Supplemental Information

Community context matters for bacteria-phage ecology and evolution

Michael Blazanin[1,2,*], Paul E. Turner[1,2,3]

[1]Department of Ecology and Evolutionary Biology, Yale University, New Haven, Connecticut, USA

[2]BEACON Center for the Study of Evolution in Action, Michigan State University, East Lansing, Michigan, USA

[3]Program in Microbiology, Yale School of Medicine, New Haven, Connecticut, USA

* Correspondence: Mike Blazanin, mike.blazanin@yale.edu

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

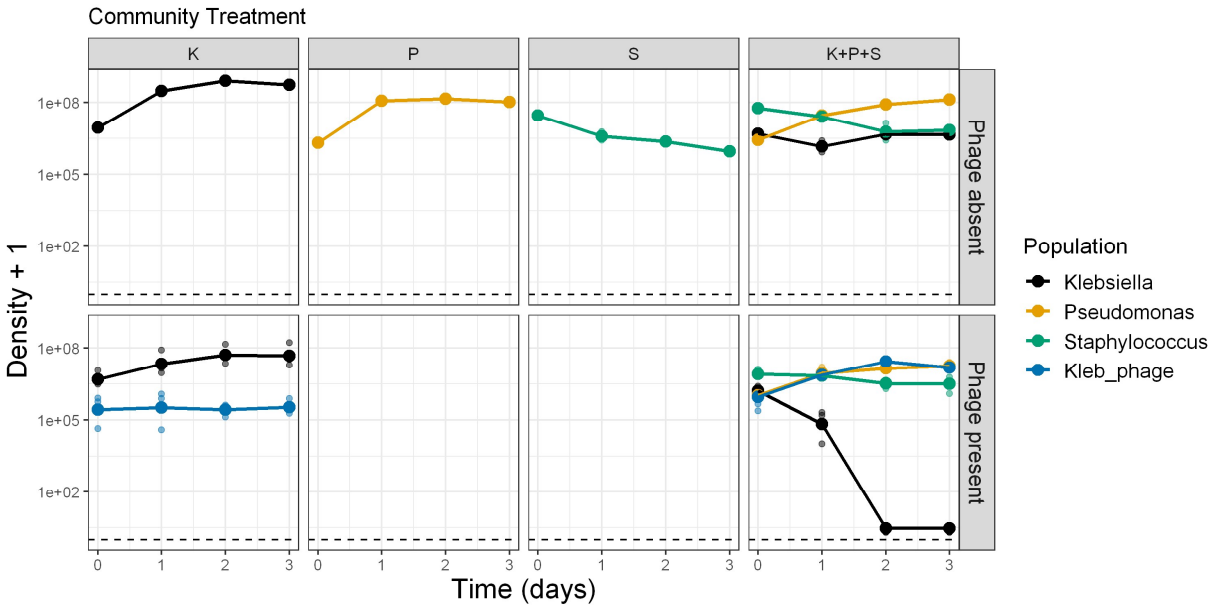

Figure S1. Density dynamics of wastewater community, related to Table 1 and Box 1. Data taken from [1]. Community treatments along the top denote which species were included in treatment (K is *Klebsiella*, P is *Pseudomonas*, and S is *Staphylococcus*). Presence versus absence of the *Klebsiella*-specific 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).

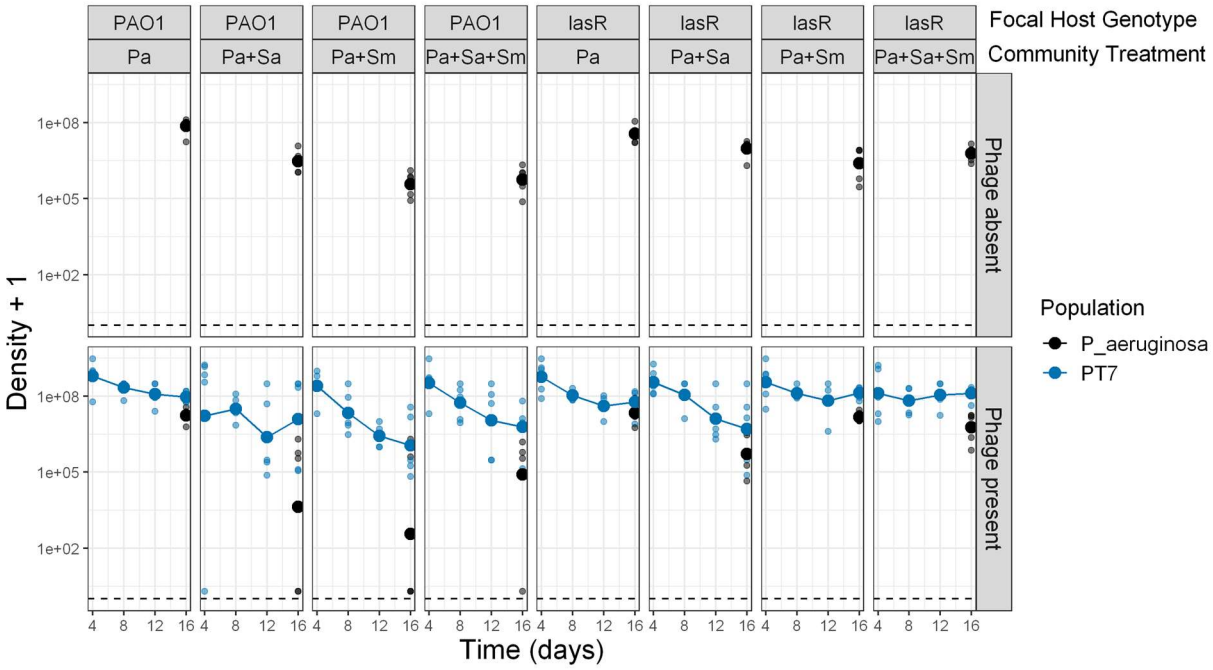

Figure S2. Density dynamics of model three-species wound community, related to Table 1 and Box 1. Data taken from [2], where the focal host genotype of *P. aeruginosa* was either wildtype PAO1 or a quorum sensing-deficient mutant *lasR.* Community treatments differed by inclusion of bacterial species (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).

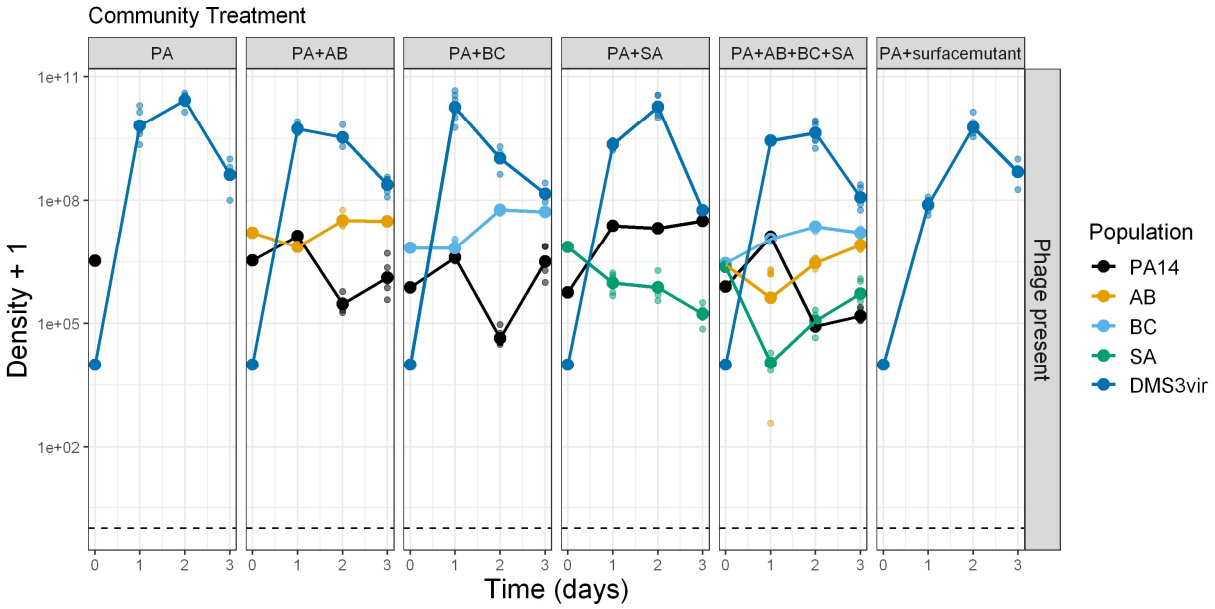

Figure S3. Density dynamics of model four-species wound community, related to Table 1 and Box 1. Data taken from [3], where treatments containing *P. aeruginosa* (PA) and a PA-specific phage were manipulated for community presence of other species (AB is *A. baumannii*, BC is *Burkholderia cenocepacia*, 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).

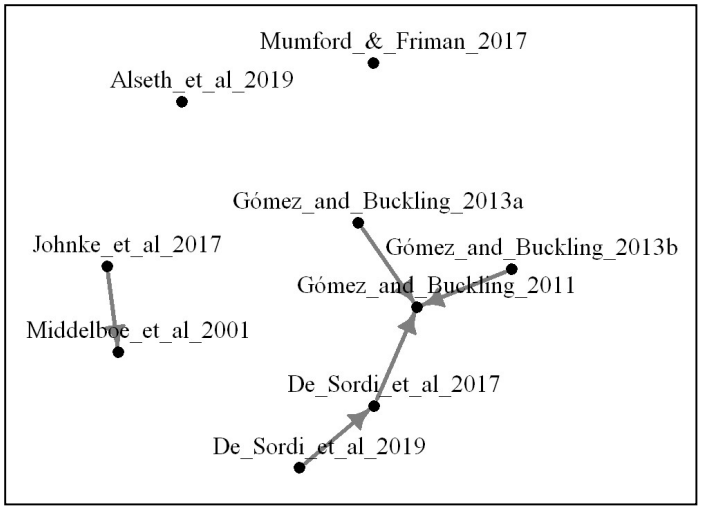

Figure S4. Citation network of papers reviewed in this article reveals minimal connectedness. Directed graph shows how the nine papers reviewed in this article cite each other, with arrows emanating from citing articles to cited articles.

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.
2. Mumford R, Friman VP. Bacterial competition and quorum-sensing signalling shape the eco-evolutionary outcomes of model in vitro phage therapy. *Evol Appl* 2017; **10**: 161–169.
3. Alseth EO, Pursey E, Lujan AM, McLeod I, Rollie C, Westra ER. Bacterial biodiversity drives the evolution of CRISPR-based phage resistance in *Pseudomonas aeruginosa*. *Nature* 2019; **574**: 549–574.
4. Wickham H, François R, Henry L, Müller K. dplyr: A Grammar of Data Manipulation. 2020.
5. Wickham H. tidyr: Tidy Messy Data. 2020.
6. Wickham H. ggplot2: Elegant Graphics for Data Analysis. 2016. Springer-Verlag New York.
7. R Core Team. R: A Language and Environment for Statistical Computing. 2020. R Foundation for Statistical Computing, Vienna, Austria.
8. Plummer M. rjags: Bayesian Graphical Models using MCMC. 2019.
9. Plummer M. JAGS: A program for analysis of Bayesian graphical models using Gibbs sampling. *Proc. 3rd Int. Workshop Distrib. Stat. Comput.* 2003. Vienna, Austria, pp 1–10.
10. Cai W, Wang H, Tian Y, Chen F, Zheng T. Influence of a bacteriophage on the population dynamics of toxic dinoflagellates by lysis of algicidal bacteria. *Appl Environ Microbiol* 2011; **77**: 7837–7840.
11. Friman VP, Buckling A. Effects of predation on real-time host-parasite coevolutionary dynamics. *Ecol Lett* 2013; **16**: 39–46.
12. Friman VP, Buckling A. Phages can constrain protist predation-driven attenuation of Pseudomonas aeruginosa virulence in multienemy communities. *ISME J* 2014; **8**: 1820–1830.
13. Hiltunen T, Kaitala V, Laakso J, Becks L. Evolutionary contribution to coexistence of competitors in microbial food webs. *Proc R Soc B* 2017; **284**: 7–9.
14. Örmälä-Odegrip AM, Ojala V, Hiltunen T, Zhang J, Bamford JK, Laakso J. Protist predation can select for bacteria with lowered susceptibility to infection by lytic phages. *BMC Evol Biol* 2015; **15**: 1–7.
15. Šimek K, Kasalický V, Horňák K, Hahn MW, Weinbauer MG. Assessing niche separation among coexisting Limnohabitans strains through interactions with a competitor, viruses, and a bacterivore. *Appl Environ Microbiol* 2010; **76**: 1406–1416.
16. Zhang J, Ketola T, Örmälä-Odegrip AM, Mappes J, Laakso J. Coincidental loss of bacterial virulence in multi-enemy microbial communities. *PLoS ONE* 2014; **9**.
17. Zhang J, Örmälä-Odegrip AM, Mappes J, Laakso J. Top-down effects of a lytic bacteriophage and protozoa on bacteria in aqueous and biofilm phases. *Ecol Evol* 2014; **4**: 4444–4453.
18. Johnke J, Cohen Y, de Leeuw M, Kushmaro A, Jurkevitch E, Chatzinotas A. Multiple micro-predators controlling bacterial communities in the environment. *Curr Opin Biotechnol* 2014; **27**: 185–190.
19. Miki T, Jacquet S. Complex interactions in the microbial world: Underexplored key links between viruses, bacteria and protozoan grazers in aquatic environments. *Aquat Microb Ecol* 2008; **51**: 195–208.
20. Brockhurst MA, Fenton A, Roulston B, Rainey PB. The impact of phages on interspecific competition in experimental populations of bacteria. *BMC Ecol* 2006; **6**: 1–7.
21. Fazzino L, Anisman J, Chacón JM, Heineman RH, Harcombe WR. Lytic bacteriophage have diverse indirect effects in a synthetic cross-feeding community. *ISME J* 2020; **14**: 123–134.
22. Harcombe WR, Bull JJ. Impact of phages on two-species bacterial communities. *Appl Environ Microbiol* 2005; **71**: 5254–5259.
23. Wang X, Wei Z, Li M, Wang X, Shan A, Mei X, et al. Parasites and competitors suppress bacterial pathogen synergistically due to evolutionary trade-offs. *Evolution* 2017; **71**: 733–746.

24. González S, Fernández L, Campelo AB, Gutiérrez D, Martínez B, Rodríguez A, et al. The Behavior of Staphylococcus aureus Dual-Species Biofilms Treated with Bacteriophage phiIPLA-RODI Depends on the Accompanying Microorganism. *Appl Environ Microbiol* 2017; **83**: 1–14.
25. Geredew Kifelew L, Mitchell JG, Speck P. Mini-review: efficacy of lytic bacteriophages on multispecies biofilms. *Biofouling* 2019; **35**: 472–481.
26. Guyader S, Burch CL. Optimal foraging predicts the ecology but not the evolution of host specialization in bacteriophages. *PLoS ONE* 2008; **3**.
27. Lehman SM, Donlan RM. Bacteriophage-mediated control of a two-species biofilm formed by microorganisms causing catheter-associated urinary tract infections in an in vitro urinary catheter model. *Antimicrob Agents Chemother* 2015; **59**: 1127–1137.
28. Middelboe M, Riemann L, Steward GF, Hansen V, Nybroe O. Virus-induced transfer of organic carbon between marine bacteria in a model community. *Aquat Microb Ecol* 2003; **33**: 1–10.
29. Gutiérrez D, Vandenheuvel D, Martínez B, Rodríguez A, Lavigne R, García P. Two Phages, phiIPLA-RODI and phiIPLA-C1C, Lyse Mono- and Dual-Species Staphylococcal Biofilms. *Appl Environ Microbiol* 2015; **81**: 3336–3348.
30. Kaur S, Chhibber S, Bansal S. Disrupting the mixed-species biofilm of Klebsiella pneumoniae B5055 and Pseudomonas aeruginosa PAO using bacteriophages alone or in combination with xylitol. *Microbiology* 2015; **161**: 1369–1377.
31. Sillankorva S, Neubauer P, Azeredo J. Phage control of dual species biofilms of *Pseudomonas fluorescens* and *Staphylococcus lentus*. *Biofouling* 2010; **26**: 567–575.
32. Davies EV, James CE, Williams D, O'Brien S, Fothergill JL, Haldenby S, et al. Temperate phages both mediate and drive adaptive evolution in pathogen biofilms. *Proc Natl Acad Sci* 2016; **113**: 8266–8271.
33. Rollins D, Clements CV, Rodrigues JLM, Duerkop BA, Hooper LV. A composite bacteriophage alters colonization by an intestinal commensal bacterium. *Proc Natl Acad Sci* 2012; **109**: 17621–17626.
34. Barbosa C, Venail P, Holguin AV, Vives MJ. Co-Evolutionary Dynamics of the Bacteria Vibrio sp. CV1 and Phages V1G, V1P1, and V1P2: Implications for Phage Therapy. *Microb Ecol* 2013; **66**: 897–905.
35. Betts A, Gray C, Zelek M, MacLean RC, King KC. High parasite diversity accelerates host adaptation and diversification. *Science* 2018; **360**: 907–911.
36. Betts A, Gifford DR, Maclean RC, King KC. Parasite diversity drives rapid host dynamics and evolution of resistance in a bacteria-phage system. *Evolution* 2016; 969–978.
37. Gulbudak H, Weitz JS. Heterogeneous Viral Strategies Promote Coexistence in Virus-Microbe Systems. *J Theor Biol* 2019; **462**: 65–84.
38. Hosseinidoust Z, Tufenkji N, van de Ven TGM. Predation in homogeneous and heterogeneous phage environments affects virulence determinants of Pseudomonas aeruginosa. *Appl Environ Microbiol* 2013; **79**: 2862–2871.
39. Koskella B, Lin DM, Buckling A, Thompson JN. The costs of evolving resistance in heterogeneous parasite environments. *Proc R Soc B* 2012; **279**: 1896–1903.
40. Wei Y, Kirby A, Levin BR. The population and evolutionary dynamics of vibrio cholerae and its bacteriophage: Conditions for maintaining phage-limited communities. *Am Nat* 2011; **178**: 715–725.
41. Jensen EC, Schrader HS, Rieland B, Thompson TL, Lee KW, Nickerson KW, et al. Prevalence of Broad-Host-Range Lytic Bacteriophages of Sphaerotilus natans, Escherichia coli, and Pseudomonas aeruginosa. *Appl Environ Microbiol* 1998; **64**: 575–580.
42. Sant DG, Woods LC, Barr JJ, McDonald MJ. Host diversity slows bacteriophage adaptation by selecting generalists over specialists. *Nat Ecol Evol* 2021.
43. Hernandez CA, Koskella B. Phage resistance evolution in vitro is not reflective of in vivo outcome in a plant-bacteria-phage system. *Evolution* 2019; **73**: 2461–2475.

44. Vorburger C, Perlman SJ. The role of defensive symbionts in host–parasite coevolution. *Biol Rev* 2018; **93**: 1747–1764.
45. Duan Y, Llorente C, Lang S, Brandl K, Chu H, Jiang L, et al. Bacteriophage targeting of gut bacterium attenuates alcoholic liver disease. *Nature* 2019.
46. Febvre H, Rao S, Gindin M, Goodwin N, Finer E, Vivanco J, et al. PHAGE Study: Effects of Supplemental Bacteriophage Intake on Inflammation and Gut Microbiota in Healthy Adults. *Nutrients* 2019; **11**: 666.
47. Hsu BB, Gibson TE, Yeliseyev V, Liu Q, Lyon L, Bry L, et al. Dynamic Modulation of the Gut Microbiota and Metabolome by Bacteriophages in a Mouse Model. *Cell Host Microbe* 2019; **25**: 803-814.e5.
48. Lennon JT, Martiny JBH. Rapid evolution buffers ecosystem impacts of viruses in a microbial food web. *Ecol Lett* 2008; **11**: 1178–1188.
49. Spus M, Li M, Alexeeva S, Wolkers-Rooijackers JCM, Zwietering MH, Abee T, et al. Strain diversity and phage resistance in complex dairy starter cultures. *J Dairy Sci* 2015; **98**: 5173–5182.
50. Auguet JC, Montanié H, Hartmann HJ, Lebaron P, Casamayor EO, Catala P, et al. Potential Effect of Freshwater Virus on the Structure and Activity of Bacterial Communities in the Marennes-Oléron Bay (France). *Microb Ecol* 2009; **57**: 295–306.
51. Braga LPP, Spor A, Kot W, Breuil MC, Hansen LH, Setubal JC, et al. Impact of phages on soil bacterial communities and nitrogen availability under different assembly scenarios. *Microbiome* 2020; **8**: 1–14.
52. Fuhrman JA, Schwalbach M. Viral influence on aquatic bacterial communities. *Biol Bull* 2003; **204**: 192–195.
53. Jardillier L, Bettarel Y, Richardot M, Bardot C, Amblard C, Sime-Ngando T, et al. Effects of viruses and predators on prokaryotic community composition. *Microb Ecol* 2005; **50**: 557–569.
54. Schwalbach MS, Hewson I, Fuhrman JA. Viral effects on bacterial community composition in marine plankton microcosms. *Aquat Microb Ecol* 2004; **34**: 117–127.
55. Weinbauer MG, Hornák K, Jezbera J, Nedoma J, Dolan JR, Šimek K. Synergistic and antagonistic effects of viral lysis and protistan grazing on bacterial biomass, production and diversity. *Environ Microbiol* 2007; **9**: 777–788.
56. Wilcox RM, Fuhrman JA. Bacterial viruses in coastal seawater: Lytic rather than lysogenic production. *Mar Ecol Prog Ser* 1994; **114**: 35–46.
57. Morella NM, Gomez AL, Wang G, Leung MS, Koskella B. The impact of bacteriophages on phyllosphere bacterial abundance and composition. *Mol Ecol* 2018; **27**: 2025–2038.
58. Reyes A, Wu M, McNulty NP, Rohwer FL, Gordon JI. Gnotobiotic mouse model of phage-bacterial host dynamics in the human gut. *Proc Natl Acad Sci* 2013; **110**: 20236–20241.
59. Baumgartner M, Bayer F, Buckling A, Hall AR. Resident microbial communities inhibit growth and antibiotic resistance evolution of Escherichia coli in human gut microbiome samples. *bioRxiv* 2020; 741439.
60. Castledine M, Padfield D, Buckling A. Experimental multi-species microbial (co)evolution results in local maladaptation. *bioRxiv* 2020.
61. Evans R, Beckerman AP, Wright RCT, McQueen-Mason S, Bruce NC, Brockhurst MA. Eco-evolutionary Dynamics Set the Tempo and Trajectory of Metabolic Evolution in Multispecies Communities. *Curr Biol* 2020; **30**: 4984-4988.e4.
62. Fiegna F, Moreno-Letelier A, Bell T, Barraclough TG. Evolution of species interactions determines microbial community productivity in new environments. *ISME J* 2015; **9**: 1235–1245.
63. Gorter FA, Manhart M, Ackermann M. Understanding the evolution of interspecies interactions in microbial communities. *Philos Trans R Soc B* 2020; **375**.

64. Jousset A, Eisenhauer N, Merker M, Mouquet N, Scheu S. High functional diversity stimulates diversification in experimental microbial communities. *Sci Adv* 2016; **2**.
65. Klümper U, Recker M, Zhang L, Yin X, Zhang T, Buckling A, et al. Selection for antimicrobial resistance is reduced when embedded in a natural microbial community. *ISME J* 2019; **13**: 2927–2937.
66. Lawrence D, Fiegna F, Behrends V, Bundy JG, Phillimore AB, Bell T, et al. Species interactions alter evolutionary responses to a novel environment. *PLoS Biol* 2012; **10**.
67. Scheuerl T, Hopkins M, Nowell RW, Rivett DW, Barraclough TG, Bell T, et al. Bacterial adaptation is constrained in complex communities. *Nat Commun* 2020; **11**.
68. Tan J, Kerstetter JE, Turcotte MM. Eco-evolutionary interaction between microbiome presence and rapid biofilm evolution determines plant host fitness. *Nat Ecol Evol* 2021.
69. O'Brien S, Hodgson DJ, Buckling A. The interplay between microevolution and community structure in microbial populations. *Curr Opin Biotechnol* 2013; **24**: 821–825.
70. Bottery MJ, Pitchford JW, Friman V-P. Ecology and evolution of antimicrobial resistance in bacterial communities. *ISME J* 2021; **15**: 939–948.
71. Barraclough TG. How Do Species Interactions Affect Evolutionary Dynamics Across Whole Communities? *Annu Rev Ecol Evol Syst* 2015; **46**: 25–48.