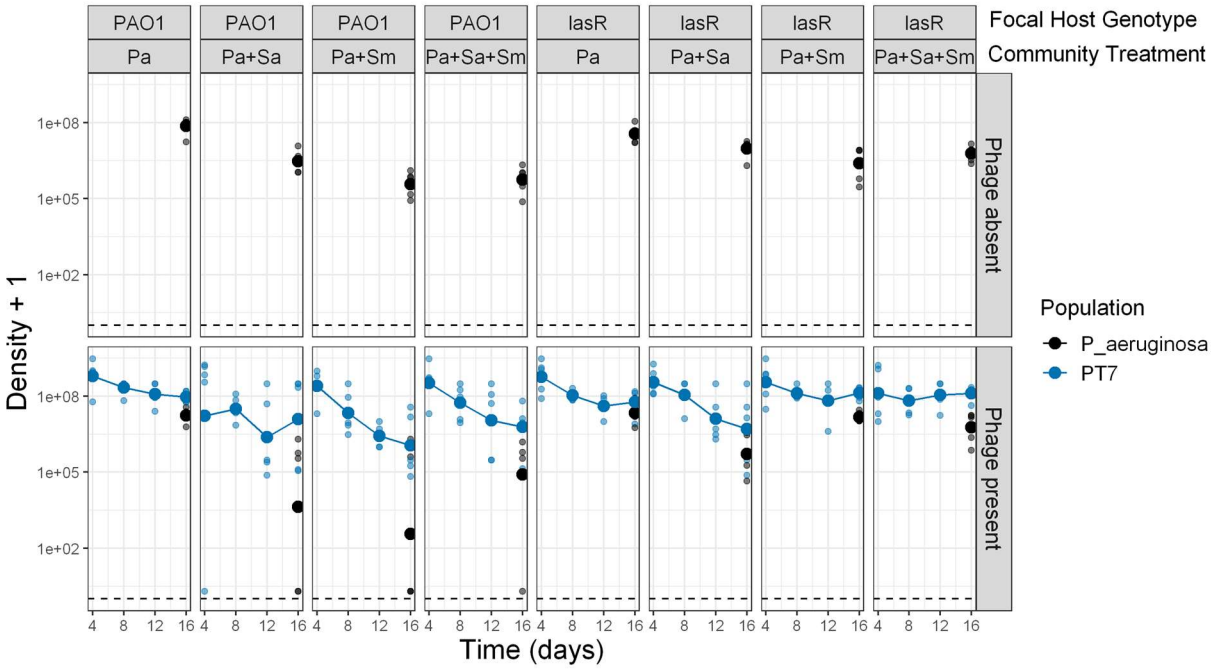

Figure S2. Density dynamics of model three-species wound community, related to Table 1 and Box 1. Data taken from [2], where the focal host genotype of *P. aeruginosa* was either wildtype PAO1 or a quorum sensing-deficient mutant *lasR.* Community treatments differed by inclusion of bacterial species (Pa is *P. aeruginosa*, Sa is *S. aureus*, Sm is *S. maltophilia*), and by presence/absence of *P. aeruginosa*-specific phage PT7. Small filled circles denote the density of individual populations, while large filled circles and lines denote mean densities (in log-space).

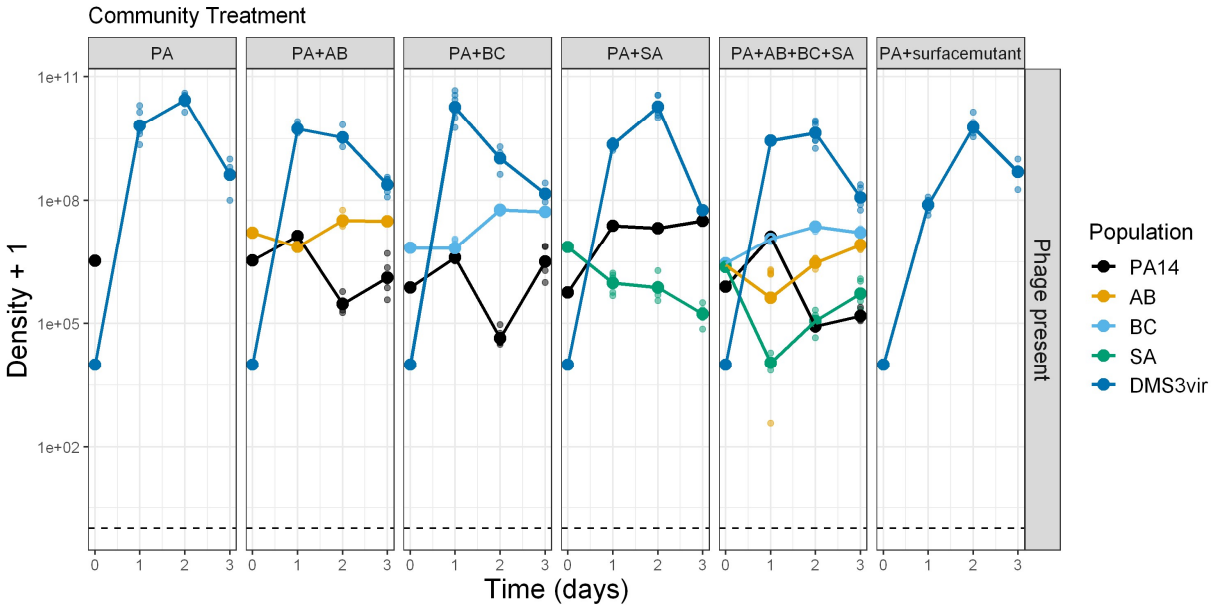

Figure S3. Density dynamics of model four-species wound community, related to Table 1 and Box 1. Data taken from [3], where treatments containing *P. aeruginosa* (PA) and a PA-specific phage were manipulated for community presence of other species (AB is *A. baumannii*, BC is *Burkholderia cenocepacia*, and SA is *S. aureus)*, and a *de novo* surface mutant of PA. Small filled circles denote the density of individual populations, while large filled circles and lines denote mean densities (in log-space).

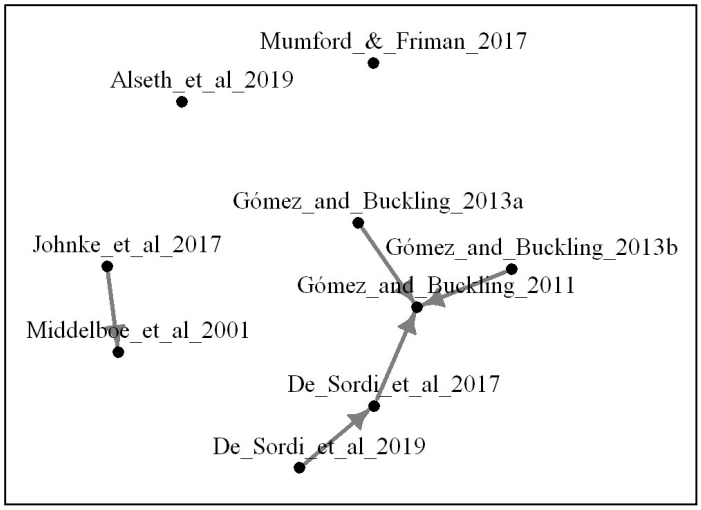

Figure S4. Citation network of papers reviewed in this article reveals minimal connectedness. Directed graph shows how the nine papers reviewed in this article cite each other, with arrows emanating from citing articles to cited articles.

Tables

Table S1. Non-exhaustive list of papers related to multispecies bacteria-phage communities but not reviewed here. These papers were assessed for inclusion in this review but excluded as outside our scope and instead falling into the categories listed. This is not an exhaustive listing of all published papers falling into each category. Note that some papers fall into multiple categories.

Category	References
Communities with microbial eukaryotes, bacteria, and phages	[10–17]
Related review(s):	[18, 19]
Communities with one phage, one phage-host bacterial species, and one non-phage-host bacterial species	[15, 20–24]
Related review(s):	[25]
Communities with two phages and two bacterial species	[13, 20, 26–31]
Communities with one bacterial host and multiple phages cocultured	[27, 32, 33]
Communities with one bacterial host cocultured with multiple phages singly or in combinations	[13, 34–40]
Communities with one phage and two bacterial hosts	[41, 42]
Related review(s):	[25]
Bacteria-phage communities with non-microbial eukaryotes	[43]
Related review(s):	[44]
Addition of a defined phage or phage mixture to a complex microbial community	[45–49]
Addition or depletion of an undefined phage mixture to/from a complex microbial community	[50–58]
Communities where bacterial community context is manipulated to observed effects on focal bacterial evolution	[59–68]
Related review(s):	[69–71]

Table S2. Analysis of alternative ecological hypotheses for bacterial density, related to Box 1. A Markov chain Monte Carlo approach was used to generate posterior likelihood distributions for the density of the focal bacterial population in each treatment. The density of the focal bacterial population in co-culture with other competitors was then contrasted with: the density in the community-absent treatment, and the predicted co-culture density (by dividing community-absent density by the total number of bacterial species). Shown are the likelihoods that co-culture density is less than community-absent density or the predicted density. Bolded values are those with >90% likelihood for an effect in either direction. For [1], P is *Pseudomonas*, and S is *Staphylococcus*; for [2], Sa is *S. aureus*, Sm is *S. maltophilia*.

Reference	Host Genotype	Phage Presence	Competitor	L(comm-present < comm-absent)	L(comm-present < prediction)
[1]		-	P+S	**0.97**	0.94
		+	P+S	0.83	0.67
[2]	PAO1	-	Sa	0.94	0.89
	PAO1	-	Sm	**1.00**	**0.99**
	PAO1	-	Sa+Sm	**0.99**	**0.97**
	PAO1	+	Sa	**1.00**	**1.00**
	PAO1	+	Sm	**1.00**	**1.00**
	PAO1	+	Sa+Sm	**1.00**	**0.98**
	lasR	-	Sa	0.76	0.64
	lasR	-	Sm	0.91	0.84
	lasR	-	Sa+Sm	0.82	0.64
	lasR	+	Sa	**0.97**	0.94
	lasR	+	Sm	0.58	0.44
	lasR	+	Sa+Sm	0.74	0.54

Table S3. Analysis of alternative ecological hypotheses for phage density, related to Box 1. A Markov chain Monte Carlo approach was used to generate posterior likelihood distributions for the density of the focal phage population in each treatment. The density of the focal phage population in co-culture with the focal host and other bacterial species was then contrasted with: the density in the community-absent treatment, and the predicted co-culture density (by dividing community-absent density by the total number of bacterial species). Shown are the likelihoods that co-culture density is less than community-absent density or the predicted density. Bolded values are those with >90% likelihood for an effect in either direction. For [3], AB is *A. baumannii*, BC is *Burkholderia cenocepacia*, and SA is *S. aureus*; for [1], P is *Pseudomonas*, and S is *Staphylococcus*; for [2], Sa is *S. aureus*, Sm is *S. maltophilia*.

Reference	Host Genotype	Phage Presence	Competitor	L(comm-present < comm-absent)	L(comm-present < prediction)
[3]		+	AB	0.67	0.46
		+	BC	0.22	**0.09**
		+	SA	0.59	0.36
		+	AB+BC+SA	0.77	0.35
[1]		+	P+S	**0.01**	**<0.01**
[2]	PAO1	+	Sa	**0.97**	0.94
	PAO1	+	Sm	0.47	0.32
	PAO1	+	Sa+Sm	0.48	0.26
	lasR	+	Sa	0.35	0.22
	lasR	+	Sm	0.68	0.53
	lasR	+	Sa+Sm	0.88	0.72

Supplementary References

1. Johnke J, Baron M, de Leeuw M, Kushmaro A, Jurkevitch E, Harms H, et al. A generalist protist predator enables coexistence in multitrophic predator-prey systems containing a phage and the bacterial predator Bdellovibrio. *Front Ecol Evol* 2017; **5**: 1–12.
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