Species interactions determine plasmid persistence in a 3-member bacterial community

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Abstract. Microbial communities are shaped by complex forces, including interspecies interactions and the effects of mobile genetic elements such as plasmids. How these forces interact to affect community responses to environmental perturbations remains unclear, particularly considering the qualitatively different natures of competitive and plasmid transfer interactions. We investigated the role of bacterial and plasmid interaction networks in community responses to single and multiple environmental perturbations, using a model community of three bacteria and two plasmids grown in rich media over five 2-day transfers. Bacterial interactions were the primary driver of community response, to the extent that plasmids were not always retained in the community even when they carried relevant resistance genes. Overall, our results indicate that while bacterial and plasmid interactions both shape community responses to environmental perturbations, bacterial interactions may be the primary drivers of community dynamics.

Introduction

Microbial communities are governed by diverse interactions. Prominent amongst these are the interactions between different species and strains. Organisms can interact antagonistically, competing for the limited resources necessary for growth and replication, or producing factors that kill or impede the growth of competitors (Granato et al. 2019). Organisms can also act mutualistically, facilitating one another's growth by cross-feeding and cooperation to utilise resources (Lawrence et al. 2012; Evans et al. 2020; van den Berg et al. 2022; Bisesi et al. 2025). However, interactions in microbiomes are not restricted to interactions between cellular organisms. Superimposed on the various species- and strain-level interactions are interactions between cells and their cognate mobile genetic elements (MGEs), which include bacteriophage and plasmids (Hall et al. 2022). Through such interactions, MGEs can exert significant effects on a community. MGEs can act as parasites, burdening community members in a manner that can prevent a species or strain from dominating (as is the case with so-called Kill-The-Winner dynamics in bacteriophage) (Thingstad 2000; Castledine and Buckling 2024). Conversely, MGEs can confer context-dependent beneficial traits on the microbes that host them, including resistance to environmental perturbations like antibiotics or heavy metals, the ability to metabolise novel resources, or traits allowing colonisation of new niches (Eberhard 1990; Scanlan et al. 2015).

Central to MGE-microbe interactions is the concept of host range: the ability of an MGE to enter and replicate within a given recipient cell type (its 'host'). In the context of beneficial MGE-host interactions, strains that are susceptible to acquiring MGEs can rapidly gain new traits which might accelerate adaptation to changing environments. Hosts of MGEs act as reservoirs of adaptive traits that can be disseminated to others in the community (Hall

et al. 2016), enhancing community-wide adaptation, and strains which can acquire multiple MGEs ('hub' or 'bridge' strains) constitute a location where recombination between MGEs could enable new combinations of functionality (Lam et al. 2019; Wyres et al. 2019). However, bridge strains may suffer excessively from metabolic and other burdens associated with carriage of multiple MGEs, ultimately resulting in their out-competition by other organisms, and local extinction (San Millan et al. 2014; Igler et al. 2022), with potential consequences for MGE persistence.

To experimentally address how the presence of a bridge strain affects community dynamics and the persistence of MGEs, we developed a simple microbial community consisting of three bacterial strains (*Pseudomonas fluorescens* SBW25, *Escherichia coli* MG1655, and *P. putida* KT2440) and two plasmids (pQBR57, which confers mercury resistance, and pKJK5, which confers kanamycin resistance) (Figure 1A). Under our experimental conditions, each plasmid can be hosted by two strains: pQBR57 by *P. fluorescens* SBW25 and *P. putida* KT2440, and pKJK5 by *E. coli* MG1655 and *P. putida* KT2440. As *P. putida* KT2440 can host both plasmids (and can harbour both at the same time) it can be considered a bridge strain, whereas the other two strains are considered 'peripheral' strains. We tracked bacterial and plasmid populations, under non-selective conditions, selective levels of mercury, selective levels of kanamycin, and both stressors in combination, for ~38 generations. We compared the results with communities lacking plasmids, lacking the bridge strain, or lacking both, to ascertain the contribution of each partner to the outcome (Figure 1B). We found that the presence of plasmids increased community resilience in the face of environmental stresses, but species interactions played a predominant role in the dynamics, occasionally driving plasmid extinction even under positive selection.

Methods

Bacterial strains and plasmids. We used *Pseudomonas fluorescens* SBW25, *Pseudomonas putida* KT2440, and *Escherichia coli* MG1655. SBW25 (Rainey et al. 1994; Bailey et al. 1995) and the plasmid pQBR57 (Lilley et al. 1994, 1996; Lilley and Bailey 1997) were isolated independently from a sugar beet field in Oxfordshire, UK. KT2440 is a derivative of *P. putida* mt-2 (Bagdasarian et al. 1981). MG1655 is a derivative of *E. coli* K-12 (Blattner et al. 1997). Interactions between SBW25 and KT2440 have been previously studied, as well as dynamics between the two strains involving pQBR57, which carries the *merA* gene for mercury resistance (Hall et al. 2015, 2016). pQBR57 is considered a narrow host range plasmid, as transconjugants have only been isolated using Pseudomonads (Hall et al. 2015). pKJK5 is a broad host range plasmid known to infect both KT2440 and various *E. coli* strains

(Sengeløv et al. 2001; Bahl et al. 2007) including MG1655 (Klümper et al. 2015). Though pKJK5 has been reported to conjugate into SBW25 (Sünderhauf et al. 2023), we have been unable to retrieve SBW25(pKJK5) transconjugants either under the experimental conditions outlined in this study, or an extended range of conditions including broth and filter mating, and selection for pKJK5-encoded traits. We therefore consider SBW25 as incapable of hosting pKJK5 in our experimental system. Both plasmids are relevant for soil microbial communities (Hall et al. 2015; Klümper et al. 2015).

We used derivatives of each strain to enable enumeration of different populations: SBW25-Sm^R*lacZ* (Hall et al. 2015), KT2440-Gm^R (Hall et al. 2016), and wild-type MG1655 (which carries the *lacZ* gene). We used pQBR57::tdTomato (Wright et al. 2024) and pKJK5::GFP, which has the GFP gene inserted into the tetracycline resistance gene and is resistant to kanamycin but not tetracycline (Klümper et al. 2015). This allowed us to track the three bacterial strains and plasmid carriage throughout the experiment: the plasmids by transfluorescence on a blue light box, and the strains by plating on agar with 250 µg/ml streptomycin and incubating at 28°C (for SBW25) and on agar with 50 µg/ml X-Gal and incubating at 37°C (for KT2440 and MG1655).

Sub-population construction. For brevity, we named our different community treatments SMK (for SBW25 + MG1655 + KT2440) or SM (for SBW25 + MG1655) for communities containing or lacking the bridge strain respectively, appended with '/p' for communities in which plasmids were initially present. Our experimental design (Figure 1) used a link-balanced initial community composition (Table 1), where all populations in treatments including plasmids start 50% plasmid-free, with the remaining 50% evenly comprised of plasmid-carrying sub-populations (one each for SBW25 and MG1655, three for KT2440). We therefore constructed all possible donor strains (Table 2) prior to starting the experiment, using the patch conjugation method from the Barrick lab (Leonard and Perreau 2024). To test effects of plasmid carriage on host growth rate, we grew overnight cultures of each plasmid-free and plasmid-carrying strain, transferred them by pin replicator (~1.5 µl volume) into 200 µl LB broth in a 96-well plate, and performed a 48-hr growth curve at 28 °C in a Tecan Infinite F Nano+ plate reader, reading OD_{600} every 15 minutes.

Table 1. Initial community compositions

Community	Population	Fraction	Sub-population	Fraction
SM	SBW25	0.5		
	MG1655	0.5		
SMK	SBW25	0.33		
	MG1655	0.33		
	KT2440	0.33		
SM/p	SBW25	0.5	SBW25	0.25
			SBW25(pQBR57)	0.25
	MG1655	0.5	MG1655	0.25
			MG1655(pKJK5)	0.25
SMK/p	SBW25	0.33	SBW25	0.165
			SBW25(pQBR57)	0.165
	MG1655	0.33	MG1655	0.165
			MG1655(pKJK5)	0.165
	KT2440	0.33	KT2440	0.165
			KT2440(pQBR57)	0.055
			KT2440(pKJKJ5)	0.055
			KT2440(pKJK5, pQBR57)	0.055

Table 2. Plasmid-hosting bacterial sub-populations

Strain	Plasmid(s)
SBW25-Sm^R*lacZ*	pQBR57::tdTomato
MG1655	pKJK5::GFP
KT2440-Gm^R	pQBR57::tdTomato
KT2440-Gm^R	pKJK5::GFP
KT2440-Gm^R	pQBR57::tdTomato and pKJK5::GFP

Experiment. We streaked out all strains on LB agar plates and incubated overnight at 28°C. We selected 6 independent colonies of each and grew them overnight in LB liquid at 28°C and 180 RPM in 50-ml Falcon tubes in an Innova 42 incubator shaker. We standardized overnight cultures to OD_{600} 1 in LB then diluted 10x, and assembled communities by combining strains in the ratios shown in Table 2 in a total volume of 600 µl. We prepared a 96-well plate with 180 µl LB per well and inoculated each replicate community four times (once per environmental treatment), to a total of 200 µl. Communities were started with 5 x 10^7 CFU. The plating pattern was randomized. No environmental stresses were added during the first cycle. We incubated the experimental plate for 2 days at 28°C and 640 RPM on a Stuart Microtitre SSL-5 orbital shaker then transferred to the next cycle by Boekel Scientific pin replicator (transfer volume ~1 µl). All subsequent 96-well plates were prepared with 200 µl LB per well, with ¼ of wells containing 10 µM HgCl2 [Hg(II)], ¼ containing 12.5 µg/ml kanamycin sulfate, and ¼ containing 10 µM HgCl2 [Hg(II)] and 12.5 µg/ml kanamycin sulfate; the remaining ¼ received no perturbation. Concentrations of mercury and kanamycin were the minimum inhibitory concentrations for the non-plasmid-hosting strains. We passaged the cultures 4 times over a total of 10 days. We quantified population and sub-populations of the initial communities and after cycles 1, 3, and 5 (days 2, 6, and 10), with a lower limit of detection of 5 x 10^5, or >0.5% of community carrying capacity. With a transfer volume of 1% of the community, we expected sub-populations below our limit of detection to be removed from the community across transfers.

Statistical analysis. We summarized overall dynamics of bacteria and plasmids for statistical analysis by calculating trapezoidal area under the curve ('AUC', Figures S2-3), to avoid analytical complications that would have been introduced in timecourse analysis by autocorrelation between timepoints. To assess the effects of community structure and environmental stresses, we used general linear models followed by Tukey HSD tests. To assess extinction probability of SBW25, we ranked extinction as a binary event and performed a Fisher's exact test. We analysed the data using R version 4.3.3 (R Core Team 2024) and RStudio version 2024.12.1+563 (RStudio Team 2020). We organized the data using the *tidyr* (Wickham et al. 2024) and *dplyr* (Wickham et al. 2023) packages, and visualized them using *ggplot2* (Wickham 2016) and *viridis* (Garnier et al. 2024).

Results

Plasmids rescue total community population size from stress. First, we considered communities lacking the bridge strain. In the absence of plasmids, exogenous stresses (mercury, kanamycin, or both) decreased cumulative community size (Figure S4; ANOVA main effect of environment on area under the curve, $F_{[3,80]} = 72.54$, *p*-value < 0.0001), an effect principally driven by the impact of kanamycin (post-hoc Tukey tests effect of kanamycin *p*-values < 0.0002; effect of mercury *p*-value = 0.055). As expected, adding resistance-encoding plasmids to the community ameliorated these effects (ANOVA main effect of plasmids, $F_{[1,80]} = 173.4$, *p*-value < 0.0001), effectively restoring pre-stress community population size (post-hoc Tukey tests, *p*-values > 0.89). Plasmid addition had no significant effect on total community size in the absence of stress (post-hoc Tukey test, *p*-value = 1). At the community level, therefore, plasmids provided robustness to stress.

Plasmids stabilized the two-member community. Under non-stressful conditions, plasmid addition did not significantly alter the dynamics of either of the two community members (Figure 2; area under the curve analysis, post-hoc Tukey HSD tests, *p*-value > 0.9 for all comparisons), possibly because the presence of plasmid-free subpopulations for each strain could mask any negative effect of plasmid burden on the overall population counts. Stresses particularly impacted SBW25, which performed worse under all stress treatments (post-hoc Tukey HSD tests, Hg *p* = 0.03, Kn and Hg + Kn *p*-values < 0.001). As predicted given the host ranges of the plasmids, for SBW25, plasmid addition mitigated only the effect of mercury stress (SM community vs SM/p community, *p*-value = 0.0006), and could not rescue the population when faced with kanamycin stress (SM community vs SM/p community, *p*-values = 1), where SBW25's population fell below the detection limit of the experiment in each case.

By contrast, MG1655 was primarily affected only by kanamycin stress (Hg *p* = 0.25, Kn and Hg + Kn *p*-values < 0.003), and though kanamycin significantly reduced MG1655's population size (post-hoc Tukey tests, *p*-value < 0.0001), the strain remained at appreciable levels even when plasmids were absent from the community (AUC difference from non-stressful conditions of -7.34 for Kn vs. -21.41 for Hg + Kn), possibly by emergence of a *de novo* mutant with increased kanamycin resistance. Plasmid addition improved MG1655's growth under kanamycin conditions, even rescuing MG1655 from dual stress (*p*-values < 0.0001).

In a two-member community with no between-species HGT, plasmids were therefore able to rescue their hosts from relevant stresses. Despite the inability to host the plasmid conferring mercury resistance, MG1655 demonstrated an ability to survive under mercury

202 conditions regardless of plasmid presence, or under dual stress when it carries the plasmid
203 conferring kanamycin resistance (Figure 2).
204
205 *Adding the bridge strain destabilized the community.* We predicted that community members
206 present in the host ranges of multiple plasmids (i.e. bridge strains, Figure 1) may experience
207 higher plasmid burden, altering their dynamics and influence on the overall community. We
208 therefore investigated the dynamics of communities which included a third member, *P. putida*
209 KT2440, which can host both pQBR57 and pKJK5 and thus acts as a bridge strain when the
210 plasmids are present.
211 First, we considered the effect of the bridge strain on plasmid-free communities. The
212 presence of the bridge strain did not significantly influence total population size (Figure S4;
213 ANOVA, $F_{[1,80]} = 2.2$, *p*-value = 0.13), and MG1655 was unaffected by KT2440's presence
214 across environmental treatments (Figures 2 & 3, ANOVA, $F_{[1,80]} = 0.095$, *p*-value = 0.75).
215 However, SBW25 was significantly impacted, experiencing population decline over the
216 course of the experiment when the bridge strain was present (Figures 2 & 3, ANOVA, main
217 effect of bridge strain, $F_{[1,80]} = 150.7$ *p*-value < 0.0001). This may have been due to niche
218 overlap between the two *Pseudomonas*, resulting in nutrient competition (Zhang et al. 2012).
219 The effect of KT2440 on SBW25 was observed both in non-stressful and mercury conditions
220 (post-hoc Tukey HSD tests, *p*-values < 0.002) and drove SBW25 below the detection limit
221 under non-stressful conditions (Figures 2 & 3, first column; Fisher's Exact test, *p*-value =
222 0.004). (We did not detect an effect in treatments containing kanamycin (*p*-values > 0.99)
223 because SBW25 quickly fell below the detection limit regardless of KT2440 presence (Figure
224 2)). The presence of the bridge strain therefore shaped the overall community principally by
225 influencing the dynamics and persistence of specific community members, in this case
226 SBW25.
227
228 *Stress type determines plasmid effects on community stability*. Next, we investigated how
229 the presence of plasmids and bridge strain interacted to affect community members. Hosting
230 pKJK5 imposes a cost relative to hosting pQBR57 (Figure S1; ANOVA for differences in
231 growth dynamics of KT2440 sub-populations, $F_{[3,23]} = 3.86$, *p*-value = 0.023; post-hoc Tukey
232 HSD test for pKJK5 vs pQBR57, *p*-value = 0.034). However, we expected plasmids to
233 facilitate growth of strains under conditions that selected for the cognate resistance traits.
234 Under mercury conditions, in contrast to the two-species communities, the presence
235 of plasmids in the three-species communities did not improve survival of the pQBR57-
236 hosting strains, SBW25 and KT2440, . In fact, while there was no significant effect on
237 SBW25 survival (Figure 3; post-hoc Tukey HSD tests for SMK vs SMK/p, *p*-value >0.99),
238 plasmid presence exerted a negative effect on KT2440, with loss from the community more

239 likely when plasmids were present than when they were absent (Figure S5; Fisher's Exact
240 test, *p*-value = 0.015). This outcome suggests that the interaction of plasmids and
241 environmental conditions can influence strain interactions in non-intuitive ways.
242 By contrast, kanamycin stress did enhance the growth of strains capable of hosting
243 the plasmid carrying the cognate kanamycin resistance gene, MG1655 and KT2440.
244 Plasmids improved MG1655 survival under both conditions containing kanamycin (Figures 2
245 & 3 "Kn" and "Hg + Kn"; post-hoc Tukey HSD tests, *p*-value < 0.0001), while plasmids
246 rescued KT2440 under kanamycin stress (Figure 3 "Kn"; post-hoc Tukey HSD test, $p <$
247 0.001), but not dual stress, even though KT2440 is capable of hosting both plasmids and
248 thus expressing resistances to both stresses (post-hoc Tukey HSD test, *p*-value > 0.99).
249 Overall, the bridge strain appears to have destabilized the community via its effect on
250 SBW25, and the plasmids could in some cases exacerbate this effect, resulting in loss of
251 species diversity from the community.
252
253 *Plasmids were not always rescued by the corresponding stress*. Communities which
254 contained plasmids had increased robustness to environmental stressors to which those
255 plasmids conferred resistance. We therefore expected plasmids to increase in frequency
256 within host populations experiencing a stress for the plasmid carries a resistance gene (i.e.
257 plasmid selection (Hall et al. 2020)). However, we did not always find this to be the case. For
258 pQBR57, which encodes resistance to mercury, dynamics were determined by the hosting
259 strain, presence of the bridge strain in the community, and environmental conditions (Figure
260 4; ANOVA, $F_{[1,63]} = 32.41$ strain, $F_{[1,63]} = 23.32$ bridge, $F_{[3,63]} = 25.28$ stress, $F_{[3,63]} = 21.98$
261 bridge x stress interaction, *p*-values < 0.0001). As expected, plasmid frequency declined in
262 the absence of selection, likely owing to fitness cost. But while mercury selection was able to
263 prevent decline of pQBR57 (Figure 4, post-hoc Tukey HSD tests, *p*-values < 0.0001), this
264 was true only for the 2-member community, where SBW25 was the only available host (post-
265 hoc Tukey HSD tests, *p*-values < 0.0001). The presence of the bridge strain removed any
266 benefit provided by environmental selection (post-hoc Tukey HSD tests, comparison
267 between SM/p and SMK/p for Hg, *p*-value < 0.0001), and pQBR57 declined similarly across
268 all environmental treatments (post-hoc Tukey HSD tests, *p*-values > 0.3).
269 By contrast, while host strain had a significant impact on pKJK5 (Figure 4; ANOVA,
270 $F_{[1,60]} = 285.03$, *p*-value < 0.0001), the overall presence of the bridge strain in the community
271 did not (ANOVA, $F_{[1,60]} = 0.072$, *p*-value = 0.82). Kanamycin selection had a significant effect
272 on pKJK5 (post-hoc Tukey HSD tests comparing kanamycin and non-kanamycin
273 environments, *p*-values < 0.0002), and though there was a quantitative difference between
274 the kanamycin and dual selection in the three-member (but not the two-member) community,
275 thanks to the contribution by the bridge strain (*p*-values < 0.0002), the overall dynamics were

276 qualitatively similar. In most cases pKJK5 dynamics follow those of MG1655, its predominant
277 host, with little contribution from the bridge strain.
278
279 **Discussion**
280 Here, we explored the interaction of several forces — inter-species interactions, mobile
281 genetic elements, and environmental change — to assess their interacting effects on
282 microbial community composition and dynamics. We found that interactions between strains,
283 known to have a predominant role in community dynamics, fundamentally impacted the
284 success of their resident MGEs. Such effects could even overwhelm any benefits provided
285 by the MGEs in changing, stressful environments. For example, we found that the mercury
286 resistance plasmid pQBR57 was lost even from environments containing mercury because
287 its resident hosts were outcompeted, while *E. coli* MG1655 persisted (allowing maintenance
288 of its plasmid pKJK5) regardless of environmental selection pressures. Such patterns
289 challenge our intuition as to drivers of MGEs in communities, indicating that MGE dynamics
290 are fundamentally determined by internal community interactions, and not only by extrinsic
291 selective pressures.
292 We manipulated community composition by the addition of a bridge strain, *P. putida*
293 KT2440, that could harbour both possible plasmids. The main effect of the bridge strain on
294 community dynamics was through competition with *P. fluorescens* SBW25, which suffered
295 from reduced abundances across environmental and plasmid conditions. Previous work
296 found that competitive interactions between these species exerted only a mild effect on
297 abundances in a structured soil environment (Hall et al. 2018), or in heterogenous liquid
298 culture, and though the effects were intensified in well-mixed rich media ((Zhang et al. 2012),
299 as used in the current study) they tended not to result in local extinction of SBW25.
300 Additional competitive pressure from the other community member, MG1655, may have
301 been sufficient to exclude SBW25 from stressful or bridge-strain-containing conditions in our
302 experiments; such competition may be further intensified in minimal media, as opposed to
303 the rich medium used here (Goldford et al. 2018). Natural communities, evolving to
304 specialize on a single labile carbon source, tend to converge on a conserved composition at
305 the Family level, consisting principally of Enterobacteriaceae and Pseudomonadaceae owing
306 to the metabolic capabilities of with each family (Goldford et al. 2018). Similar species-
307 sorting interactions may be at work in our simpler communities, resulting in the exclusion of
308 the less competitive Pseudomonad.
309 While we expected that the relative success of the bridge strain ought to promote the
310 persistence of both plasmids, plasmid maintenance was generally reduced in communities
311 containing the bridge strain. Previous work found that *P. putida* KT2440 was a less
312 favourable host than *P. fluorescens* SBW25 for plasmid pQBR57, even if the fitness effects

313 in each strain are similar ((Hall et al. 2016; Kottara et al. 2018), Fig. S2). The alternative
314 plasmid pKJK5 was likewise more costly to the bridge strain than to its peripheral strain *E.*
315 *coli* MG1655 (Fig. S2). Competitive interactions between organisms, and variance in MGE
316 stability across species and strains (possibly depending on co-infection status of the host
317 (San Millan et al. 2014; Igler et al. 2022)), thus interact to have consequential effects for
318 maintenance and spread of MGEs that could not be predicted from MGE host ranges alone
319 (Alonso-del Valle et al. 2021). Similarly, we expected that selection for plasmid-borne
320 resistances would have promoted the corresponding plasmid, and the organisms able to
321 host that plasmid. However, our results suggest that selection for plasmid-borne resistances
322 was insufficient to rescue plasmids, or hosts, in a community context — most strikingly in
323 communities treated with mercury, where both hosts of the mercury resistance plasmid went
324 extinct. Higher concentrations of mercury may have rescued pQBR57 (Hall et al. 2020),
325 suggesting that local plasmid fate may vary across heterogeneous environments.
326 Embedding bacterial-plasmid interactions in a community has been shown to impact
327 plasmid fate. Walker-Sünderhauf et al. (2023) showed that maintenance of pKJK5 in two
328 different microbes isolated from potting soil (species of *Stenotrophomonas* and *Variovorax*)
329 was disrupted in the context of a five-species community, leading to plasmid loss from these
330 organisms. Kottara et al. (2021) similarly showed that plasmid prevalence in a focal species
331 could be reduced by the epidemiological dilution effect of living in a multi-species community,
332 as conjugation events were more likely to result in plasmid transfer to non-focal organisms.
333 Besides shaping microbial community composition, strain competition (i.e. features of the
334 bacterial interaction matrix 'A', Fig. 1) can exert consequential influences on subsequent
335 opportunities for HGT, by driving the dynamics of the vectors of gene transfer, sometimes to
336 extinction. The influence of strain competition on plasmid survival might explain why some
337 plasmids may have evolved to manipulate expression of secreted competitive molecules (Di
338 Venanzio et al. 2019; Billane et al. 2022).
339 In a reciprocal manner, microbial community dynamics can be profoundly altered in
340 the context of horizontal gene transfer. HGT can have a positive effect on community
341 diversity and microbiome stability by enabling beneficial resistances to transfer across
342 community members, facilitating their survival (Coyte et al. 2022). The transfer of costly
343 genes can also maintain community stability in a process of 'dynamic neutrality', reducing
344 variance in growth rates between species and mitigating competition (Zhu et al. 2024),
345 although this process may be influenced by changes in species interactions. Our work
346 suggests that structure in the HGT network can have a critical effect modulating the impact
347 of HGT on communities, allowing – or preventing – mobile genes to hitch-hike on
348 competitive community members.

The non-*Pseudomonas* species in our experiment, *E. coli* MG1655, offers a contrast. MG1655 was successful at competition regardless of the presence or absence of the bridge strain, and was able to maintain pKJK5 successfully. Moreover, MG1655 was successful at surviving in the face of single stresses even when plasmids were absent. Our concentrations of selective agents were determined based on single-species MIC measurements; growth in a multi-species community — together with the large population size and standing genetic diversity gained during the first growth cycle (from which stresses were absent) — likely allowed survival beyond these minimal concentrations. Moreover, mercury resistance is achieved by environmental detoxification (Barkay et al. 2010), a 'public good' which could benefit other, nearby community members lacking specific mercury resistance genes. Interestingly, MG1655 could survive dual stresses only in communities with plasmids, even though it couldn't maintain both plasmids itself, suggesting that resistance to one stress improved the capacity of this strain to cope with a heterologous stress, possibly by enabling sufficient population size for *de novo* mutants to emerge.

Overall, our work demonstrates the importance of considering multiple factors to understand community dynamics. Further, we suggest that plasmids are at risk of being lost from a community due to impacts on their hosts, even when environmental conditions would be expected to select for them. This idea suggests additional complexity that may be at play in plasmid evolution.

Acknowledgements

This work was supported by a Human Frontiers Science Programme Project Grant to SP, MdeD, JPJH (RGY0064/2022). JPJH is supported by an MRC Career Development Award (MR/W02666X/1). We thank Uli Klümper (TU Dresden) for providing pKJK5::GFP, and Mike Bottery (University of Manchester) for *E. coli* MG1655.

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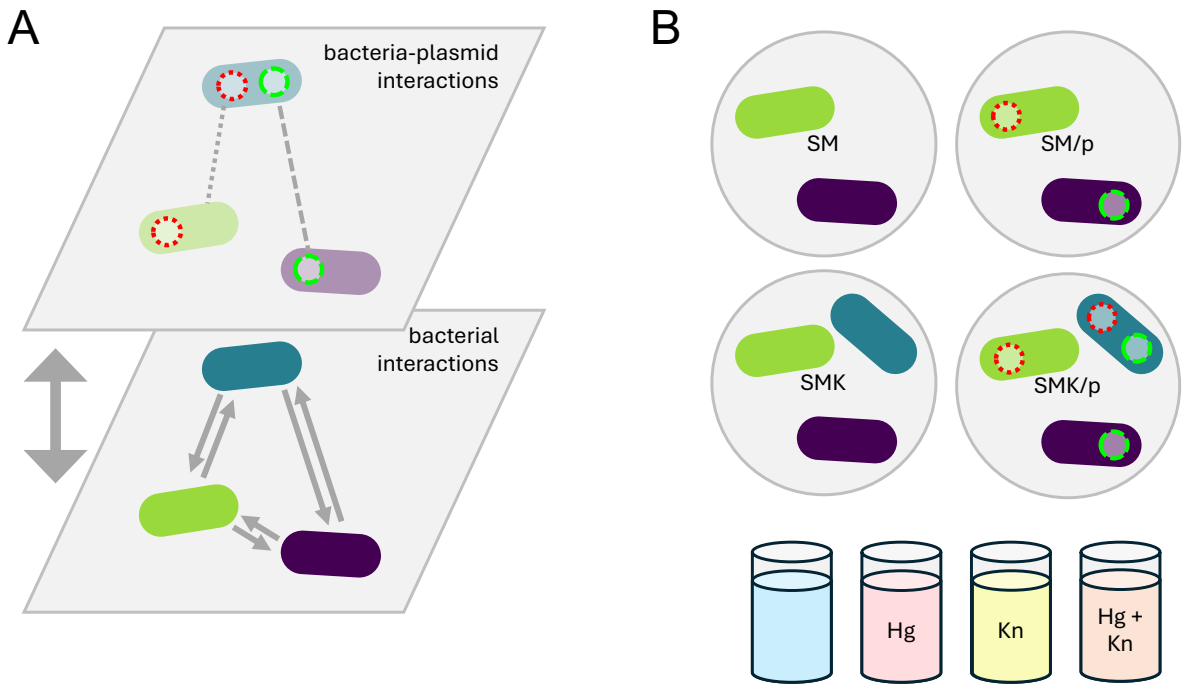

Figure 1. Experimental design. The community interaction network (A) consists of three bacterial strains (lower layer) and two plasmids (upper layer). The bacteria are *P. fluorescens* SBW25 (green oval), *P. putida* KT2440 (blue oval), and *E. coli* MG1655 (purple oval). The plasmids are pQBR57 (red dotted ring) and pKJK5 (green dashed ring). KT2440 is able to host both plasmids, making it a bridge strain within the community. We analysed dynamics of four communities under four environmental conditions, including three stress treatments (B).

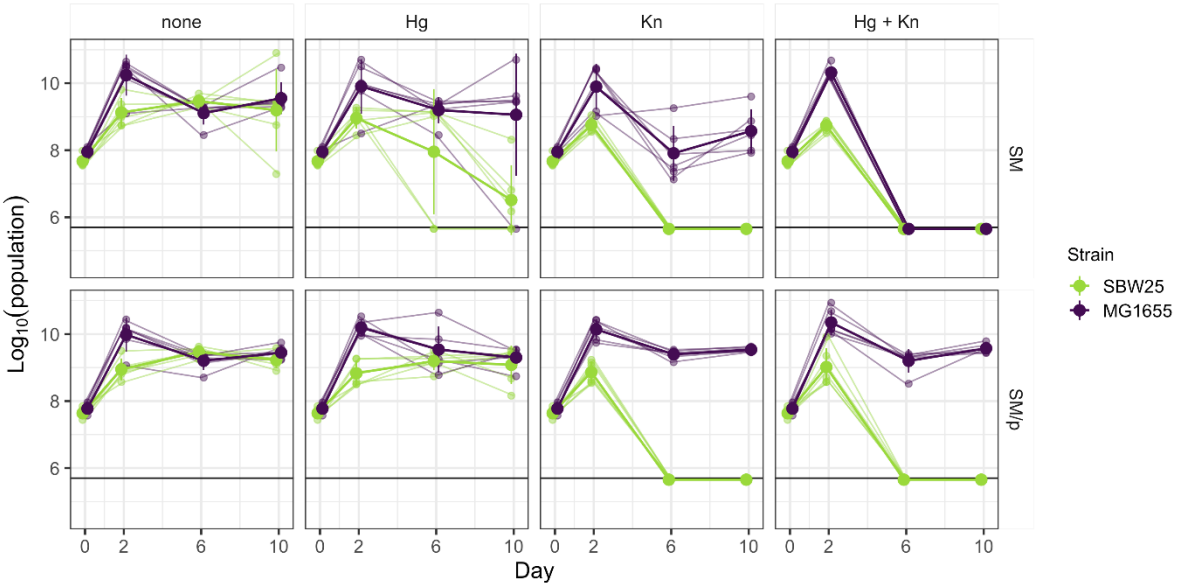

Figure 2. Bacterial dynamics in the 2-member community. Population sizes of SBW25 and MG1655 over 10 days under different environmental conditions (environmental perturbations were included from day 2 onward). The upper row of panels shows dynamics when plasmids were absent from the community, and the lower row shows bacterial dynamics when plasmids were present. Small dots represent replicate values, large dots represent means of 6 biological replicates, and vertical lines represent 95% confidence intervals of the means. Stresses were added after day 2.

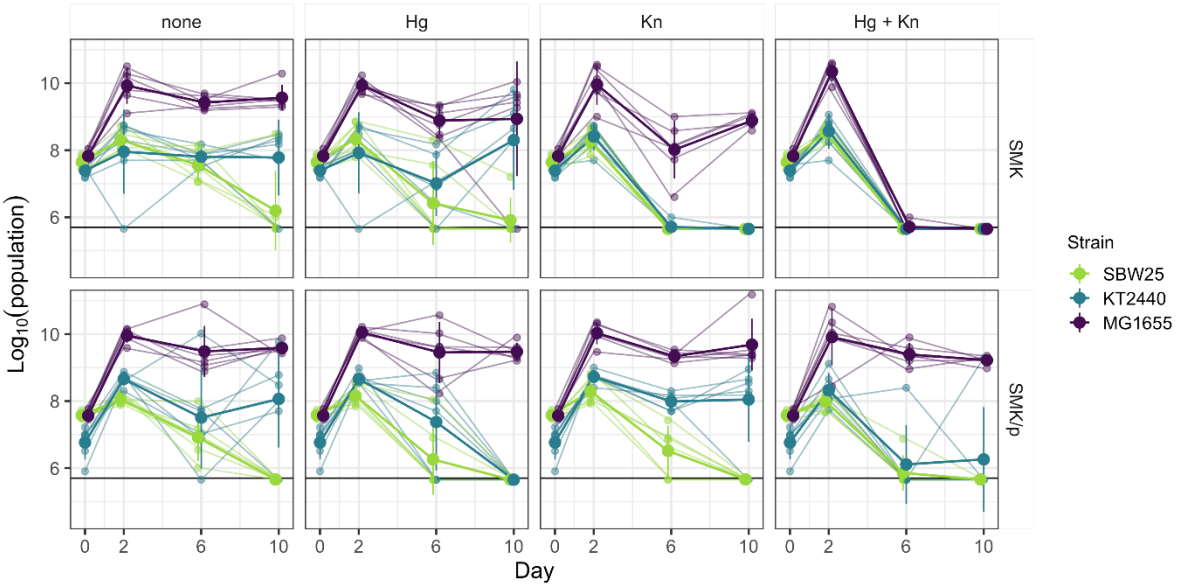

Figure 3. Bacterial dynamics in the 3-member community. Population sizes of SBW25, KT2440, and MG1655 over 10 days under different environmental conditions (environmental perturbations were included from day 2 onward). The upper row of panels shows dynamics when plasmids were absent from the community, and the lower row shows bacterial dynamics when plasmids were present. Small dots represent replicate values, large dots represent means of 6 biological replicates, and vertical lines represent 95% confidence intervals of the means. Stresses were added after day 2.

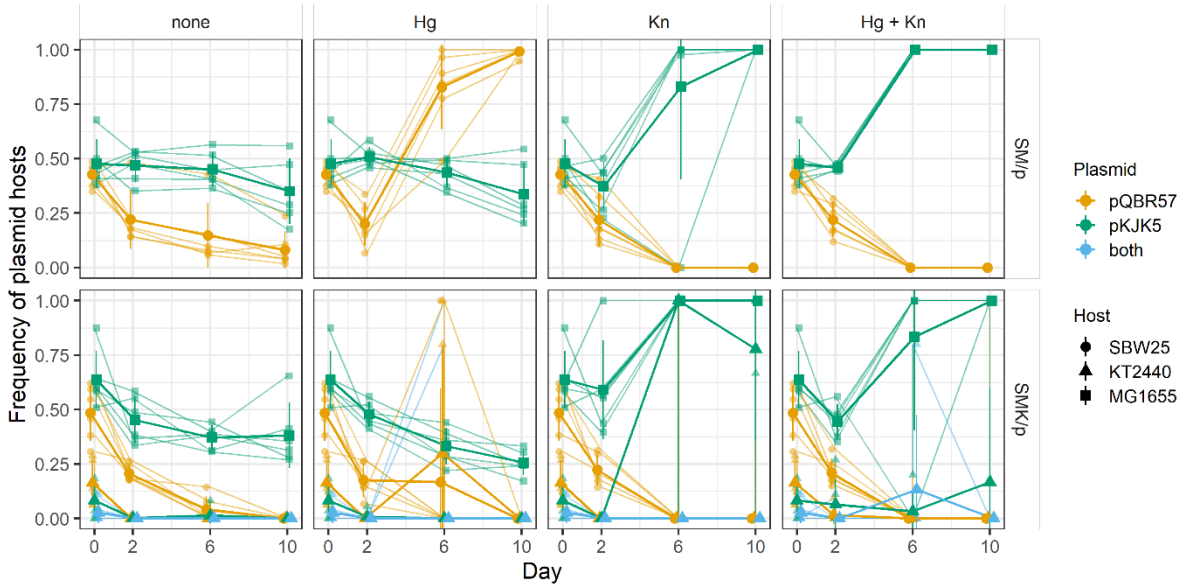

Figure 4. Plasmid frequency dynamics. Frequencies of plasmid-carrying subpopulations of the host bacteria over 10 days under different environmental conditions, where pQBR57 confers Hg resistance and pKJK5 confers Kn resistance. A frequency of 1 indicates that all cells of that host contain the plasmid. Colors represent plasmid status (hosting one plasmid, the other, or both plasmids) and shapes represent host species. The upper row of panels shows plasmid frequency dynamics in the 2-member community, and the lower row shows dynamics in the 3-member community. Small dots represent replicate values, large dots represent means of 6 biological replicates, and vertical lines represent 95% confidence intervals of the means

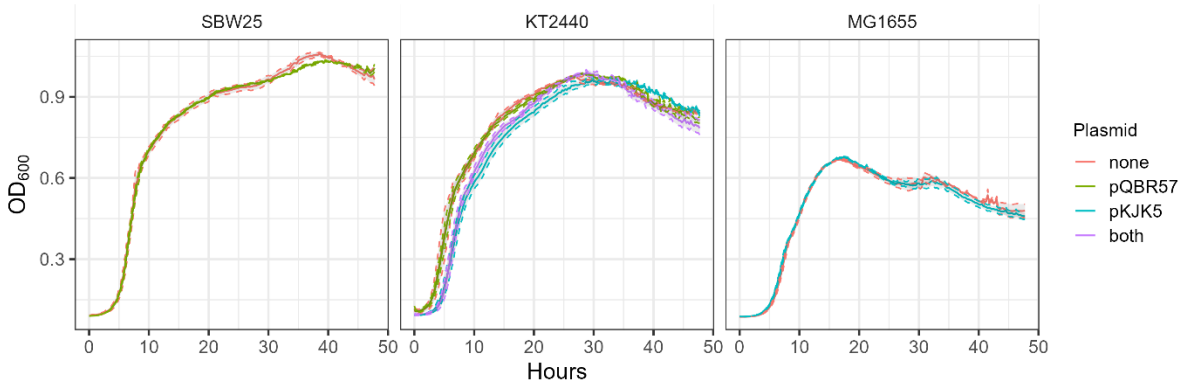

Figure S1. Plasmid fitness costs across hosts. Growth profiles of all possible subpopulations (each bacterial strain with each possible plasmid status) over 48 hours, showing the effect of the plasmids on host growth dynamics. Each time step represents 15 minutes. There are 3 biological replicates of each subpopulation.

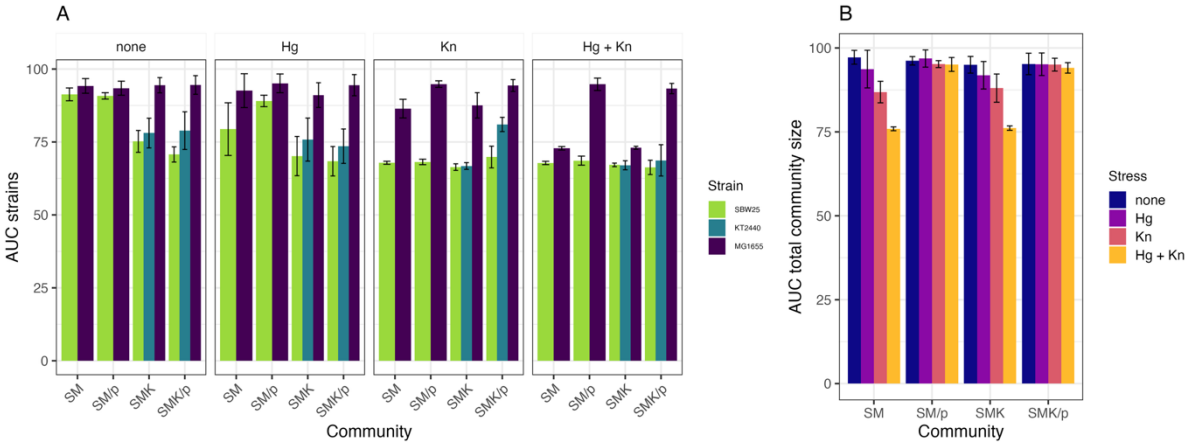

Figure S2. Areas under the curve (AUCs) for bacterial populations. (A) AUCs for strain dynamics across communities and environmental conditions. (B) AUCs for total community dynamics across environmental conditions. Bars are averages of six biological replicates; error bars show 95% confidence intervals.

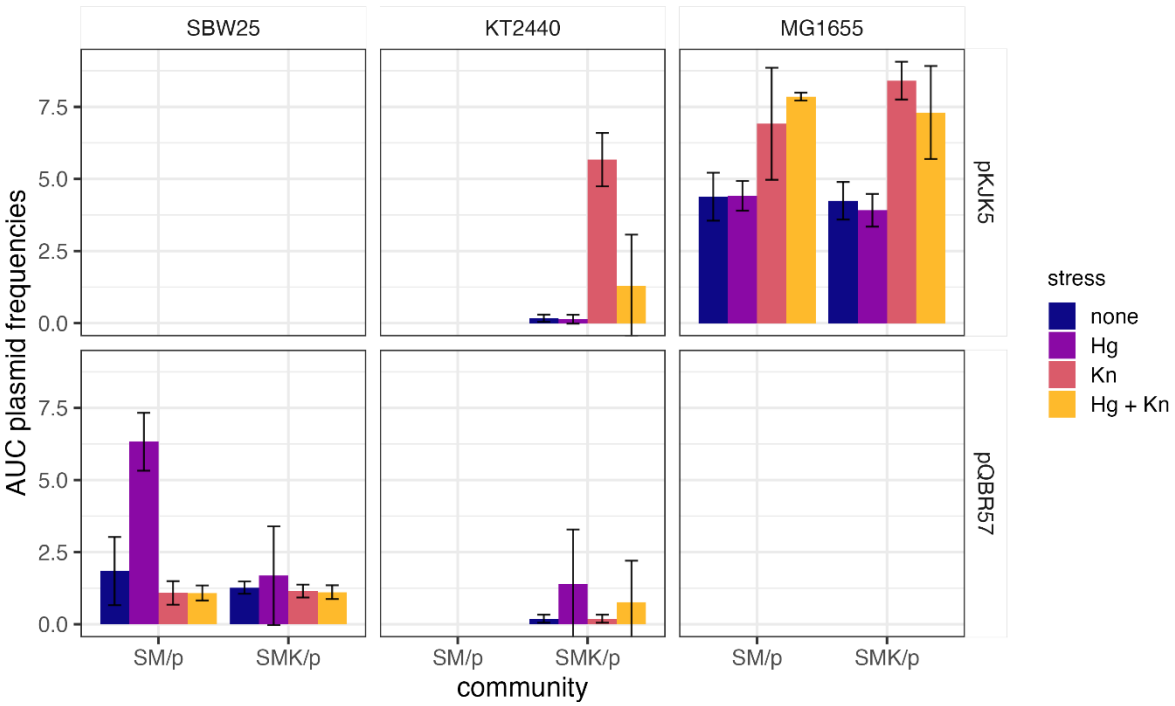

Figure S3. Areas under the curve (AUCs) for plasmids. AUCs for plasmids by host strain, across communities and environmental stresses. Bars are averages of six biological replicates; error bars show 95% confidence intervals.

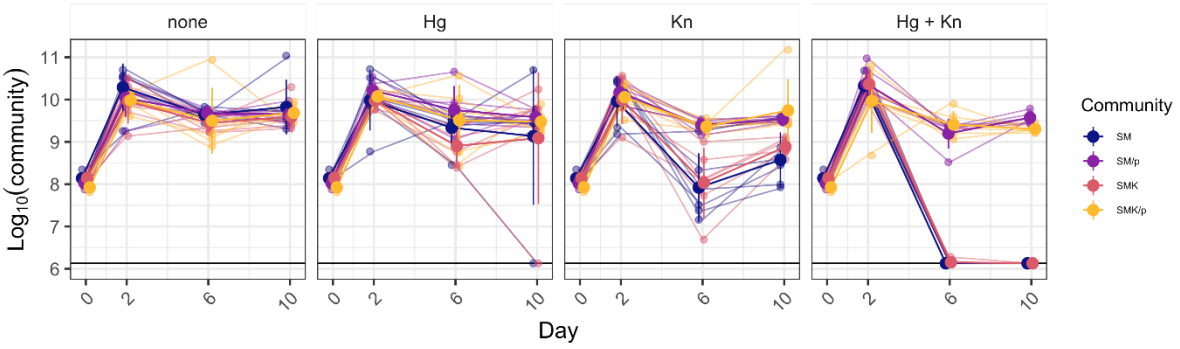

Figure S4. Total community size across environments. The total size of all bacterial populations in each community over 10 days, as affected by the environmental conditions. Small dots represent replicate values, large dots represent means of 6 biological replicates, and vertical lines represent 95% confidence intervals of the means

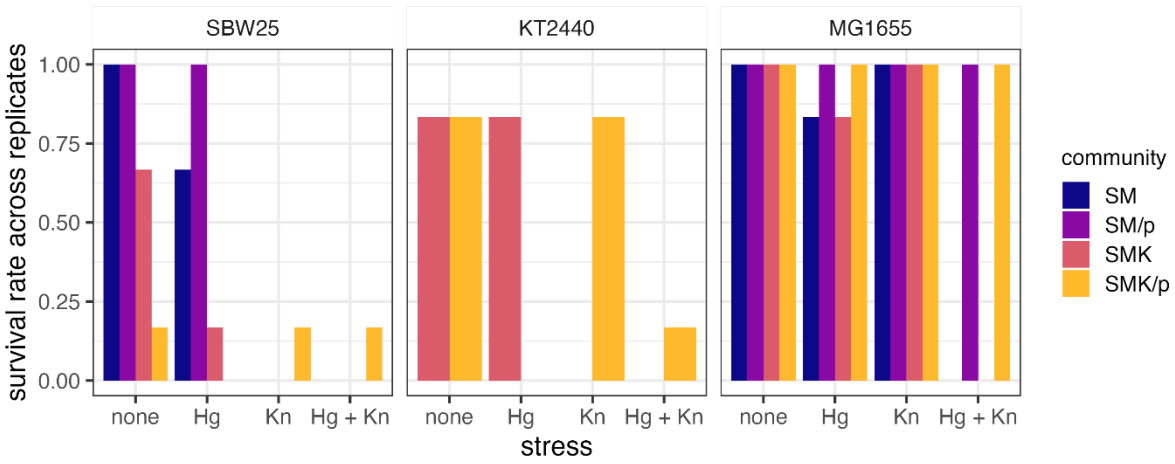

Figure S5. Strain survival. Fraction of replicates, out of six total replicates, in which each strain was detected at the final timepoint, across communities and environmental conditions. Limit of detection was 5 x 10^5 CFUs.