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1 Pre-exposure of abundant species to disturbance improves resilience in
2 microbial metacommunities

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16 Abstract

17

18 Understanding factors influencing community resilience to disturbance is critical for mitigating
19 harm at various scales, including from medication to gut microbiota and from human activity
20 to global biodiversity, yet there is a lack of data from large-scale controlled experiments.
21 Factors expected to boost resilience include prior exposure to the same disturbance and
22 dispersal from undisturbed patches. Here we set up an *in vitro* system to test the effect of
23 disturbance pre-exposure and dispersal represented by community mixing. We performed a
24 serial passage experiment on a 23-species bacterial model community, varying pre-exposure
25 history and dispersal rate between three metacommunity patches subjected to different levels
26 of disturbance by the antibiotic streptomycin. Expectedly, pre-exposure caused evolution of
27 resistance, which prevented decrease in species abundance. The more abundant the pre-
28 exposed species had been in the undisturbed community, the less the entire community changed.
29 Pre-exposure of the most dominant species also decreased abundance change in off-target
30 species. In the absence of pre-exposure, increasing dispersal rates caused increasing spread of
31 the disturbance across the metacommunity. However, pre-exposure kept the metacommunity
32 close to the undisturbed state regardless of dispersal rate. Our findings demonstrate that pre-
33 exposure is an important modifier of ecological resilience in a metacommunity setting.

34 Introduction

35

36 Ecological disturbances are events causing ecosystem change.¹ They vary in magnitude,
37 frequency and extent, with durations ranging from discrete, short-term pulse disturbances to
38 long-term or continuous press disturbances.^{2,3} Currently, ecosystems on Earth are experiencing
39 unprecedented anthropogenic disturbances owing to human activity. These include
40 disturbances associated with global climate change, such as atmospheric increases in carbon
41 dioxide, as well as those caused by using pesticides, herbicides and pharmaceuticals in
42 agriculture and medicine.

43

44 To mitigate unwanted effects of ecological disturbances, it is critical to develop a mechanistic
45 understanding of disturbance response.⁴ In particular, this means understanding the conditions
46 where disturbances compromise the structure or function, that is, the resilience of ecosystems.
47 There exist two frameworks on resilience. The engineering resilience framework is focused on
48 the return of a system to its pre-disturbance state, and can be partitioned into withstanding
49 change during a disturbance (*i.e.*, ecological resistance) and post-disturbance recovery.⁵ The
50 ecological resilience framework is focused on the degree and type of disturbance required to
51 drive a system into a different state (*i.e.*, tipping point, causing a regime shift).⁶ In this study,
52 we examine a system experiencing a constant press disturbance and therefore adopt the latter
53 framework, seeking to identify conditions driving or preventing clear shifts in the system. It is
54 critical to understand when a clear shift occurs in the state of an ecosystem as this can impair
55 ecosystem functioning or even result in community collapse.⁷⁻⁹

56

57 Prior research has identified numerous factors influencing resilience in species communities,
58 including disturbance intensity, frequency, timing, and spatial extent, and the biological level
59 affected.^{9,10} Here we focus on two key factors: pre-exposure and dispersal. First, past
60 disturbances (*i.e.*, pre-exposure) can prime communities to better cope with future disturbances
61 through mechanisms including rapid trait evolution, epigenetics and maintenance of trait
62 diversity (via genetic heterogeneity or phenotypic plasticity).⁹ In this study, we utilize rapidly
63 evolving microbes, stressing the first (and potentially last) of these mechanisms. While
64 microbes are known to rapidly evolve resistance to various stressors in one- and two-species
65 setups, only a few controlled studies have examined rapid microbial evolution or its ecological
66 effects in larger communities.¹¹⁻¹⁵ We recently subjected a multispecies microbial community

67 to antibiotic pulse disturbance and found that the intrinsic competitive fitness and antibiotic
68 susceptibility traits of the species primarily drove ecological changes despite the emergence of
69 antibiotic resistance mutations.¹⁶ However, in line with ecological literature, such evolutionary
70 trait changes could affect the community response to future disturbances. To test this in the
71 study at hand, we individually pre-exposed each species in a 23-species model bacterial
72 community to gradually increasing and ultimately high levels of antibiotic disturbance, using
73 the aminoglycoside antibiotic streptomycin. This was followed by phenotyping and whole-
74 genome sequencing thus obtained populations to identify associated trait evolution. We then
75 constructed communities with different pre-exposure histories for use in a serial passage
76 experiment to test for disturbance response (Figure 1A).

77
78 Dispersal is another critical factor affecting resilience. Communities are typically nested within
79 patches in a metacommunity with varying magnitudes of dispersal (*i.e.*, connectivity).¹⁷
80 Dispersal drives diversity, increasing local patch (alpha) and decreasing metacommunity (beta)
81 diversity.^{14,18} Dispersal varies in rate and scale, from small subpopulations to entire
82 communities (*i.e.*, community coalescence) common to microbes, with higher dispersal levels
83 expected to strengthen dispersal effects.^{19,20} However, not many studies have investigated the
84 effect of dispersal rate on the disturbance response of communities. Findings from a recent
85 study suggest that dispersal between communities experiencing low-level disturbance can
86 improve community resilience (*e.g.*, restoring lost species), while dispersal between
87 communities experiencing high-level disturbance can decrease community resilience (*e.g.*,
88 driving extinction of weaker competitors).²¹ Dispersal from an undisturbed to a disturbed patch
89 can boost resilience in a manner akin to source-sink dynamics.^{16,22} Contrariwise, dispersal from
90 a disturbed to an undisturbed patch can spread the eco-evolutionary effects of the disturbance
91 to undisturbed communities.^{23,24}

92
93 While the effects of pre-exposure to disturbance and dispersal on the community response to
94 disturbance have received some attention, there is virtually no experimental evidence on the
95 combined influence of these two factors. In the absence of pre-exposure, dispersal from
96 disturbed patches should spread effects of the disturbance across the metacommunity. A higher
97 dispersal rate should strengthen this effect. However, by boosting community resilience, pre-
98 exposure to disturbance should also prevent change of the metacommunity. Higher dispersal
99 rates should have little bearing on the outcome, potentially decreasing metacommunity
100 diversity.

101

102 To test the effect of pre-exposure and dispersal rate on ecological resilience, we performed a
103 full-factorial serial passage experiment for the 23-species model bacterial community with five
104 pre-exposure histories: communities containing (1) only naïve (i.e., ancestral) species, (2–4) a
105 streptomycin pre-exposed population of one of three abundant species, or (5) disturbance pre-
106 exposed populations of all species (Figure 1A). We divided each of the communities into three
107 patches, subjected to either no disturbance, low disturbance level, or high disturbance level
108 (different concentrations of streptomycin). To model the effect of connectivity level, we
109 subjected the sets of patches to three rates of community mixing (global connectivity, with
110 entire communities from all three patches mixed): no, low (every sixth transfer) and high (every
111 third transfer). The three patches subject to mixing constitute metacommunities. We collected
112 ecological and phenotypic data for species frequencies and antibiotic resistance for
113 communities at the end-point of the serial transfer experiment, allowing us to test the conditions
114 driving or preventing community change (Figure 1B).

115

116 Results

117

118 Pre-exposure to disturbance caused trait evolution

119

120 We used a synthetic community of 23 gram-negative bacterial species isolated from soil,
121 aquatic, plant, animal, and human sources, as described earlier²⁵. Most community members
122 display quasi-stable coexistence over dozens of serial transfers^{16,25,26}. As the species have been
123 isolated from different environments, the presence of species interactions such as cross-feeding
124 is uncertain. All the species can be cultured individually in uniform laboratory conditions, have
125 reference genomes, and have been phenotyped for various traits. These include the model
126 disturbance for this study, streptomycin, with community members displaying a wide range of
127 intrinsic susceptibility levels (Figure 2A).

128

129 Pre-exposure of three abundant community members as monocultures to increasing levels of
130 streptomycin led to increased disturbance resistance for two of the species: *Aeromonas* and
131 *Pseudomonas chlororaphis* (Figure 2A; *t*-tests on IC₅₀ values of ancestral vs. pre-exposed
132 populations with Bonferroni correction, $P < 0.001$; Supplementary Table 1). In turn, the species
133 *Citrobacter* was already intrinsically resistant prior to pre-exposure (Figure 2A).

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Whole-genome sequence data for pre-exposed populations of the 23 species supported trait evolutionary change (Figure 2B). The predominant target of recurrent nonsynonymous mutations reaching fixation or high allele frequency was the gene *rpsL*, encoding the streptomycin binding site in the small subunit of the ribosome, a known target of high-level streptomycin resistance mutations^{27,28}. These were also observed in two of the three abundant species used in the pre-exposure treatments: *Aeromonas* and *Pseudomonas chlororaphis*. Moreover, recurrent mutations occurred in *rsmG* previously associated with low-level streptomycin resistance.²⁹

One of the abundant species used in the pre-exposure treatment, *Citrobacter*, lacked mutations in *rpsL*, consistent with its intrinsic resistance and lacking the selection pressure to evolve *de novo* resistance (Figure 2A). Its phenotypic resistance is supported by genomic data, as it contains four genes (*APH(3'')-Ib*, *APH(6)-Id*, *strA*, and *strB*) encoding aminoglycoside (including streptomycin) inactivating enzymes and seven genes (*acrD*, *baeR*, *baeS*, *cpxA*, *cpxR*, *kdpE*, and *tolC*) encoding aminoglycoside efflux pumps.²⁵ Nevertheless, the pre-exposed population of *Citrobacter* did contain one low-frequency (7.5 %) mutation in the multidrug efflux encoding gene *oprM*.³⁰ In addition, there could be mutations such as structural variants that were not detected due to the use of short-read sequencing data.

Stronger disturbance led to stronger community change

In this study, we examine system change through changes in species abundance (relative abundance which is also a proxy for biomass), including alpha (within-community) diversity and beta (between-community) diversity. We examine alpha diversity through species richness and Shannon diversity, incorporating both species richness and evenness. We examine beta diversity, as previously¹⁶, through Kullback–Leibler (KL) divergence which measures the relative entropy between two distributions (here, relative abundance vectors of communities) assuming values between 0 (perfect match) and ∞ . KL divergence is more sensitive to small compositional changes at low abundances than Manhattan- (e.g., Bray-Curtis dissimilarity) and Euclidean-based measures.³¹

166 In the absence of dispersal, across the different pre-exposure histories, stronger disturbance led
167 to a stronger change in community composition relative to the disturbance-free condition
168 (PERMANOVA model on community composition excluding community mixing treatments:
169 disturbance level $r^2 = 0.42$, $P = 0.01$, pairwise comparisons for streptomycin level all $P < 0.04$;
170 Supplementary Table 2; community composition in the different treatments is visualized in
171 Figure 3 top rows and Extended Data Figures 1–3). In the presence of streptomycin, most
172 species decreased in abundance but some species with higher resistance level increased in
173 abundance (Figure 3C top row; Extended Data Figures 3 & 4; linear model for relationship
174 between intrinsic resistance level and change in frequency in the absence of streptomycin pre-
175 exposure or community mixing: coefficient of determination $R^2 \sim 0.25$ and $P < 0.001$ for both
176 low and high streptomycin levels). These frequency changes approximate changes in absolute
177 abundance owing to relatively constant community biomass levels in our experiment (Extended
178 Data Figures 5 & 6). Consistent with resistant cells being favored with streptomycin, in the
179 absence of dispersal, streptomycin level explained most of the variation (68.0 %) in the half-
180 maximal inhibitory concentration (IC_{50}) values of eight clones isolated at random from each
181 experimental end-point community (Extended Data Figure 7; Supplementary Tables 3–6).

182

183 Streptomycin level also influenced community diversity. Higher Shannon diversity occurred at
184 low streptomycin level compared to no streptomycin or high level (least diversity; Extended
185 Data Figure 8; Supplementary Table 7). This was strongly influenced by the dominant species
186 *Aeromonas* whose population collapsed with streptomycin. This increased evenness at low
187 streptomycin level, while high streptomycin level expectedly decreased diversity by driving
188 species extinctions and competitive dominance of particular resistant species. This finding is
189 consistent with the intermediate disturbance hypothesis positing that increasing disturbance
190 levels initially increase diversity by reducing the abundance of competitively dominant
191 species³². Nevertheless, the effect only holds for the *Aeromonas* species, as its pre-exposure to
192 streptomycin removes the effect (Extended Data Figure 8). Similarly, the effect was removed
193 when all species were pre-exposed to streptomycin, without Shannon diversity reduction even
194 at high disturbance level. Species richness alone displayed a gradual drop at increasing
195 streptomycin level with the extinction of susceptible species (Extended Data Figure 9;
196 Supplementary Table 8). Similar to its effect on Shannon diversity, in the presence of
197 streptomycin, pre-exposure of all species in the community maintained species richness.

198

199 **Pre-exposure decreased impact of disturbance on community**

200

201 Although streptomycin drove most of the variation in community composition, pre-exposing
202 community members to streptomycin itself also had a minor effect on composition (Extended
203 Data Figures 1, 2 & 10; Supplementary Table 9). Since this study focuses on streptomycin as a
204 model disturbance, pre-exposure was not treated as a disturbance. Instead, to control for the
205 effect of pre-exposure, community data was examined relative to the streptomycin-free
206 composition within each pre-exposure treatment.

207

208 Pre-exposure led to maintaining the abundance of more susceptible species that otherwise
209 declined (Supplementary Figure 1; ANOVA for linear model on frequency change of focal
210 species in the absence of community mixing: streptomycin level $F_{2,54} = 238.4$, $P < 0.001$;
211 absence/presence of pre-exposure $F_{1,54} = 1778$, $P < 0.001$; species $F_{2,54} = 1195$; all interactions
212 $P < 0.001$; Supplementary Table 10). Without pre-exposure, the population of only one
213 relatively susceptible species (Figure 2A), *Aeromonas*, collapsed at low streptomycin level. In
214 turn, regardless of pre-exposure, the population of the intrinsically resistant species *Citrobacter*
215 increased in the presence of streptomycin.

216

217 At the community level, pre-exposure of a abundant susceptible species (*Aeromonas* and
218 *Pseudomonas chlororaphis*) caused a decrease in compositional change at high streptomycin
219 level compared to the absence of pre-exposure (Figure 4). This was caused by two factors. First,
220 maintaining abundance of the focal species itself decreased total community change (Figure
221 4A; Supplementary Figure 2). Second, for three out of four replicate communities for the most
222 abundant and relatively susceptible *Aeromonas* species, the non-focal community fraction was
223 also protected from change (Figure 4B; Tukey post-hoc test for non-focal community:
224 *Aeromonas* vs. *Pseudomonas chlororaphis*, $P = 0.061$; *Aeromonas* vs. *Citrobacter*, $P = 0.042$;
225 Supplementary Table 11). The same result was found for compositional (i.e., directional)
226 change as for the magnitude of change, such that only for pre-exposed *Aeromonas*, composition
227 in the non-focal community fraction was significantly altered compared to the absence of pre-
228 exposure (PERMANOVA model on community composition in the control condition without
229 mixing or streptomycin: *Citrobacter* pre-exposure $r^2 = 0.12$, $P = 0.40$; *Aeromonas* pre-exposure
230 $r^2 = 0.56$, $P = 0.02$; *Pseudomonas chlororaphis* pre-exposure $r^2 = 0.18$, $P = 0.30$;
231 Supplementary Table 2). This effect includes, for example, better maintenance of *Hafnia alvei*
232 and *Kluyvera intermedia* (Figure 3C top right).

233

234 Since *Aeromonas* occupies up to 80 % of the community without streptomycin, it is likely to
235 strongly influence the resource environment. Its loss with streptomycin would represent a
236 major additional disturbance, explaining why its maintenance with pre-exposure protects also
237 certain other species from change. Consistent with this, at high streptomycin level without
238 dispersal, the pre-exposure of *Aeromonas* led to the second lowest level of total community
239 change after the pre-exposure of all community members (Supplementary Figure 3; ANOVA
240 for linear model on KL divergence of communities from pre-exposure treatment specific
241 baseline at experimental end-point at high streptomycin level in the absence of community
242 mixing: pre-exposure treatment $F_{4,15} = 867$, $P < 0.001$; Tukey post-hoc test on all species pre-
243 exposed vs. other treatments and *Aeromonas* pre-exposed vs. other treatments, all comparisons
244 $P < 0.001$; Supplementary Table 12).

245

246 **Dispersal spread patch features to metacommunity**

247

248 Expectedly, dispersal spread patch features into the three-patch metacommunity (Figure 3;
249 Extended Data Figures 1–3). This caused intermediate species richness across the
250 metacommunity when compared to the patches in the absence of dispersal, with Shannon
251 diversity differences between the patches decreasing for most pre-exposure treatments from
252 low to high dispersal rates (Extended Data Figures 8 & 9; Supplementary Tables 7 & 8).
253 Consistent with this, dispersal spread streptomycin resistant cells across the metacommunity
254 from the high-streptomycin patch, such that the level of variation in IC_{50} values in clones
255 isolated from the experimental end-point explained by streptomycin in the individual patches
256 decreased from 68.0 % at no mixing through 26.6 % at low mixing rate to 8.5 % at high mixing
257 rate (Extended Data Figure 7; Supplementary Tables 3–6). Therefore, consistent with theory,
258 dispersal decreased metacommunity (beta) diversity (Supplementary Figure 3; ANOVA for
259 linear model on KL divergence of communities from pre-exposure treatment specific baseline:
260 community mixing rate $F_{2,135} = 44.7$, $P < 0.001$; community mixing rate \times streptomycin level
261 $F_{4,135} = 24.3$, $P < 0.001$; community mixing rate \times pre-exposure treatment $F_{8,135} = 25.0$, $P <$
262 0.001 ; Tukey HSD for pairwise comparisons on community mixing rate, no mixing vs.
263 low/high mixing rate $P < 0.001$; Supplementary Table 13).

264

265 To examine the effect of dispersal rate on resilience, we computed the mean composition of the
266 communities across the three streptomycin levels for each pre-exposure treatment. This
267 represents a null scenario where the dispersal treatment composition is simply the average of
268 the composition in the three patches. We then tested whether community composition in the
269 low or high dispersal rate treatments differed from this null scenario. If the composition
270 significantly differs from the average composition at a particular dispersal rate, the dispersal
271 rate disproportionately favors the spread of particular patch effects across the metacommunity
272 rather than evenly homogenizing composition across the patches. In the absence of pre-
273 exposure, the low dispersal rate corresponded to the null scenario whereas the high dispersal
274 rate differed significantly from the null model and low dispersal rate (PERMANOVA model
275 for all ancestral community: dispersal rate $r^2 = 0.63$, $P = 0.01$; pairwise comparisons: null
276 model vs. low $P = 0.228$, null vs. high $P = 0.003$, low vs. high $P = 0.003$; Supplementary Table
277 2). This corresponded to a high magnitude of change from the streptomycin- and dispersal-free
278 baseline community at high dispersal rate, representing decreased resilience at high compared
279 to low mixing rate (Figure 5A left; ANOVA for linear model on KL divergence of communities
280 from the streptomycin- and dispersal-free condition: dispersal rate $F_{1,44} = 78.2$, $P < 0.001$;
281 Supplementary Table 14). When comparing against the other dispersal-free patches, the
282 dispersal rate leads all patches closer to the high streptomycin scenario (Figure 3B, left). This
283 also applied to some individual replicates within the low dispersal rate communities, seen as
284 heightened variance between replicates with low dispersal rate, suggesting that the low
285 dispersal rate used in this study was close to a community tipping point (Figure 5B; ANOVA
286 for linear model on effect of streptomycin disturbance and dispersal on variance between
287 replicate communities: dispersal rate $F_{2,36} = 6.1$, $P = 0.005$; Tukey post-hoc test for low vs.
288 no/high dispersal rate both $P = 0.02$, no vs. high $P = 1.0$; Supplementary Table 15).

289

290 **Pre-exposure removed dispersal-resilience relationship**

291

292 Protection of the community through pre-exposure removed the negative association between
293 dispersal rate and resilience (results shown for *Aeromonas* in Figure 5A; Supplementary Figure
294 3). For the pre-exposure of the dominant *Aeromonas caviae* species or all species, this was seen
295 as only minor community change at low dispersal rate compared to the baseline
296 (Supplementary Figure 3) as well as lack of compositional difference between the low and high
297 dispersal rate treatments (PERMANOVA model for *Aeromonas*: dispersal rate $r^2 = 0.31$, $P =$
298 0.01 ; pairwise comparisons: null model vs. low $P = 0.009$, null vs. high $P = 0.003$, low vs. high

299 $P = 0.249$; PERMANOVA model for all pre-exposed: dispersal rate $r^2 = 0.23$, $P = 0.02$;
300 pairwise comparisons: null model vs. low $P = 0.192$, null vs. high $P = 0.033$, low vs. high $P =$
301 0.194 ; Supplementary Table 2). Therefore, pre-exposure of *Aeromonas* or all species avoided
302 spread of the streptomycin scenario across the metacommunity at high dispersal rate (for
303 *Aeromonas*, see Figure 3B, right). For pre-exposed *Citrobacter* and *Pseudomonas chlororaphis*,
304 where some community change occurred at low mixing rate (Supplementary Figure 3), this
305 was seen as smaller compositional change from the baseline at high compared to low dispersal
306 rate (Supplementary Figure 3), with low dispersal rate composition being closer to the equal
307 mixing ratio null model (PERMANOVA model for *Citrobacter*: community mixing rate $r^2 =$
308 0.31 , $P = 0.01$; pairwise comparisons: null model vs. low $P = 1.00$, null vs. high $P = 0.006$, low
309 vs. high $P = 0.030$; PERMANOVA model for *Pseudomonas chlororaphis*: community mixing
310 rate $r^2 = 0.34$, $P = 0.02$; pairwise comparisons: null model vs. low $P = 1.00$, null vs. high $P =$
311 0.009 , low vs. high $P = 0.030$; Supplementary Table 2). Therefore, pre-exposure of abundant
312 species can strongly influence the relationship between dispersal and resilience.

313

314 Discussion

315

316 Here we tested how pre-exposure to disturbance and dispersal influence disturbance response
317 in 23-species metacommunities. As predicted, pre-exposure caused trait evolution (Figure 2),
318 decreasing the effect of the disturbance on the disturbed communities⁹ (Figure 4) and thereby
319 also on the metacommunity (Figure 5A). Moreover, expectedly, dispersal homogenized species
320 composition and traits across the metacommunity, reducing beta diversity compared to lack of
321 dispersal (Figure 3A).^{14,18,21} Since the different patches experienced different levels of
322 disturbance, this resulted in decreased diversity in the undisturbed and increased diversity in
323 the high-disturbance patch (Extended Data Figures 8 & 9; Supplementary Tables 7 & 8).^{16,22-24}
324 In the absence of pre-exposure, higher dispersal rates facilitated spread of disturbance effects,
325 decreasing metacommunity resilience (cf. ²¹) but this was cancelled by pre-exposure of
326 abundant community members (Figure 5A). These results show that the dispersal-resilience
327 relationship depends on dispersal rate, and that the relationship is critically altered by pre-
328 exposure of important community members.

329

330 There are several limitations in the current study that warrant future investigation. First, the
331 mechanism underlying the negative relationship between dispersal rate and community

332 resilience in uncertain. It could, for instance, be caused by decreased recovery time for the
333 undisturbed patch between dispersal events at high dispersal rate. As we only collected end-
334 point data, future studies including sampling over time are needed to test this hypothesis.
335 Second, we found that pre-exposure of the dominant *Aeromonas* species to disturbance altered
336 the abundance of also the other community members, protecting the community from change
337 with disturbance, but the reason for this should be addressed by future studies. A focal species
338 could alter community-wide species composition through competition for shared resources,
339 altering the resource landscape for the other species or through species interactions such as
340 producing useful or harmful metabolites³³⁻³⁵. Stress conditions may also change the nature of
341 species interactions.^{36,15,37,38}

342
343 Third, our decision to choose focal species for the pre-exposure treatments based on their
344 abundance could have led to the oversight of important species. Although in our setup changes
345 in the single dominant *Aeromonas* species explained most of the variance in all experimental
346 outcomes, it has generally been established that low-abundance species can also be critical for
347 community functioning, such as cross-feeding networks.³⁹ Therefore, in the future,
348 compositional data should be complemented by functional (e.g., transcriptomic or
349 metabolomic) data to inspect how species loss and evolutionary change alters metabolic
350 pathways.

351
352 Fourth, in our study setup, dispersal was modeled by mixing entire communities. The high
353 magnitude of dispersal is likely to have influenced the study results. For instance, very low
354 dispersal rates may lead to species-poor communities with vacant niches^{20,40}, which was likely
355 averted in the low dispersal rate treatment in this study owing mixing entire communities.
356 Future studies varying both the magnitude and rate of dispersal are required to better elucidate
357 these dynamics. An advantage of mixing entire communities is that our results may have
358 implications for extending existing community coalescence theory^{19,41,42} to a dynamic temporal
359 setting.

360
361 Fifth, we observed a strong capacity of pre-exposure, particularly for the most abundant and
362 stress susceptible community members, to decrease the effect of the disturbance, also
363 cancelling the spread of disturbance across the metacommunity at high dispersal rate (Figure
364 5A). This demonstrates the potential of rapid evolution to improve ecological resilience,
365 including in a metacommunity setting. Notably, however, we designed our experimental setup

366 to quantify the maximum potential of rapid evolution by pre-exposing the species to be highly
367 stress resistant. Moreover, we pre-exposed the species by exposing them to increasing levels
368 of stress. The capacity for rapidly evolvable species to evolve *de novo* stress resistance is likely
369 to be constrained during sudden exposure to high-level disturbance and when nested in a
370 multispecies community¹³. In a multispecies community, susceptible species can rapidly
371 become outcompeted by intrinsically resistant species and have lower population sizes
372 (potentially decreasing evolvability) compared to when cultured alone, and a community
373 context has been shown to constrain adaptation.⁴³

374

375 One reason for our setup, maximizing the impact of rapid evolution through pre-exposure to a
376 high streptomycin concentration without a community context, was that we had previously
377 failed to observe a clear effect of rapid evolution on community dynamics when communities
378 consisting of initially unevolved species were exposed to antibiotic pulses¹⁶. Similar to that
379 study, *de novo* evolution of streptomycin resistance is also likely to have occurred in multiple
380 species during our study, although we lack the phenotypic and genomic data to test this
381 explicitly. Due to these previous findings from the same community exposed to the same
382 antibiotic for a similar duration, we consider it unlikely that *de novo* evolution would be
383 important for the dynamics in this system. Nevertheless, we did find some signals potentially
384 indicating a minor influence of *de novo* evolution on community dynamics. For instance, in
385 individual replicate communities, certain species with low IC₅₀ values (*Hafnia*, *Kluyvera*, and
386 *Paraburkholderia*) increased in frequency at high streptomycin level (Figure 3C top row).
387 Moreover, in one non-pre-exposed replicate community (D, low mixing rate; Figure 3C middle
388 left), the population of the relatively susceptible species *Aeromonas* did not collapse as in all
389 other communities lacking its pre-exposure. These cases may indicate the influence of *de novo*
390 resistance evolution causing evolutionary rescue of these species in individual communities.
391 The relatively brief time frame of these studies is a limitation, since there is limited time for
392 rare *de novo* resistance mutations occurring at different times in the experiment to increase to
393 high allele frequency and exert community-wide impacts. This may explain why initial species
394 characteristics seems to drive these systems, and future studies with longer time frames may
395 show greater community consequences of *de novo* evolution.

396

397 Our findings are relevant for designing successful control interventions to improve ecological
398 resilience in natural communities. When there is absence of pre-exposure or low potential for
399 rapid evolutionary change, low levels of dispersal between disturbed and undisturbed patches

400 may facilitate metacommunity resilience. Our previous study suggests that one-way
401 immigration from an undisturbed (*i.e.*, source) to a disturbed patch is ideal for resilience, but
402 this may not always be achievable¹⁶. However, our findings in this study suggest that with high
403 levels of dispersal, there is a risk of spreading eco-evolutionary effects of disturbances across
404 metacommunities (see also ²¹). Nevertheless, when there is high potential for evolutionary
405 change in response to the disturbance, or if pre-exposed populations of abundant taxa are
406 introduced into a community, rapid trait evolution can be harnessed to protect from the
407 disturbance at both local (patch) and global (metacommunity) levels.

408

409 Exploiting these features of rapid evolution and dispersal could be an effective tool for
410 promoting compositional resilience in species communities facing environmental change.
411 Nevertheless, this approach is also accompanied by risks. Reduced within-species diversity¹⁶
412 or pleiotropic effects of resistance mutations^{44,45} in an evolved species may cause community-
413 wide changes in composition (as observed here) and function, or affect the viability and
414 resilience of the evolved species. Exploiting evolution and connectivity may still be considered
415 worthwhile, as it is increasingly acknowledged that control interventions to steer ecology and
416 evolution virtually always carry associated costs due to the complexity of biological systems^{4,9}.
417 An optimal control strategy for a given eco-evolutionary system, including one seeking to
418 improve resilience, is one that strikes a balance between the importance of achieving a
419 particular target and the importance of minimizing associated costs.

420

421 Methods

422

423 **Synthetic bacterial community and experimental evolution**

424

425 All experiments used synthetic assemblages of 23 different soil, water, and host-associated
426 bacterial species (Supplementary Table 16). Before the main experiment, all 23 species were
427 experimentally evolved in two steps to have a maximum range of potential phenotypic and
428 genotypic diversity derived from streptomycin exposure, thereby mimicking natural
429 communities containing a legacy of past disturbance exposure within genetically
430 heterogeneous populations. In the first high-resistance generation step, monocultures of each
431 species were grown in sub-minimal inhibitory concentrations (sub-MICs) of streptomycin
432 (MICs from ²⁵) in Protease Peptone Yeast Extract (PPY) medium for 24–72 hours at 28 °C.

433 Monocultures were then serially transferred (96 deep well plates; 1,500 μ L PPY; 3 % transfer
434 volume, 48-hour transfer interval; 28 $^{\circ}$ C, shaking at 1000 rpm) with streptomycin
435 concentrations doubling every transfer. The transfer series was stopped when bacterial optical
436 density (OD) fell below 0.1 OD units (Supplementary Table 16), and 1 mL of the previous
437 culture was frozen in 30 % glycerol. In the second diversity generation step, evolved
438 populations and ancestral forms of each species were revived from -80 $^{\circ}$ C and precultured (6
439 mL Reasoner's 2A medium, R2A; 28 $^{\circ}$ C; shaking at 50 rpm; 96 hours in total), then mixed in
440 an equal ratio, and grown for 48 hours in duplicate (96 deep well plates; 1,500 μ L PPY; 3 %
441 transfer volume; 28 $^{\circ}$ C; shaking at 1,000 rpm) at 12 different streptomycin concentrations (0,
442 1, 5, 10, 20, 50, 100, 200, 500, 1000, 2000 and 5000 μ g mL $^{-1}$). After this round of growth, 100
443 μ L from each streptomycin concentration was combined per species. Each evolved population
444 was frozen in 40 % glycerol for later experimental use.

445

446

447 **Serial passage experiment and measurements**

448

449 The main experiment was initiated with bacterial mixtures manually assembled into five pre-
450 exposure histories: only ancestral forms of the 23 species (“all ancestral”), 22 ancestral species
451 plus one experimentally pre-exposed population from one of three abundant species
452 (*Citrobacter koseri* HAMBI 1287, *Aeromonas caviae* HAMBI 1972, or *Pseudomonas*
453 *chlororaphis* HAMBI 1977), and experimentally pre-exposed populations of all 23 species (“all
454 pre-exposed”). These bacterial mixtures were assembled by first reviving the ancestral form
455 and the pre-exposed population of each species from $-80\text{ }^{\circ}\text{C}$ and preculturing them for 24 hours
456 in R2A (6 mL; $25\text{ }^{\circ}\text{C}$; 50 rpm). One mL from each preculture was combined into one of the five
457 pre-exposure treatments above, briefly mixed by vortexing, and then divided into four parts.
458 These four parts were then used as the four replicate inoculae per evolutionary history for the
459 main experiment. The initial species compositions differed slightly between the pre-exposure
460 treatments (Supplementary Figure 4). Notably, the species *Brevundimonas bullata* HAMBI 262
461 and *Chitinophaga sancti* HAMBI 1988 were not present at detectable levels in the community
462 stock used to initiate the all pre-exposed treatment. Among these species, *Brevundimonas*
463 *bullata* HAMBI 262 was detected in the all pre-exposed treatment at the experimental end-
464 point (Extended Data Figure 1). *Chitinophaga sancti* HAMBI 1988 was present in the other
465 treatments at very low levels at the experimental end-point, suggesting that its absence from
466 the all pre-exposed treatments is unlikely to have influenced the findings in the study.
467 Originally, the pre-exposure treatment included also the species *Pseudomonas putida* HAMBI
468 6, *Agrobacterium tumefaciens* HAMBI 105, and *Sphingobacterium spiritivorum* HAMBI 1896.
469 However, whole-genome sequence analysis of the pre-exposed populations of these species
470 used to initiate the serial passage experiment showed them to be contaminated with other pre-
471 exposed species. They were therefore omitted from the analysis.

472

473 Next, three different streptomycin concentrations (0, 20, or $1,000\text{ }\mu\text{g mL}^{-1}$) representing three
474 levels of disturbance were applied to each replicate per evolutionary history. Finally, a
475 community mixing treatment was nested within replicates of each evolutionary history to
476 simulate differing amounts of connectivity between streptomycin “patches.” In the no-mixing
477 treatment, each streptomycin patch was serially transferred to the same streptomycin patch. In
478 the low mixing rate treatment, the three streptomycin patches were thoroughly mixed every 12
479 days (six transfers), and this mixture was used to inoculate all streptomycin patches in the next
480 transfer. The high mixing rate treatment was the same as the low mixing rate treatment, but

481 mixing occurred every six days (three transfers). The mixing treatment consisted of mixing
482 equal volumes of all three streptomycin levels (0/20/100 $\mu\text{g mL}^{-1}$), resulting in a concentration
483 in the mix of 353 $\mu\text{g mL}^{-1}$. The protocol leads to a maximum streptomycin concentration of
484 approximately 10 $\mu\text{g mL}^{-1}$ (assuming no degradation of streptomycin during previous culture
485 cycle) after the first transfer following mixing, and negligible amounts thereafter prior to the
486 next mixing event (e.g., 0.3 $\mu\text{g mL}^{-1}$ after second transfer). Such a residual streptomycin level
487 may have imposed some selection in the streptomycin-free patch in the first culture cycle after
488 community mixing, as it exceeds the IC_{50} value of three low-abundance species in the
489 community (*Acinetobacter lwoffii* HAMBI 97, *Microvirga lotononidis* HAMBI 3237, and
490 *Paraburkholderia caryophylli* HAMBI 2159; Figure 2A). However, this residual level should
491 not affect the competitive dynamics of the two dominant species *Citrobacter koseri* HAMBI
492 1287 (high-resistance) and *Aeromonas caviae* HAMBI 1972 (more susceptible), as the growth
493 of the latter is not impaired until concentrations exceeding 16 $\mu\text{g mL}^{-1}$ (Supplementary Figure
494 5). Therefore, in the community mixing treatment, the dominance of either strain should be
495 driven purely by density-dependent effects based on the intrinsic or evolved traits of the strains
496 (growth and resistance) in the different streptomycin patches.

497

498 Experimental microcosms were maintained for 23 serial transfers (96 deep well plates; 1,500
499 μL R2A; 3% transfer volume; 48-hour transfer interval; 25 $^{\circ}\text{C}$, shaking at 1,000 rpm) in the
500 appropriate streptomycin concentration. For the low and high mixing rate treatments, 780 μL
501 from the three streptomycin concentrations were pooled, vortexed, and used as the next
502 inoculum in the series. Optical density (600 nm) was measured every 48 hours (Extended Data
503 Figure 5). After the 23rd transfer, an aliquot was cryopreserved (40 % glycerol) to be revived
504 later for the dose-response analysis (see below). The remainder of each sample was
505 destructively harvested to collect material for DNA extraction and amplicon sequencing.

506

507 **Sequencing and bioinformatics**

508

509 Bulk DNA was extracted from a 500 μL aliquot of experimental samples (cryopreserved in
510 40 % glycerol) using the DNeasy 96 Blood & Tissue Kit (Qiagen) according to the
511 manufacturer's instructions. The V3–V4 hypervariable region of the 16S rRNA gene was
512 amplified from total community DNA following the standard Illumina 16S Metagenomic
513 Sequencing Library Preparation protocol (Illumina, San Diego). Briefly, the protocol uses the
514 primer pair PCR1_Forward (50 bp): 5'–

515 TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG–3’,
516 PCR1_Reverse (55 bp): 5’–
517 GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC–
518 3’ in a limited-cycle PCR reaction, then attaches Nextera XT barcodes using a dual-index
519 arrangement. The libraries were then pooled and sequenced on an Illumina MiSeq using paired
520 300-bp reads and MiSeq v3 reagents at the Finnish Institute of Molecular Medicine (FIMM).
521 Library indices were subsequently demultiplexed using bcl2fastq v2.2. Paired-end 16S rRNA
522 amplicon reads were then quality trimmed, merged, filtered, and mapped to a reference of the
523 16S rRNA gene from the 23 species as previously described⁴⁶. Prior to data analysis, species
524 counts were normalized by species-specific 16S rRNA gene copy number.

525

526 Genomic DNA from ancestral forms and one replicate of the evolved populations was extracted
527 using the DNeasy 96 Blood & Tissue Kit (Qiagen) from 24-hour overnight cultures grown in
528 PPY medium. Sequencing of genomic DNA was performed at SeqCenter
529 (<https://www.seqcenter.com/>). Sample libraries were prepared using the Illumina DNA Prep kit
530 and IDT 10 bp UDI indices and sequenced on an Illumina NextSeq 2000, producing 2 × 151
531 bp reads. Demultiplexing, quality control, and adapter trimming were performed with bcl-
532 convert (v3.9.3).

533

534 To ensure the pre-exposed starting populations were axenic, reads were competitively mapped
535 against a set of closed reference genomes using bbsplit (sourceforge.net/projects/bbmap/). This
536 tool simultaneously maps reads against several reference genomes and identifies the best-
537 matching genome for each read pair. We excluded all read pairs mapping ambiguously to more
538 than one reference genome (i.e., multiple mapping positions within a containment threshold of
539 the top-scoring mapping position) but kept reads that mapped ambiguously within a single
540 genome. Starting populations with significant contamination from other species were discarded
541 from further analysis. The purity of the starting populations was then verified via PCR of the
542 16S rRNA gene (primers 27F, 1492R) and Sanger sequencing, to ensure that only one template
543 was present in the sequencing reaction. Sanger sequencing traces of all replicates of *Citrobacter*
544 *koseri* 1287, *Aeromonas caviae* 1972, and *Pseudomonas chlororaphis* 1977 did not have
545 multiple peaks at any position, confirming the taxonomic purity from competitive read
546 mapping.

547

548 The competitive mapping process generated a set of read pairs unique to the expected species
549 from each experimentally evolved population. Evolved species with <25× coverage of the
550 target genome (HAMBI 97, 105, 262, 1988, and 3237; Table S16) were excluded from
551 downstream analysis. Taxonomically verified read pairs were mapped to closed reference
552 genomes for each species⁴⁷ using BWA-mem v0.7.17⁴⁸. Alignment files were preprocessed
553 with GATK v4.4 following best practices.⁴⁹ Mutect2 from GATK v4.4⁵⁰ was used to call
554 genomic variants using default parameters, and mutect calls were filtered to exclude spurious
555 calls using FilterMutectCalls with the --microbial-mode option. Filtered variants were
556 annotated using SnpEff v4.3.⁵¹ Gene calls were from Prokka v1.14.6.⁵² Functional annotations
557 of genes were derived from the Prokka internal database and the eggNOG 6.0 database⁵³ using
558 eggNOG-mapper v2.1.10.⁵³

559

560 **Inference of IC₅₀ values**

561

562 Using a dose-response curve analysis, half maximal inhibitory streptomycin concentrations
563 (IC₅₀) were estimated for the ancestral forms of each bacterial species, the pre-exposed
564 populations of the three abundant species used in the pre-exposure treatment (*Citrobacter*
565 *koseri* HAMBI 1287, *Aeromonas caviae* HAMBI 1972, and *Pseudomonas chlororaphis*
566 HAMBI 1977), and for clones randomly picked from the final time point in the experiment.
567 Clones of ancestral/evolved forms of each species and from the experiment end point (i.e., day
568 46) were picked from agar plates and precultured for 24 hours in PPY medium, then inoculated
569 at a density of 0.01 OD₆₀₀ into 200 μL R2A medium at streptomycin concentrations ranging
570 from 0, 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024, 2048, 4096, and 8192 μg mL⁻¹. Cultures
571 were grown for 48 hours at 25 °C in 96 well-plates with shaking (1,000 rpm), and culture
572 density was assessed at 48 hours using optical density (OD₆₀₀). For the strains used to initiate
573 the experiment, four replicate dose-response experiments were performed for each ancestral
574 species (four clones per species) and 64 for each population of the three pre-exposed species.
575 For the experimental end point communities, eight clones were isolated and tested from each
576 of the 180 communities (1,440 clones in total).

577

578 Dose-response curves were fit to the resulting blank-corrected optical density data from each
579 species or clone following Sebaugh⁵⁴ but using a four-parameter log-logistic function of the
580 form

581

582

$$f(x) = c + \frac{d - c}{1 + \exp(b(\log(x) - \log(e)))}$$

583

584 where c is the lower asymptote, d is the upper asymptote, b is the slope at the inflection point,
585 and e is the IC_{50} value or the antibiotic concentration where the growth (optical density) is at
586 half the maximum value. The log-logistic function was fit to the optical density measurements
587 using the Levenberg-Marquardt Nonlinear Least-Squares Algorithm implemented in
588 `minpack.lm v1.2-4` in R v4.2.2. IC_{50} values were set to the maximum tested streptomycin
589 concentration when optical density was always greater than 0.2 OD_{600} units and did not
590 decrease across the assayed streptomycin concentration range in a sigmoid shape with a clearly
591 defined upper and lower asymptote.

592

593 **Downstream data analyses**

594

595 All downstream analyses were performed in the R v4.2.3 environment⁵⁵. The t-distributed
596 stochastic neighbor embedding (t-SNE) map for Figure 3B and Extended Data Figure 2 was
597 created using the `Rtsne` package⁵⁶ with the options `perplexity = 20` and `theta = 0.5`.
598 Permutational analysis of variance (PERMANOVA)⁵⁷ as implemented in the `adonis` function
599 in the `vegan` package⁵⁸ was used to test whether the antibiotic level, community mixing rate or
600 pre-exposure treatment affected community composition. The method tests the probability that
601 the observed distances between groups could arise by chance by comparing them with random
602 permutations of the raw data.⁵⁹ The influence of the experimental treatments on IC_{50} and KL
603 divergence values relative to the pre-exposure history specific baseline (streptomycin- and
604 mixing-free condition) was investigated using linear regression models.

605

606 **Data availability**

607

608 Raw sequence data (fastq files) has been deposited in the NCBI Sequence Read Archive (SRA)
609 under the accession PRJNA1126612. Pre-processed data on the growth of pre-exposed species
610 at different streptomycin concentrations, genomic variants of pre-exposed species, community
611 size in the main experiment, and community composition in the main experiment is available
612 in Zenodo: <https://doi.org/10.5281/zenodo.14015860>.⁶⁰

613

614 **Code availability**

615

616 All code needed to reproduce the downstream analyses and figures are available in Zenodo:

617 <https://doi.org/10.5281/zenodo.14015860>.⁶⁰

618

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620

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624

625 **Author Contributions Statement**

626

627 Design of serial passage experiment: TH, SH. Performing serial passage experiment: EA.

628 Design of data analysis: JC, SH, VM. Performing data analysis: JC, SH. Interpreting results:

629 JC, TH, SH, VM. JC wrote the first manuscript draft, with contributions from all authors. All

630 authors approve the final version of the manuscript.

631

632 **Competing Interests Statement**

633

634 The authors state that they have no competing interests.

635 Figure legends

636

637 **Figure 1 | Design of experiment to test effect of pre-exposure and community mixing rate on disturbance**
638 **response in multispecies community. (A)** The experimental system consisted of a 23-species bacterial model
639 community where evolutionary history H was modified by pre-exposing each species in a monoculture to the
640 disturbance, the antibiotic streptomycin, and constructing communities with no pre-exposed species, one among
641 three abundant species being pre-exposed, or all 23 species being pre-exposed. **(B)** These communities were each
642 subjected to a regime of three rates of community mixing M (no, low or high) between three patches experiencing
643 different levels of disturbance (*i.e.*, patch harshness D : no, low or high antibiotic level), thereby constituting
644 metacommunities. Each unique treatment combination was replicated four times, making up a total of 180
645 communities (five pre-exposure treatments, three rates of community mixing, three disturbance patches, and four
646 replicates). Communities were serially passaged (1 % volume) to fresh medium every 48 hours for 22 transfers
647 (46 days). Low and high rates of community mixing (modeling connectivity and migration) were implemented by
648 globally mixing communities from all three patches prior to serial transfer every three or six transfers, respectively.
649 **(C)** To test the study questions, high-throughput sequencing was used to quantify the community state C at the
650 experimental end-point, and clones were isolated to test for resistance phenotypes in the different treatments. This
651 data was used to estimate divergence of communities ΔC from the no-disturbance, no-mixing baseline (dashed
652 horizontal line as measured at the experimental end-point) within each pre-exposure history as a proxy for
653 ecological resilience.

654

655 **Figure 2 | Streptomycin susceptibility of experimental species and nonsynonymous mutations in pre-**
656 **exposed species. (A)** Streptomycin susceptibility (IC_{50}) for 23 experimental species. Four (4) distinct clones from
657 the ancestral species were phenotyped (transparent points) and are shown with the mean (muted red) and a
658 nonparametric bootstrap for 95 % confidence limits of the population mean (line range) across those replicate
659 clones. Streptomycin IC_{50} of pre-exposed populations (muted blue) are shown for the three abundant species (axis
660 labels highlighted with grey) in the community used in the pre-exposure treatment of the community experiment.
661 For these, 16 clones were randomly phenotyped from each of the 4 biological replicates for populations of each
662 species following exposure to increasing concentrations of streptomycin (64 clones in total). **(B)** Genes hit by
663 nonsynonymous mutations (SNPs or indels) at min. 5 % allele frequency in pre-exposed populations. In addition
664 to the gene, the amino acid change is indicated in the y -axis label. The streptomycin resistance associated genes
665 *rpsL* and *rsmG* are highlighted with grey background. Fixed or near-fixed mutations (allele frequency min. 0.95)
666 are indicated with a white cross.

667

668 **Figure 3 | Effect of disturbance, community mixing and pre-exposure of single most abundant community**
669 **member (*Aeromonas caviae* HAMBI 1972) on community composition and disturbance resistance. (A)**
670 Relative abundance of species at the end-point of 46-day serial passage experiment ($N = 4$ replicates per unique
671 treatment combination). Subcolumns show data for the three disturbance levels (no, low or high streptomycin, or
672 Sm, level; deepening shades of red) in two key pre-exposure treatments, separated by black vertical lines from
673 left to right as follows: (1) ancestral populations used for all species (“All anc.”); (2) a population used for the
674 most abundant species in the undisturbed community, *Aeromonas caviae* HAMBI 1972, that had evolved to be
675 highly resistant to the disturbance as a result of pre-exposure (“Pre-exp. 1972”). A model bacterial community
676 consisting of 23 gram-negative species was employed in the experiment. The three streptomycin disturbance
677 patches (no, low, or high level) mixed at low or high rate have a shared history and can be identified by the
678 replicate number shown on the x -axis. **(B)** A t-SNE map showing *de novo* community clustering at the end-point
679 of serial passage experiment. All data points originate from the same t-SNE analysis and have been separated into
680 panels (with same arbitrary axis units) to illustrate how experimental treatments influence compositional
681 divergence. The t-SNE map is a 2D projection of a manifold in high-dimensional space, and only the relationship
682 between the points is meaningful, not point positioning, with the axes given in arbitrary units. **(C)** Frequency of
683 each species relative to the frequency of the same species in the pre-exposure history specific control condition
684 with no antibiotic or community mixing (*i.e.*, upper left-hand white corner) at the end-point of serial passage
685 experiment. Data are presented as mean values \pm SEM ($N = 4$ replicates for each treatment condition). The data
686 points in the control condition (top row with white background) represent variation of the four control replicates
687 around their mean (zero) and therefore deviate from zero. The species have been ordered by increasing
688 streptomycin resistance level (IC_{50} value) of the ancestral species.

689

690 **Figure 4 | Effect of streptomycin pre-exposure on community resilience.** The y -axis shows community
691 resilience quantified as KL divergence of community composition from the streptomycin-free condition relative
692 to the pre-exposure-free environment. Therefore, the lower the value, the less community change occurs at high

693 streptomycin level and the more resilient the community is. **(A)** Resilience of the entire community at high
694 streptomycin level. **(B)** Resilience of the non-focal community fraction at high streptomycin level (i.e., the pre-
695 exposed species has been removed). Pre-exposed species have been ordered from left to right by increasing
696 abundance in the control condition (antibiotic and pre-exposure free environment), with the exception of placing
697 the all pre-exposed treatment in front of the list in **A** (absent from **B** as all community members have been pre-
698 exposed). For both **A** and **B** panels, box plot bars and circles indicate medians and data points, respectively. The
699 boxes indicate the interquartile range (25–75th percentile) and whiskers indicate lower and upper quartiles minus
700 or plus 1.5 times the interquartile range.

701
702 **Figure 5 | Effect of community mixing rate and disturbance pre-exposure on resilience across**
703 **metacommunities.** **(A)** KL divergence from the streptomycin- and mixing-free environment at low and high
704 community mixing rate for communities containing only ancestral species (left) and communities where the most
705 dominant community member *Aeromonas caviae* HAMB1 1972 had been pre-exposed to streptomycin (right).
706 Metacommunity refers to the three disturbance level patches (no, low, or high streptomycin level) subject to
707 community mixing. The 12 points overlaid above each box plot include the four replicates from each of the three
708 streptomycin patches (streptomycin-free, low level, and high level) comprising metacommunities. Each point
709 indicates the compositional distance (KL divergence on *y*-axis) of an individual community within the
710 metacommunity from the streptomycin- and mixing-free (i.e., no metacommunity) control condition (mean of
711 four replicates) at the experimental end-point. **(B)** Variance in KL divergence between replicate communities in
712 each treatment at different community mixing rates ($N = 180$ communities / 4 replicates = 45 replicate sets). The
713 45 points overlaid above each box plot show variation among the four replicate communities in identical
714 conditions (one of three streptomycin levels and one of five pre-exposure treatments) for each of the three
715 community mixing rates. The *y*-axis shows variance in KL divergence quantified as in panel **A**, indicating the
716 level of variation among replicates in community change from the baseline (no streptomycin or community
717 mixing) as a function of community mixing rate. For both **A** and **B** panels, box plot bars and circles indicate
718 medians and data points, respectively. The boxes indicate the interquartile range (25–75th percentile) and whiskers
719 indicate lower and upper quartiles minus or plus 1.5 times the interquartile range.

720

721

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