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Repeatable ecological dynamics govern the response of experimental communities to antibiotic pulse perturbation

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1. Extended Data

Figure #	Figure title One sentence only	Filename This should be the name the file is saved as when it is uploaded to our system. Please include the file extension. i.e.: <i>Smith_ED Fig1.jpg</i>	Figure Legend If you are citing a reference for the first time in these legends, please include all new references in the Online Methods References section, and carry on the numbering from the main References section of the paper.
Extended Data Fig. 1	Extended Data Fig. 1	Fig_S1.tiff	Figure S1. A t-SNE map showing <i>de novo</i> community clustering before, during and after recovery from antibiotic pulse at different antibiotic levels with or without immigration (N = 190). The different antibiotic levels are indicated by color coding, and the time points relative to the antibiotic pulse are indicated by different shapes. Low, intermediate and high antibiotic levels correspond to 4, 16 and 128 $\mu\text{g ml}^{-1}$ streptomycin, respectively. All data points originate from the same t-SNE analysis and have been separated into two panels (with same arbitrary axis units) only for the sake of visual clarity of immigration effect (at high antibiotic level, post-recovery communities indicated by diamonds more often resume pre-disturbance composition in upper left-hand region).
Extended Data Fig. 2	Extended Data Fig. 2	Fig_S2.tiff	Figure S2. The extinction probability of species as a function of antibiotic level and the presence/absence of immigration (binomial glm estimate \pm 95 % confidence intervals). Extinction is defined as the absence of a species after the antibiotic pulse (day 32 onwards) that was present prior to the pulse (day 16), and has been computed only for the species fulfilling these criteria in at least one experimental community (in total, 146 cases of

			extinction were observed). Low, intermediate and high antibiotic levels correspond to 4, 16 and 128 $\mu\text{g ml}^{-1}$ streptomycin, respectively.
Extended Data Fig. 3	Extended Data Fig. 3	Fig_S3.tiff	Figure S3. Bacterial biomass estimated by optical density (OD) at 600 nm at different levels of antibiotic pulse (expressed in $\mu\text{g ml}^{-1}$) in the pre-disturbance (day 16), post-disturbance (day 32) and post-recovery (day 48) phases (mean \pm standard deviation).
Extended Data Fig. 4	Extended Data Fig. 4	Fig_S4.tiff	Figure S4. Global comparative view of community composition as shown by Kullback-Leibler (KL) divergence across all samples (N = 190). The color scale from blue to red indicates the degree to which community composition differs between two communities. KL divergence has been computed from species compositional data. The heat map has been color-annotated for the different immigration and antibiotic treatments and experimental phases. Low, intermediate and high antibiotic levels correspond to 4, 16 and 128 $\mu\text{g ml}^{-1}$ streptomycin, respectively.
Extended Data Fig. 5	Extended Data Fig. 5	Fig_S5.tiff	Figure S5. Competitive fitness landscapes across all samples during the antibiotic pulse and recovery phases (N = 126). The color scale from blue to red indicates the degree to which the competitive fitness landscapes are correlated between two communities. Correlations have been computed from competitive fitness data for each species in the communities. The heat map has been color-annotated for the different immigration and antibiotic treatments and experimental phases. Low, intermediate and high antibiotic levels correspond to 4, 16 and 128 $\mu\text{g ml}^{-1}$ streptomycin, respectively. The black boxes show the data presented at species-level detail in Figure 4 in the main text.
Extended Data Fig. 6	Extended Data Fig. 6	Fig_S6.tiff	Figure S6. Correlation of competitive fitness landscapes

			<p>within replicates in each experimental treatment (mean + 95 % confidence interval). Correlations have been computed from competitive fitness data for each species in the communities. The figure aggregates the pairwise correlation values shown for each replicate pair within treatments in Figure S4. Low, intermediate and high antibiotic levels correspond to 4, 16 and 128 $\mu\text{g ml}^{-1}$ streptomycin, respectively.</p>
Extended Data Fig. 7	Extended Data Fig. 7	Fig_S7.tiff	<p>Figure S7. Percentage of variance in the competitive fitness of species explained by the experimental treatments (antibiotic level and presence / absence of species immigration) and species traits (antibiotic MIC and intrinsic growth rate). The variance partitioning is based on ANOVA on competitive fitness performed separately for the antibiotic pulse and recovery phases (detailed results are presented in Tables S1 and S2).</p>
Extended Data Fig. 8	Extended Data Fig. 8	Fig_S8.tiff	<p>Figure S8. Illumina read recruitment (median per species) in whole genome alignments for deep sequencing data (two upper panels) or raw 16S rRNA amplicon data (bottom panel). The HAMBI codes of the species are indicated in the horizontal axis, with two exceptions: K12 and RP4 denote the chromosome and plasmid sequence, respectively, from <i>E. coli</i> JE2571. Read recruitment in whole genome alignments is indicated as number of reads (100 bp) in 1,000 bp blocks, and needs to be divided by 10 $((100 \text{ bp} \times \text{read count}) / (1,000 \text{ bp block}))$ to obtain an estimate of genome coverage. For instance, a median read count of 1,000 corresponds to roughly 100\times genome coverage. In the uppermost panel, deep sequencing data was mapped separately to the genome of each individual species, and in the middle panel, the data was mapped to a multi-FASTA file containing all the genomes, producing comparable results. 16S rRNA amplicon read</p>

			counts have been normalized to 15,000 reads per sample.
Extended Data Fig. 9	Extended Data Fig. 9	Fig_S9.tiff	Figure S9. Deep sequencing read recruitment across the genomes of abundant species. Genomic position is indicated as relative position (0–1) across the whole chromosome for closed genomes or largest contig for draft genomes. Read recruitment in whole genome alignments is indicated as number of reads (100 bp) in 1,000 bp blocks, and needs to be divided by 10 ((100 bp × read count)/(1,000 bp block)) to obtain an estimate of genome coverage.

5 **2. Supplementary Information:**

6 **A. Flat Files**

7

Item	Present?	Filename This should be the name the file is saved as when it is uploaded to our system, and should include the file extension. The extension must be .pdf	A brief, numerical description of file contents. i.e.: <i>Supplementary Figures 1-4, Supplementary Discussion, and Supplementary Tables 1-4.</i>
Supplementary Information	Yes	SI.pdf	Supplementary Tables S1 to S2
Reporting Summary	Yes	nr-reporting-summary.pdf	
Peer Review Information	Yes	PRFile_Cairns.pdf	

8 Repeatability ecological dynamics govern the response of experimental com-
9 munities to antibiotic pulse perturbation

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31 **Abstract**

32

33 In an era of pervasive anthropogenic ecological disturbances, there is a pressing need to understand
34 the factors constituting community response and resilience. A detailed understanding of disturbance
35 response needs to go beyond associations and incorporate features of disturbances, species traits,
36 rapid evolution and dispersal. Multispecies microbial communities experiencing antibiotic perturba-
37 tion represent a key system with important medical dimensions. However, previous microbiome
38 studies on the theme have relied on high-throughput sequencing data from uncultured species with-
39 out the ability to explicitly account for the role of species traits and immigration. Here we serially
40 passaged a 34-species defined bacterial community through different levels of pulse antibiotic dis-
41 turbance, manipulating the presence or absence of species immigration. To understand the ecologi-
42 cal community response measured by amplicon sequencing, we combined initial trait data measured
43 for each species separately and metagenome sequencing data revealing adaptive mutations during
44 the experiment. We found that the ecological community response was highly repeatable within the
45 experimental treatments, which could be partly attributed to key species traits (antibiotic suscepti-
46 bility and growth rate). Increasing antibiotic levels were also coupled with increasing species ex-
47 tinction probability, making species immigration critical for community resilience. Moreover, we
48 could detect signals of antibiotic resistance evolution occurring within species at the same time
49 scale, leaving evolutionary changes in communities despite recovery at the species compositional
50 level. Together these observations reveal a disturbance response which appears as classic species
51 sorting but is nevertheless accompanied by rapid within-species evolution.

52

53 Introduction

54

55 In the Anthropocene¹ characterized by anthropogenic perturbations of environments ranging in
56 scale from the individual organism (e.g. gut microbiota of mammal) to the global ecosystem (e.g.
57 climate change and loss of biodiversity), it is paramount to understand factors determining biologi-
58 cal resilience². Understanding how the effects of perturbations percolate through the ecosystem is
59 vital to better understand the risks and benefits of human driven control efforts in the restoration
60 and conservation of populations. For instance, antibiotic treatment affects not only the pathogen
61 population but also off-target species in the microbiota of the patient, promoting the spread of anti-
62 microbial resistance³. Rational interventions require a detailed, ideally mechanistic, understanding
63 that goes beyond associations, integrating community dynamics, species traits, environmental vari-
64 ables, evolutionary events and stochasticity. However, we are far from such an understanding,
65 which has in part been attributed to the sparsity of controlled studies amidst *in vivo*, field and theo-
66 retical studies⁴. Nevertheless, recent advances in predictive modeling suggest that such an under-
67 standing is possible for certain rapidly evolving systems⁵.

68

69 A notable case of interest is the response of multispecies bacterial communities to perturbations by
70 antibiotics, pharmaceuticals and other compounds of an anthropogenic origin. Understanding this
71 response can be critical, among others, for rational therapeutics to mitigate unwanted effects on
72 patient health caused by changes in the gut microbiota (e.g. *Clostridioides difficile* infection⁶), man-
73 agement of waste water treatment to ensure the maintenance of key functionalities in bacterially
74 driven processes⁷, and redesigning of agricultural practices to maintain microbiota contributing to
75 crop health and productivity⁸.

76

77 Traditionally, ecological timescales have been considered shorter than evolutionary timescales,
78 causing ecological processes to drive the community response to environmental change. The com-
79 munity response to perturbation is therefore expected to be determined by selection on pre-existing
80 traits at the species level⁹. Selection may also act on the standing genetic variation within popula-
81 tions and affect the community response to perturbation¹⁰⁻¹³. However, the role of standing trait
82 variation at the species level in the bacterial disturbance response remains unclear, because large
83 population sizes and short generation times can render bacterial populations virtually unlimited by
84 mutation supply. This leads to the omnipresence and rapid generation of intraspecific trait variation,
85 which can play a major role in ecological dynamics¹⁴, especially since mutations in the bacterial
86 genome can have strong effects on traits. For instance, a single point mutation in the gene *rpsL* can
87 make a bacterium over 250-fold more resistant to the antibiotic streptomycin¹⁵. Overall, strong evi-
88 dence has emerged in recent decades showing that rapid evolution can alter ecological dynamics in
89 communities across a range of systems, even those with lower rates of evolution compared to mi-
90 crobial systems¹⁶. Similarly, a community ecology context can be important for evolutionary trajec-
91 tories¹⁷. While there is an extensive number of studies on both the species compositional effects of
92 antibiotic perturbation on the microbiome and the genetics of antibiotic resistance evolution in indi-
93 vidual species, both aspects have rarely been analyzed together. Furthermore, high-throughput se-
94 quencing based approaches allowing the investigation of mutations in longitudinal microbiome data
95 have focused on *in vivo* and field samples where the species are uncultured and the pre-existing
96 traits of the species cannot be explicitly estimated¹⁸. This may hinder the ability to assess the im-
97 portance of evolution relative to species sorting in the perturbation response of communities.

98

99 Ecological resilience describes the ability of a community not only to withstand a perturbation (eco-
100 logical resistance) but also to recover from it. Studies have shown variable results concerning the
101 resilience of the human gut microbiota to antibiotic perturbations at high, clinical concentrations¹⁹⁻²¹.

102 Effects of antibiotics that may or may not completely reverse after the perturbation include reduced
103 community stability²² and diversity²³, which have been associated with adverse health consequen-
104 ces in patients^{24,25}. However, the sparsity of longitudinal studies and the variability in time points
105 sampled since the perturbation pose challenges for comparing studies and assessing whether the
106 communities are still in a state of recovery at the time of sampling. Moreover, the conditions deter-
107 mining a particular community response remain unclear. These include factors such as the level of
108 perturbation and species immigration²⁶, a key feature of microbial communities and form of bacte-
109 riotherapy (e.g. probiotic supplementation after antibiotic treatment)²⁷. There is also a practical need
110 to understand how the disturbance and immigration responses of bacterial communities interact. For
111 example, this information is critical for the design of minimal artificial communities to replace fecal
112 microbiota transplantation (FMT), where gut microbial communities disturbed by antibiotic pulses
113 are treated by a fecal “immigration” to restore a healthy microbiota²⁸.

114

115 Here we used a 34-species model bacterial community to examine the role of ecological and evolu-
116 tionary processes in the community response to different levels of pulse disturbance by the amino-
117 glycoside antibiotic streptomycin in the absence or presence of species immigration. We performed
118 a serial passage experiment, collecting amplicon data to track ecological dynamics and deep se-
119 quencing data to track evolutionary dynamics, and combined experimental data with pre-existing
120 trait data on community members (Figure 1). We found that communities responded sensitively and
121 repeatably to the different environments. This could be linked to species sorting and selection on
122 their traits (growth rate and antibiotic susceptibility) as well as an increase in the extinction proba-
123 bility of particular species at increasing antibiotic levels in the absence of immigration. Adaptive
124 mutations also occurred but could not be linked to the ecological dynamics. Despite the sensitive
125 response to the perturbation, communities were able to recover close to the initial community state
126 in all but the highest antibiotic level. However, the loss of species as a function of antibiotic level as

127 well as the occurrence of evolutionary changes within species still left persistent changes in com-
128 munities, compromising their resilience over the long term. Importantly, immigration played a key
129 role in resilience at the species level by preventing species extinctions.

130

131 Results

132

133 **Both antibiotic level and immigration strongly determine ecological resilience**

134

135 The communities were compositionally sensitive to the different antibiotic levels and the presence
136 of immigration (Figures 2 & 3; Extended Data Figure 1). Machine learning models could be trained
137 to correctly predict the antibiotic level during the antibiotic pulse from species composition data
138 (random forest, rf, model using community composition data from all treatments immediately post-
139 perturbation to classify antibiotic level: permutation test $p < 0.001$, accuracy estimated by leave-
140 one-out cross-validation, LOOCV, 0.88). However, the ability to distinguish between the antibiotic
141 levels decreased after the recovery period (rf model using community composition data from all
142 treatments immediately post-perturbation to classify antibiotic level: permutation test $p < 0.001$,
143 LOOCV accuracy 0.53). In contrast, machine learning models could correctly classify the immigra-
144 tion treatment only after the recovery period (rf model using community composition data from all
145 treatments immediately post-perturbation to classify immigration presence/absence: permutation
146 test $p = 0.19$, LOOCV accuracy 0.53; rf model for immigration post-recovery: permutation test $p <$
147 0.001 , LOOCV accuracy 0.69). These results suggest that the antibiotic perturbation had a composi-
148 tional effect specific to the antibiotic level, this effect decreased with recovery, and the latter pro-
149 cess was influenced by species immigration.

150

151

152 To further investigate factors determining ecological resilience, we inspected the effect of the dis-
153 turbance on two measures of entropy. We used the Shannon diversity (information entropy incorpo-
154 rating species richness and evenness) after the disturbance and Kullback-Leibler (KL) divergence
155 (relative entropy comparing species composition) in individual communities over time after the
156 disturbance relative to the pre-disturbance state²⁹ (Figure 3). An analysis of this data shows that
157 diversity decreased (ANOVA for linear regression model on Shannon diversity with lowest AIC
158 value; antibiotic $F_{3,60} = 12.1, p < 0.001$; the immigration treatment did not have a significant effect
159 during the antibiotic pulse and was not included in the best model; Figure 3a) and community com-
160 position became increasingly altered as a function of antibiotic level during the pulse, and that im-
161 migration enhanced community recovery after the pulse (ANOVA for gls model on KL divergence
162 with lowest AIC value: antibiotic level $F_{3,118} = 19.8, p < 0.001$; immigration $F_{1,118} = 6.33, p = 0.013$;
163 recovery time $F_{1,118} = 8.31, p = 0.005$). Pairwise comparisons of estimated marginal means for KL
164 divergence show significant differences ($p < 0.02$) between all of the antibiotic levels except for the
165 control and lowest level.

166

167 Persistent alterations in community composition were only observed for the highest antibiotic level
168 in the absence of immigration (Figure 3b). Increasing antibiotic levels increased, however, species
169 extinction probability, which was strongly counteracted by immigration (ANOVA for binomial glm
170 model with lowest AIC value: antibiotic $\chi^2_{3,1643} = 22.3, p < 0.001$; immigration $\chi^2_{1,1646} = 27.3, p <$
171 0.001 ; species $\chi^2_{25,1618} = 166, p < 0.001$; antibiotic \times immigration $\chi^2_{2,1615} = 7.52, p = 0.057$; Extend-
172 ed Data Figure 2). Therefore, in the absence of immigration, loss of species caused alterations in
173 community composition, and the magnitude of the effect was proportional to the magnitude of the
174 perturbation. The reported community effects of antibiotic level and immigration are unlikely to
175 have been affected by changes in total bacterial biomass, since similar levels of biomass were ob-
176 served across the experimental conditions and time points (Extended Data Figure 3). This indicates

177 that the relative abundance of species is here a close approximation of absolute abundance, which is
178 not always the case in microbial community studies and can have important implications for study
179 conclusions³⁰.

180

181 **Ecological dynamics are highly repeatable within antibiotic and immigration treat-** 182 **ments**

183

184 We next investigated whether increasing antibiotic levels are coupled with decreased repeatability²²
185 in community trajectories, as this could be a signal of alternative stable states (including bi- and
186 multi-stability), stochastic species extinctions or adaptive *de novo* mutations. In contrast with this
187 expectation, we found relatively low levels of divergence in community states within each antibi-
188 otic and immigration treatment (Extended Data Figure 4). To further examine this result, we esti-
189 mated the competitive fitness of each species in the community during the antibiotic pulse or recov-
190 ery phase using the replicator equation from evolutionary game theory. In this approach, the fre-
191 quency change of one species over time is considered to be an outcome of its fitness reduced by the
192 average fitness of the community. We found that the competitive fitness of the species responded
193 repeatably to the different antibiotic levels, and that many of the species displaying a strong positive
194 response during the pulse displayed an inverse response during the recovery phase (intermediate
195 antibiotic level without immigration illustrated in Figure 4; for complete data for all treatments, see
196 Extended Data Figures 5 and 6). We also found that increasing antibiotic levels increased fitness
197 variance (particular species exhibited very high or low competitive fitness values), as hypothesized
198 previously³¹, which is reflected by increasingly correlated competitive fitness landscapes during the
199 antibiotic pulse and becomes reversed during the recovery phase (Extended Data Figures 5 and 6).
200 We could attribute 10–13 % variation in the competitive fitness of species during the antibiotic
201 pulse and recovery phases to the interplay between antibiotic level and key species traits, intrinsic

202 antibiotic susceptibility (streptomycin MIC) and intrinsic growth rate (r_{\max} ; Tables S1 and S2; Ex-
203 tended Data Figure 7). For instance, two species with high growth rate combined with high antibi-
204 otic susceptibility, *Aeromonas caviae* HAMBI 1972 (MIC = 0.75 $\mu\text{g ml}^{-1}$) and *Pseudomonas chlo-*
205 *roraphis* HAMBI 1977 (MIC = 8.0 $\mu\text{g ml}^{-1}$), decreased in abundance and competitive fitness at
206 increasing antibiotic levels (Figures 2 and 4). Potential reasons for why a higher proportion of var-
207 iation in competitive fitness could not be explained by species traits despite the highly consistent
208 ecological response include the presence of important unmeasured species traits, species interac-
209 tions, or non-linear system-level behavior (e.g. dramatic community change only after particular
210 concentration reached). Such information would be useful for predictive modelling.

211

212 To more precisely inspect the low levels of community divergence observed within treatments (Ex-
213 tended Data Figure 4), we quantified the repeatability of community trajectories within each antibi-
214 otic and immigration treatment using the diversity dissimilarity index³³ which relates diversity
215 pooled over replicate communities to the mean diversity of replicate communities. This yields a
216 value between zero and one, where zero indicates that replicate communities are identical and one
217 that they are completely different. The community trajectories were highly repeatable (close to zero)
218 in the different experimental treatments when species were weighted based on their abundance,
219 although a slight (approximately 5 %) decay in repeatability was observed for the highest antibiotic
220 level during the pulse (Shannon entropy panel in Figure 5). The high repeatability suggests that rare
221 stochastic mutational events are unlikely to have been a major driver of the ecological dynamics of
222 the abundant species. However, there was a higher decay in repeatability when species were given
223 equal weight regardless of abundance, indicating that low-abundance species account for most of
224 the loss in repeatability (species richness panel in Figure 5). When combined with the finding that
225 the extinction probability of species increased as a function of antibiotic level (Extended Data Fig-
226 ure 2), these results suggest that the stochasticity introduced by antibiotic perturbation was at least

227 partially accounted for by low-abundance species being driven extinct differentially between repli-
228 cate communities. Nevertheless, a potential role for evolutionary rescue in the stochasticity of the
229 ecological dynamics of low-abundance species cannot be ruled out.

230

231 **Antibiotic resistance mutations occur despite repeatable ecological dynamics**

232

233 To investigate mutational dynamics, we deep-sequenced three of the eight replicate communities
234 during and after recovery from the antibiotic pulse. From these community metagenomes, we could
235 extract whole-genome data for abundant species with sufficient genome coverage in a particular
236 community. We focused on genes estimated to be under selection ($N = 91$) based on containing
237 more nonsynonymous hits in independent populations than expected by chance. Most of these
238 genes contained mutations across experimental treatments, suggesting that they represent adapta-
239 tions to the general experimental conditions rather than treatment-specific adaptations (Figure 6).
240 Nevertheless, antibiotic and immigration also significantly enriched mutations for a small subset of
241 genes even when controlling for the presence or absence of whole-genome data (and thereby, in-
242 formation for a particular mutational target) for the different species in each community meta-
243 genome (PERMANOVA for binary vectors of mutated genes, i.e. mutational profiles: species $R^2 =$
244 $0.81, p < 0.001$; antibiotic $R^2 = 0.015, p < 0.001$; immigration $R^2 = 0.0082, p < 0.001$).

245

246 Among the mutational targets that occurred only in the presence of the antibiotic, several have been
247 previously associated with increased levels of streptomycin or aminoglycoside resistance or with
248 the presence of these agents, suggesting that antibiotic-related adaptations occurred during the ex-
249 periment. These genes include *rpsL* (encoding ribosomal protein S12, a common streptomycin re-
250 sistance target)³⁴, *rsmG* (ribosomal methyltransferase)³⁵, *phoQ* (PhoP-PhoQ two-component regula-
251 tory system)³⁶, *cya* (adenylate cyclase)³⁷, *cra* (catabolite repressor/activator)³⁷, *cspA* (cold shock

252 protein)³⁸, *relA* (GTP pyrophosphokinase mediating stringent response)¹⁵ and *spoT* ((p)ppGpp syn-
253 thase/hydrolase mediating stringent response)¹⁵. Most of these mutations occurred in the intermedi-
254 ate or highest antibiotic concentration, while displaying no general pattern with respect to the pres-
255 ence or absence of immigration (observed only without immigration: *rpsL* (1×) and *cspA* (2×); only
256 with immigration: *rsmG* (2×); both without and with immigration: *cra* (2×), *cya* (13×), *phoQ* (2×),
257 *relA* (4×); Figure 6). Notably, mutations in a particular gene were not required for a species to suc-
258 ceed at a given antibiotic level. For instance, an *rpsL* mutation in *Agrobacterium tumefaciens*
259 HAMBI 105 and *rsmG* mutations in *Pseudomonas chlororaphis* HAMBI 1977 only occurred in a
260 subset of the replicates where the species displayed high abundance in the respective antibiotic
261 treatment (Figure 6). The low level of within-treatment repeatability in mutational targets contrasts
262 with the high level of within-treatment repeatability in ecological trajectories for abundant species.
263 Therefore, we could not detect an influence of genomic evolution on the ecological dynamics with
264 the methods employed in this study, although we acknowledge the limitations of our approach (fo-
265 cusing on abundant species in a subset of replicates).

266

267 **Discussion**

268

269 Using a controlled setup to investigate the antibiotic response of a multispecies bacterial community
270 in the presence and absence of species immigration, we found that the replicate communities re-
271 sponded repeatably to the different treatments, with the magnitude of the community response and
272 persistent community changes increasing with increasing antibiotic levels. The community effects
273 of antibiotics expectedly included both abundance changes and extinctions of particular species.
274 Persistent community changes were linked to increasing species extinctions at increasing antibiotic
275 level, which prevented abundant high-growth-rate species sensitive to the antibiotic from rebound-
276 ing to their pre-disturbance abundance during the recovery phase, and could be counteracted by

277 species immigration. This was not a straightforward outcome, since the original community compo-
278 sition may not have recovered despite reintroduction of sensitive species if communities had
279 reached an alternative stable state during the antibiotic pulse. Overall, these findings highlight the
280 importance of classic and relatively simple ecological processes, species sorting and immigration,
281 in defining how microbial communities respond to antibiotic perturbation.

282

283 The high level of repeatability of the ecological dynamics in our study appears to contrast with pre-
284 vious studies showing decreased stability²² and bistability³⁹ in microbial communities following
285 antibiotic exposure. However, similar to these studies, the minor decay in repeatability we observed
286 did occur under high antibiotic levels (Figure 5). Furthermore, at the intermediate antibiotic level,
287 certain replicate communities experienced a more dramatic decline in diversity compared to others
288 (Figure 3a and Extended Data Figure 1), indicating that this antibiotic level may be close to a tip-
289 ping point concentration where bistable system-level behavior is possible. Quantifying these effects
290 more precisely would have required experimenting with a wider range of antibiotic concentrations.
291 Moreover, a previous study shows that high rates of immigration can fuel adaptation to antibiotics
292 by increasing the supply of genetic variation⁴⁰. Therefore, higher levels of immigration could poten-
293 tially have exacerbated the role of evolution in our immigration treatment such that replicate com-
294 munities would have diverged more during the antibiotic pulse, as species containing adaptive mu-
295 tations by chance or the timing of occurrence of these mutations would differ between them. This
296 could also have decreased the rate of recovery in the immigration treatment, since there would have
297 been an increased likelihood of communities reaching alternative stable states and larger differences
298 in post-disturbance genetic composition may have slowed down species compositional recovery
299 rate. However, establishing this would have required experimenting with different levels of immi-
300 gration. Therefore, the level of ecological repeatability observed may be influenced by a number of
301 study conditions.

302

303 We also found adaptive mutations sweeping to high frequencies in populations of individual species,
304 although we could not connect these with species abundance. This shows that the disturbance re-
305 sponse in microbial systems results from a combination of ecological and evolutionary processes
306 operating at the same timescale. However, connecting these processes may not always be straight-
307 forward⁴¹. Similar to our finding, in a recent analysis of longitudinal linked-read sequencing data
308 from human gut microbiota subjected to antibiotic treatment, antibiotic resistance mutations were
309 found to sweep to high frequencies in the populations of single species without necessarily resulting
310 in an increased abundance of the species in the community¹⁸. In a microbial community, species
311 sorting and adaptive mutations occur simultaneously in multiple species, and all these factors have
312 the potential to interact, making it challenging to disentangle ecological from evolutionary process-
313 es. Furthermore, in a number of conditions, relative fitness increases within a species based on al-
314 lele frequency changes do not translate into changes in absolute fitness (population size)³². In a
315 multispecies setup, the competitive release of an adapted species may be suppressed, for example,
316 by the presence of other abundant species with relatively low intrinsic antibiotic susceptibility and
317 equal or higher resource use ability. The occurrence of a fitness trade-off between growth rate and
318 antibiotic resistance⁴² could also make the net fitness advantage of antibiotic resistance low, causing
319 a weak ecological effect difficult to detect in a multispecies setup. Importantly, whatever the under-
320 lying mechanism, this study supports the notion that within-species adaptive evolution can occur
321 during a perturbation even when this is not readily suggested by the ecological dynamics.

322

323 Our findings have important implications for the understanding and management of ecological re-
324 siliance. Although we found similar outcomes from antibiotic perturbation as those reported in hu-
325 man gut microbiome studies, such as decreased diversity²³, these outcomes were mostly limited to
326 the community state at the end of the perturbation, and were followed by community recovery close

327 to the pre-disturbance state. This is non-trivial taken that priority effects⁴³ or the presence of alter-
328 native stable states^{44,45} could cause a perturbed community to recover to an altered state, and em-
329 phasizes the need to assess ecological resistance during perturbations separately from recovery and
330 longer-term resilience⁴⁶. More generally, since the advent of amplicon and metagenomic sequenc-
331 ing, changes in bacterial communities have been found in response to a plethora of environmental
332 factors, but our findings suggest that such changes may not persist and a need for caution in data
333 interpretation in the absence of longitudinal data. Nevertheless, despite the communities mostly
334 rebounding, species extinctions, which were more likely at increasing antibiotic levels, left persis-
335 tent marks in community composition, similar to recent findings from human gut microbiota²⁰, alt-
336 hough species immigration enabled community recovery. This indicates that storing and reintroduc-
337 ing susceptible low-abundance species with key functionalities could play a crucial role in human
338 management of ecological disturbances. Ecological resilience was most notably compromised for
339 the highest antibiotic level, suggesting that in a therapeutic context, intermediate antibiotic levels
340 may represent a desirable compromise minimizing off-target effects assuming they are sufficient to
341 treat a pathogen³. Notably, here we considered only a single pulse disturbance, while communities
342 often face multiple disturbances, with historical disturbance regimes frequently priming populations,
343 communities and ecosystems, both ecologically and evolutionarily, to similar disturbances in the
344 future^{39,47,48}. Therefore, the types of persistent ecological (lost species) and evolutionary (resistance
345 mutations) changes observed in this study may have important consequences for the response of
346 communities to future perturbations.

347

348 **Methods**

349

350 **Strains and culture conditions**

351

352 The liquid medium used in the experiment was specifically developed for complex communities
353 and a long culture cycle. An artificial bacterial community consisting of 34 species (for species list,
354 see Supplementary Materials in ⁴⁹) was almost entirely chosen from the HAMBI Culture Collection,
355 University of Helsinki, except for *Escherichia coli* K-12 strain JE2571⁵⁰. The bacteria are gram-
356 negative and represent three classes (Alpha-, Beta- and Gammaproteobacteria) in the phylum Prote-
357 obacteria and three classes (Chitinophagia, Flavobacteriia and Sphingobacteriia) in the phylum Bac-
358 teroidetes. The species are not representative of a particular natural system but were rather selected
359 based on growth in simple, uniform laboratory conditions. Different versions of the artificial com-
360 munity have been used in two previous studies^{49,51}, where details are reported regarding its con-
361 struction and the phenotypic and genomic characteristics of the species.

362

363 A medium was specifically refined for the selected community and long culture cycles. The co-
364 culture medium contains 1 g l⁻¹ R2A broth (Labema, Helsinki, Finland) and 0.5 g l⁻¹ of cereal grass
365 medium (Ward's Science, St Catharines, ON, Canada) in M9 salt solution. The cereal grass medium
366 stock was prepared by autoclaving it in deionized H₂O and filtering through 5 µl to remove particu-
367 late matter.

368

369 **Serial passage experiment**

370

371 A 48-day serial passage antibiotic pulse experiment was performed consisting of three epochs: 16
372 days without streptomycin to allow the community composition to acclimatize to experimental con-
373 ditions, 16 days with streptomycin at the concentrations 4, 16, and 128 µg ml⁻¹, and 16 days without
374 antibiotics to allow the community to recover (Figure 1). The experiment included an antibiotic-free
375 control treatment. The experiment was performed in a full-factorial design without and with immi-

376 gration consisting of adding an inoculum of the original community at each transfer. Each treatment
377 combination was replicated eight times.

378

379 The experiment was conducted in ABgene™ 96 Well 2.2 ml Polypropylene Deepwell Storage
380 Plates (Thermo Fisher Scientific, Waltham, MA, USA) in the co-culture medium. Prior to starting
381 the experiment, all the strains were transferred to the co-culture medium and cultured for 96 hours
382 at 28 °C / 50 rpm. Following this, they were pooled together in equal volumes and freeze-stored
383 with 30% glycerol at –80 °C. To start the experiment, 10 µl of 100-fold diluted freezer-stock com-
384 munity was added to each well containing 500 µl of medium and 50 µl of sterile dH₂O to compen-
385 sate the dilution caused by streptomycin additions. Equal volumes were used instead of using more
386 precise methods such as plate counting or flow cytometry to equalize cell numbers for each species
387 prior to starting the experiment since we assumed that uncertainty in the latter methods and differ-
388 ences between species in cell viability and revival from frozen inoculum would nevertheless have
389 introduced substantial initial differences in species abundances. We therefore accepted that the
390 starting conditions are biased toward high growth ability species which would in any case have
391 been likely to rise to dominance rapidly during the initial culture cycles (first 16-day acclimation
392 epoch). Culturing throughout the experiment was performed at 28 °C / 50 rpm. The experiment was
393 maintained every 96 hours by transferring 50 µl, about 10%, to fresh medium prepared as in the
394 beginning of the experiment (representing approx. 3.33 bacterial generations per culture cycle with
395 the minimal assumption that bacteria multiply until reaching carrying capacity). For the immigra-
396 tion treatment, 10 µl of 100-fold diluted freeze-stored community was also added. For cultures con-
397 taining streptomycin, the dH₂O was replaced with an equal volume of the appropriate streptomycin
398 stock solution.

399

400 **Data collection**

401

402 The pre-existing phenotypic trait data for community members used in this study, including intrinsic
403 growth rate and streptomycin minimum inhibitory concentration (MIC) values, was obtained as
404 described previously⁴⁹. To monitor bacterial density during the serial passage experiment, optical
405 density values at 600 nm wavelength (OD_{600nm}) were measured from old cultures prior to the disturbance
406 (day 16), after the disturbance (day 32), and after the recovery period (day 48) using a well
407 plate reader (Tecan Infinite M200 well-plate reader, Tecan Trading AG, Switzerland). Samples
408 from time points 16 days (before streptomycin addition), 32 days (last time point with streptomycin)
409 and 48 (final time point) days were also frozen in glycerol at $-80^{\circ}C$ for further analysis.

410

411 DNA was extracted from the original freezer-stock community and the first three (1–3) out of eight
412 experimental replicate communities from days 16, 32 and 48 in the serial passage experiment for a
413 first batch of amplicon sequencing and the deep sequencing. A second batch of DNA extraction and
414 amplicon sequencing was later performed for the remaining five replicates (4–8). DNA extraction
415 was performed directly on freeze-stored samples without regrowing with the DNeasy 96 Blood &
416 Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions using 400–600
417 μ l of defrosted sample. DNA concentrations were measured with the Qubit™ 2.0 (Life Technologies
418 Corporation, Carlsbad, CA, USA) fluorometer using the Qubit™ dsDNA HS Assay Kit (Thermo
419 Fisher Scientific, Waltham, MA, USA). Paired-end 16S rRNA amplicon sequencing (V3 and V4
420 regions, 2×300 bp; all three time points) and metagenomic deep sequencing (2×101 bp; only days
421 32 and 48) was performed by the Institute for Molecular Medicine Finland (FIMM) using the Illumina
422 MiSeq and Illumina HiSeq2500 platforms, respectively, employing in-house protocols similar
423 to those described before⁴⁹.

424

425 **Sequence data processing**

426

427 For 16S rRNA amplicon data, raw reads were first paired using the paired-end read merger Pear
428 v0.9.6⁵² with defaults settings. Adapters and primers were removed from the paired reads using
429 Cutadapt v1.10⁵³ with the options -q 28 (quality-cutoff for trimming 3' end of read), -n 2 (two
430 rounds of adapter searching), -e 0.2 (maximum error rate of 20 % for adapter identification), and --
431 minimum-length 400 (discarding reads < 400 bp after quality control steps). Read quality was con-
432 trolled with FastQC v0.11.8 (www.bioinformatics.babraham.ac.uk/projects/fastqc) and MultiQC
433 v1.7⁵⁴ before and after running Pear and Cutadapt. USEARCH v1.10⁵⁵ was used to quality filter the
434 reads using the --fastq-filter command with the options -fastq_maxee 1 (maximum expected er-
435 rors 1), -fastq_truncqual 10 (truncating reads at first incidence of quality 10), -fastq_minlen 150
436 (minimum read length after other filtration steps), and -fastq_truncflen 150 (truncating reads at
437 length 150 bp). Unique sequences were obtained by dereplicating using the VSEARCH v2.13.3⁵⁶
438 command --derep_fulllength, followed by removal of chimeric sequences using the VSEARCH
439 command --uchime_denovo with default settings. The reads were mapped to a reference database
440 containing the 16S rRNA gene sequences of the 34 experimental species with USEARCH -
441 closed_ref command with > 97 % identity requirement. Problems associated with closed reference
442 operational taxonomic unit (OTU) clustering for environmental bacterial communities⁵⁷, such as
443 false positive genus assignment, should not apply to this case as the community is defined and has
444 its own reference database. The two DNA extraction and amplicon sequencing batches (replicates
445 1–3 vs. replicates 4–8) display a minor but distinct batch effect in community composition (Figure
446 2). However, performing downstream analyses separately for the two batches did not affect the
447 qualitative findings in the study.

448

449 For deep sequencing data, Cutadapt 1.12⁵³ was used to remove sequencing adapters and quality trim
450 sequence data, with the parameters -O 10 (minimum overlap for an adapter match), -q 28 (quality

451 cutoff for the 3' end of each read), and --minimum-length 30 (minimum length of trimmed read).
452 Sequence data quality before and after Cutadapt was assessed using FastQC
453 (www.bioinformatics.babraham.ac.uk/projects/fastqc) and MultiQC⁵⁴. The deep sequencing data
454 was mapped to a multi-FASTA file containing the whole-genome sequences of all experimental
455 isolates (genome accessions indicated in ⁴⁹) except for one rare species lacking genome data (*Rose-*
456 *omonas gilardii* HAMBI 2470), using bowtie2⁵⁸ with default settings. The Picard command Mark-
457 Duplicates was used to mark duplicates in alignment (BAM) files after sorting with SAMtools⁵⁹.
458 Subsequently, BEDtools 2.2 was used to compute genome coverage in 1 kb windows⁶⁰. This pan-
459 genome mapping approach produced similar results compared to mapping the data to each genome
460 individually, indicating that genome coverage was not reduced due to biased read recruitment in
461 homologous regions, as well as cross-validating amplicon data (Extended Data Figure 8). Genome
462 coverage data also indicated a lack of major copy number aberrations (Extended Data Figure 9).

463

464 Prior to variant calling and annotation, the metagenomic alignment files were split by species using
465 SAMtools⁵⁹. Alignment files containing below 200,000 reads, representing 5× genome coverage for
466 a 4 Mb bacterial genome, were removed. Since differential genome coverage would affect variant
467 count, nucleotide diversity and allele frequency estimates and thereby act as a confounder in down-
468 stream analyses comparing experimental treatments, all remaining BAM files were downsampled to
469 200,000 reads. Following this, genomic variants (SNPs and short INDELS) were called from BAM
470 files with FreeBayes 1.1.0-60⁶¹, using a population level approach (--pooled-continuous) and call-
471 ing only one variant allele per locus (--use-best-n-alleles 1). Variants were filtered based on exceed-
472 ing Phred-scaled quality 20 ("QUAL > 20") and read depth 2 ("DP > 2") using vcfliib from vcflib
473 (<https://github.com/vcflib/vcflib>). This allowed detecting variants that had reached high frequency
474 (min. 50 %) in a total of 229 samples representing abundant species in the experiment during (day

475 32) and after recovery from (day 48) the antibiotic pulse. Variants were annotated using SnpEff
476 4.3⁶².

477

478 **Ecological analyses**

479

480 All analyses were performed in the R v3.6.1 environment⁶³. The t-distributed stochastic neighbor
481 embedding (t-SNE) map for Extended Data Figure 1 was created using the Rtsne package⁶⁴ with the
482 options perplexity = 20 and theta = 0.5. Random forest models using community composition data
483 to classify the antibiotic or immigration treatments after the antibiotic pulse or following the recov-
484 ery period were generated using the randomForest package⁶⁵. Before analyses, rare species were
485 removed based on > 80 % of values being zero, and the data was standardized by converting each
486 value into a Z-score (subtracting each sample's mean and dividing by the sample's standard devia-
487 tion). Random forest classification was performed using the function randomForest implementing
488 the Breiman's random forest algorithm, with the options importance = TRUE and proximities =
489 TRUE. Subsequently, permutation tests (1,000 permutations) were implemented using the function
490 rf.significance to test whether the models perform better than expected by chance. Following this,
491 the function train in the package caret⁶⁶ was used to systematically partition the data into training
492 and tests sets repeatedly using the leave-one-out cross-validation (LOOCV) approach to estimate
493 model performance (accuracy).

494

495 The influence of the experimental treatments on KL divergence relative to the pre-disturbance state
496 was investigated using generalized least squares models (gls) as implemented in the nlme package⁶⁷,
497 specifying a residual variance structure dependent on the antibiotic level. The stepAIC function in
498 the MASS package⁶⁸ was subsequently used to select the best model based on the Akaike infor-
499 mation criterion (AIC). The competitive fitness of species during the antibiotic pulse or the recov-

500 ery period was estimated as the logarithm of the final frequency relative to the starting frequency,
501 which can be directly derived from the replicator equation in evolutionary game theory. The strain
502 *Azospirillum brasilense* HAMBI 3172 was chosen to be the reference and its logarithm of the final
503 frequency relative to the starting frequency was subtracted from all other values. To control for
504 noise from the frequency changes of low-abundance species and to award more weight to species
505 with high abundance in at least one of the estimated time points, a pseudocount constituting 1 %
506 proportion was added to the species abundance data prior to computing competitive fitness. The
507 effect of the experimental treatments on species extinction probability was tested using the base R
508 function glm with the option family = “binomial”.

509

510 To quantify the repeatability of ecological dynamics, we used the diversity dissimilarity index³³:

511

$$\frac{\frac{D_{pooled}}{D_{mean}} - 1}{M - 1}$$

512

513 where D is a diversity index (either Shannon diversity or species richness computed using the vegan
514 package⁶⁹), and M is the number of communities whose species compositions are compared (over
515 time). If the species compositions of replicates are identical, the diversity of the pooled community
516 is equal to the mean diversity, and the diversity dissimilarity index equals 0, and if the communities
517 have no species in common, the index equals 1.

518

519 **Evolutionary analyses**

520

521 We used minimal criteria to filter raw genomic variants from the downsampled variant data prior to
522 downstream analyses. First, we removed data for one species, HAMBI 403, which had a large num-

523 ber of variants (130,000) indicative of an incorrect reference genome. Second, there were peaks
524 above 80 % in variant frequency distributions across the communities. Such a high level of parallel-
525 ism suggests that the variants are either ancestral or systematic sequencing errors, and variants oc-
526 ccurring in over 80 % of the communities were therefore removed.

527

528 From this variant data set, we extracted nonsynonymous mutations and devised a threshold for re-
529 currence. Of all the coding genes in all the genomes, we drew mutations from a multinomial distri-
530 bution with replacement. If these 588 mutations were randomly distributed over the 58,220 coding
531 genes in the genomes, we would expect only five genes mutated in two or more populations. In total,
532 there were 1092 coding nonsynonymous mutations across 47 genes independently mutated in two
533 or more populations. Therefore, we focused on multi-hit genes which were independently mutated
534 in two or more populations. This set was used for the statistical analysis below, while a larger set
535 from genomic variant data prior to downsampling, and also including the known streptomycin re-
536 sistance gene *rpsL*, was used for Figure 6 to present the maximum amount of functionally annotated
537 potential targets of selection.

538

539 We used permutational analysis of variance (PERMANOVA)⁷⁰ to test whether the antibiotic level
540 or presence/absence of immigration affected the targets of mutation. Each community was scored
541 by the presence (1) or absence (0) of a nonsynonymous mutation in each of the multi-hit genes, and
542 these data were used to calculate the Euclidian distance between populations⁷¹. Before performing
543 PERMANOVA, its assumption of homogeneity of multivariate dispersions within treatments was
544 tested with the betadisper function in the vegan package that uses the PERMDISP2 procedure as
545 described previously⁷². The adonis function in the vegan package was then used to test the probabil-
546 ity that the observed distances could arise by chance by comparing them with random permutations
547 of the raw data⁷³.

548

549

550 **Data availability**

551

552 Raw sequence data (fastq files) has been deposited in the NCBI Sequence Read Archive (SRA)
553 under the accession PRJNA632457. All code and pre-processed data needed to reproduce the
554 downstream analyses and figures are available via GitHub:
555 https://github.com/johannescairns/repeatable_dynamics (permanent doi:
556 <https://doi.org/10.5281/zenodo.3908935>).

557

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722

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730 **Author contributions**

731

732 Design of serial passage experiment: TH, JC, LB. Performing serial passage experiment: RJ. Design
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734 JC wrote the first manuscript draft, with contributions from all authors. All authors approve the fi-
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737 **Competing interests**

738

739 The authors state that they have no competing interests.

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742 **Figure legends**

743

744 **Figure 1.** Experimental design. (A) Physical setup. A serial passage experiment was conducted
745 with a 34-species artificial community in deep 96-well plates. Initial key traits of community mem-
746 bers (intrinsic growth rate, represented by growth curve with yellow background, and intrinsic anti-
747 biotic susceptibility level, represented by growth curve in purple background) were measured sepa-
748 rately for individual isolates used to construct the community. (B) Layout of antibiotic pulse experi-
749 ment. The experiment consisted of serial propagation at 4-day (96 h) intervals for three 16-day
750 epochs: an acclimation period, an antibiotic pulse period with three different levels of pulse antibi-
751 otic disturbance together with an antibiotic-free control treatment, and a recovery period. To inves-
752 tigate the role of species immigration, the full experiment was performed without and with reintro-
753 ducing a small amount (1:500 cells relative to serial transfer inoculum) of the original community at
754 each transfer. Each unique treatment combination was replicated eight times. Samples (N = 192)
755 were collected for DNA extraction prior to the pulse (T0), after the pulse (T1) and after recovery
756 (T2) to track community composition (amplicon sequencing) and genomic evolution (metagenomic
757 sequencing).
758

759 **Figure 2.** Community dynamics during antibiotic pulse experiment. The figure depicts the frequen-
760 cies of abundant species across time in eight replicate communities for each unique treatment com-
761 bination indicated on the right (low, intermediate and high antibiotic levels correspond to 4, 16 and
762 128 $\mu\text{g ml}^{-1}$ streptomycin, respectively). The shaded area shows the antibiotic pulse epoch, with
763 increasingly dark hue indicating increasing antibiotic level. The top four panels show the different
764 antibiotic levels for the immigration-free treatment and the bottom four panels for the immigration
765 treatment. The y-axis has been square root transformed and scaled 0–1 to allow visual discernment
766 of less abundant species. “Others” denotes rare taxa that fail to reach a frequency of 5 % in at least
767 one community and time point. In total, 190 experimental samples are included in the figure togeth-
768 er with one stock community sample to represent initial species composition for all communities.
769 For two communities, adequate amplicon sequence data could not be recovered for day 48.
770

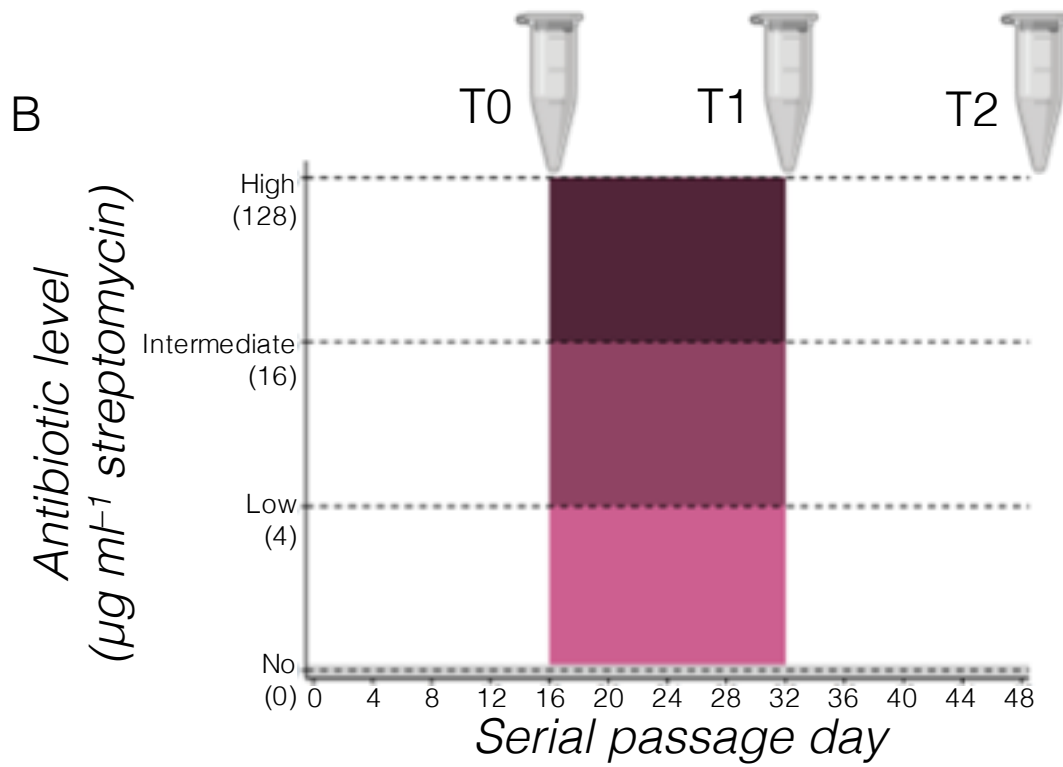
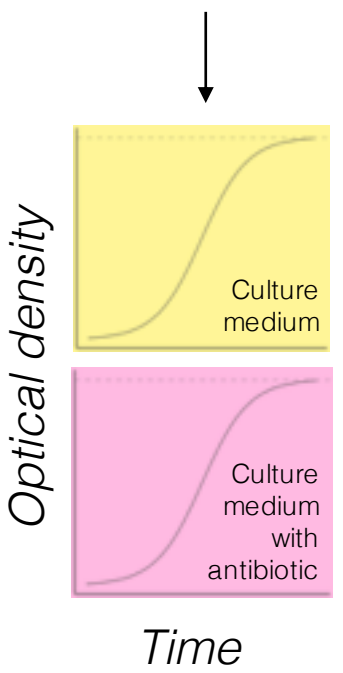
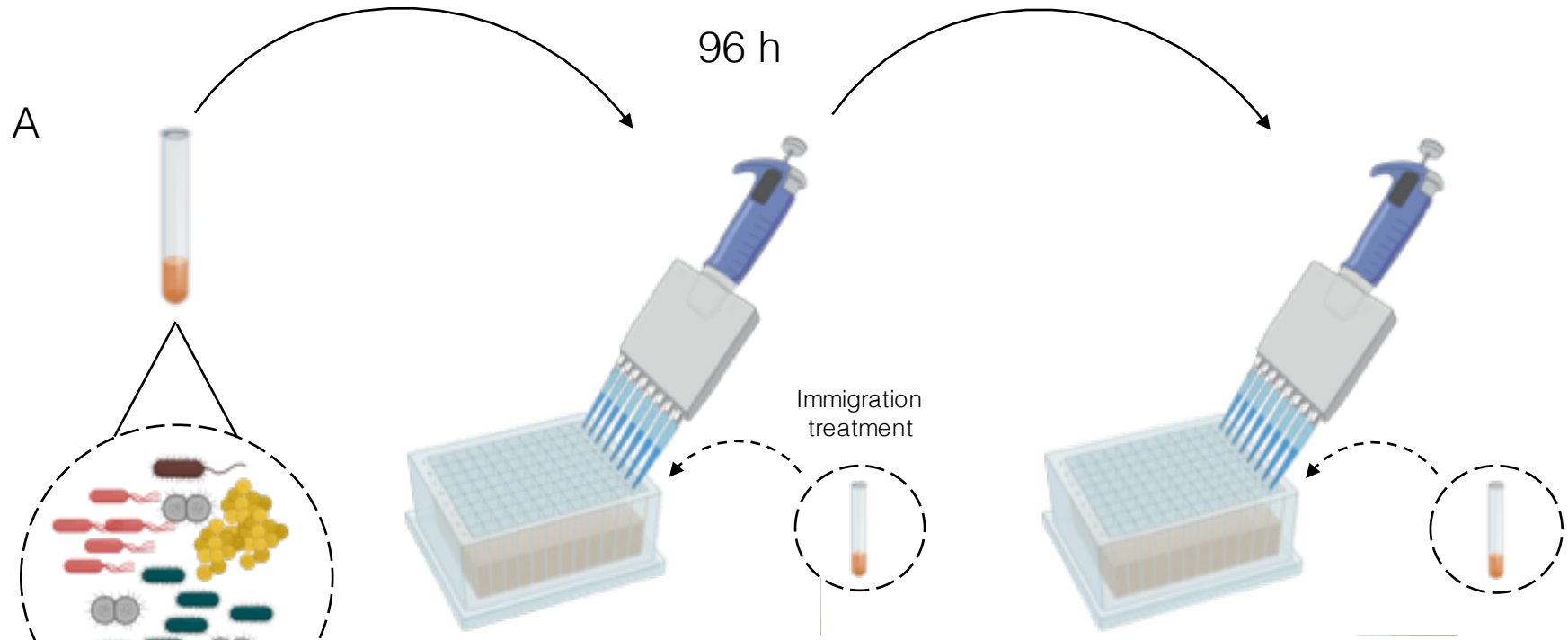
771 **Figure 3.** Community response to antibiotic perturbation. (A) Shannon diversity at the end of the
772 antibiotic pulse (N = 64). (B) Ecological resilience without (left) and with (right) species immigra-
773 tion (N = 190). Resilience has been quantified for each community separately as the Kullback-
774 Leibler (KL) divergence of community composition over time after the disturbance relative to the
775 pre-disturbance state. In both panels, the data for the respective metric (Shannon diversity or KL
776 divergence, both computed from species composition data) is displayed by a box and whiskers plot
777 overlaid by raw data points. The lower and upper hinges of the box and whiskers plot correspond to
778 the 25th and 75th percentiles, while the lower and upper and whiskers extend from the hinge to the
779 smallest or largest value, respectively (max. $1.5 \times$ interquartile range from hinge). Low, intermedi-
780 ate and high antibiotic levels correspond to 4, 16 and 128 $\mu\text{g ml}^{-1}$ streptomycin, respectively.
781

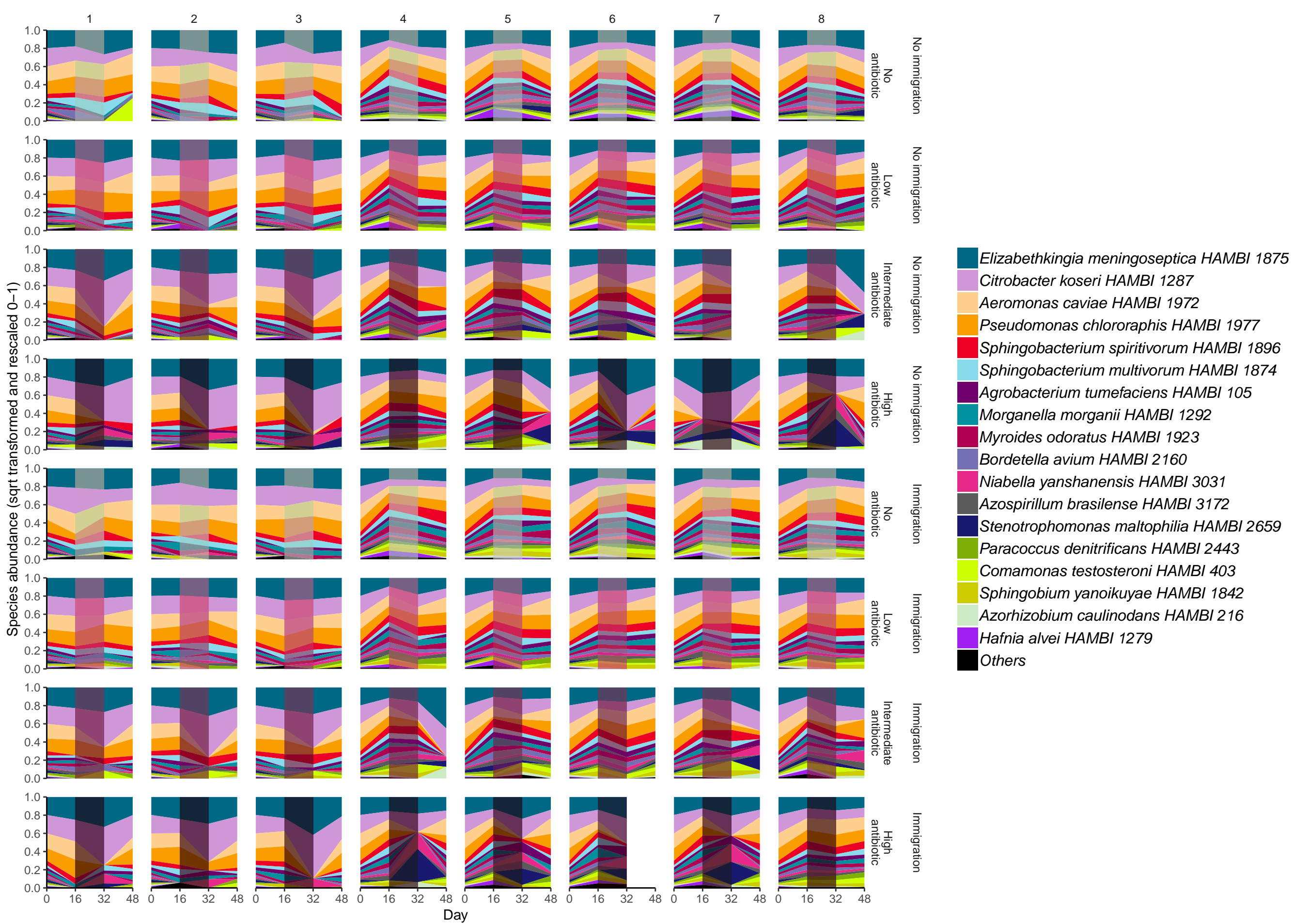
782 **Figure 4.** Competitive fitness of species in replicate communities (N = 8) during and after recovery
783 from intermediate-level antibiotic pulse (16 $\mu\text{g ml}^{-1}$ streptomycin) in the absence of immigration
784 (mean indicated by dashed line). The figure illustrates the repeatability of the species response be-
785 tween replicate communities and the inverse response for multiple species during the perturbation
786 (top panel) versus recovery (bottom panel) phases. The competitive fitness of species is estimated

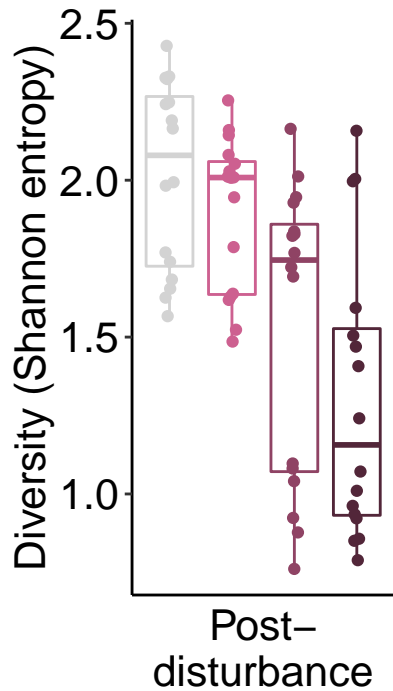
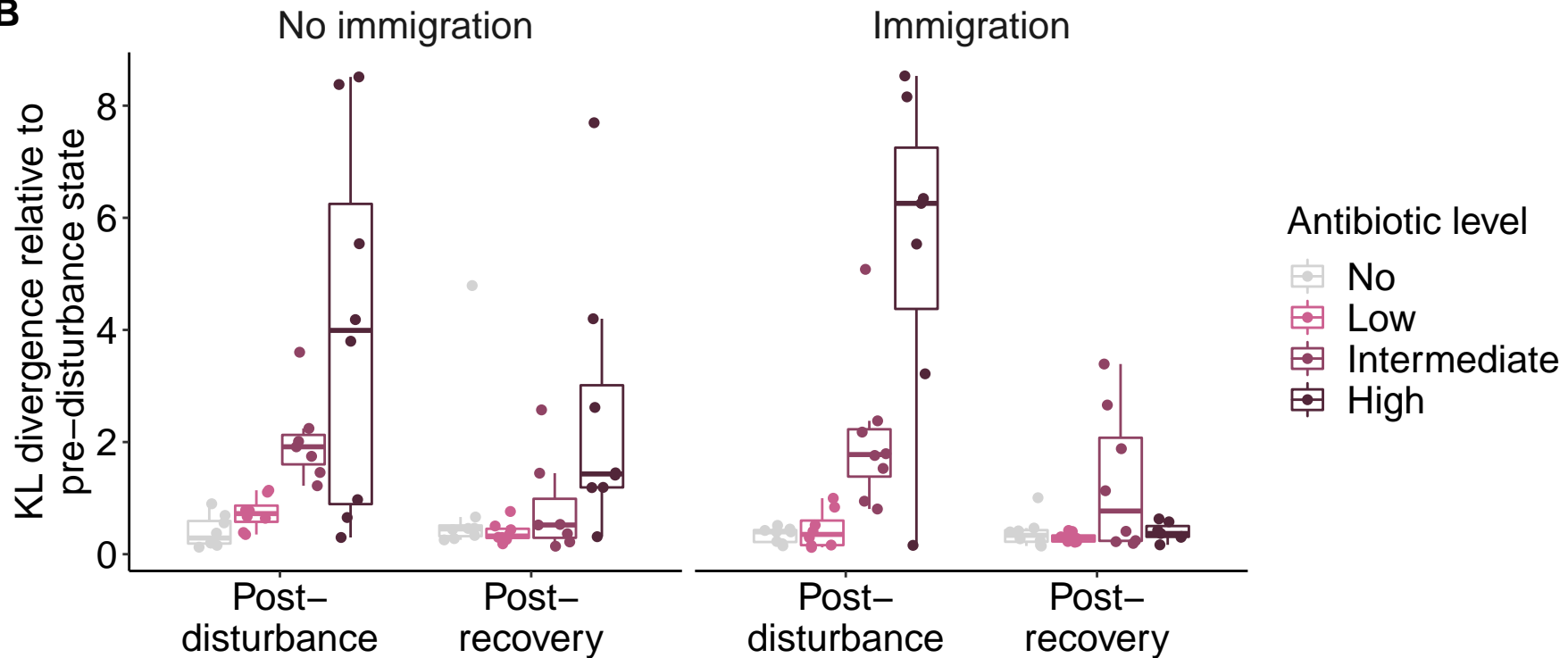
787 as the logarithm of the final frequency relative to the starting frequency, derived from the replicator
788 equation in evolutionary game theory. Data for replicate 7 is missing from the panel below because
789 of inadequate amplicon sequence data for one community sample (final time point).
790

791 **Figure 5.** Repeatability of community trajectories, assessed using the diversity dissimilarity index
792 (\pm bootstrapped standard error; $N = 190$) where zero indicates perfect identity and one complete
793 dissimilarity between replicate communities. Repeatability is shown separately for Shannon diversity
794 (top), which gives more weight to abundant species, and species richness (bottom), which gives
795 equal weight to all species. The antibiotic pulse epoch is indicated by grey shade. Low, intermediate
796 and high antibiotic levels correspond to 4, 16 and 128 $\mu\text{g ml}^{-1}$ streptomycin, respectively. The di-
797 versity dissimilarity index has been computed from species compositional data.
798

799 **Figure 6.** Targets of adaptive mutations reaching high frequencies (> 0.3 to fixation) in high-
800 abundance species during or after recovery from antibiotic pulse. The same mutations were mostly
801 observed in both time points when the species was detectable (i.e. mutation not lost during recovery).
802 The heat map shows functionally annotated targets of recurrent nonsynonymous mutations, as
803 well as the known streptomycin resistance gene *rpsL* which is only mutated in a single community.
804 Since the genomic variants were recovered from deep sequencing data, they could only be con-
805 firmed for a subset of the three sequenced replicates and experimental treatments owing to differen-
806 tial abundance of species and volume of sequence data. Color coding is used to indicate the number
807 of the replicates where a genomic target of interest was mutated relative to the number for which
808 genomic variant data could be recovered in a particular experimental treatment.
809





A**B**

Antibiotic level

No

Low

Intermediate

High

