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

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Abstract

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

Introduction

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

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

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

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

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

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

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

Results

Pre-exposure to disturbance caused trait evolution

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

Pre-exposure of three abundant community members as monocultures to increasing levels of streptomycin led to increased disturbance resistance for two of the species: *Aeromonas* and *Pseudomonas chrororaphis* (Figure 2A; *t*-tests on IC_{50} values of ancestral vs. pre-exposed populations with Bonferroni correction, $P < 0.001$; Supplementary Table 1). In turn, the species *Citrobacter* was already intrinsically resistant prior to pre-exposure (Figure 2A).

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 previously[16], 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]

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

Streptomycin level also influenced community diversity. Higher Shannon diversity occurred at low streptomycin level compared to no streptomycin or high level (least diversity; Extended Data Figure 8; Supplementary Table 7). This was strongly influenced by the dominant species *Aeromonas* whose population collapsed with streptomycin. This increased evenness at low streptomycin level, while high streptomycin level expectedly decreased diversity by driving species extinctions and competitive dominance of particular resistant species. This finding is consistent with the intermediate disturbance hypothesis positing that increasing disturbance levels initially increase diversity by reducing the abundance of competitively dominant species[32]. Nevertheless, the effect only holds for the *Aeromonas* species, as its pre-exposure to streptomycin removes the effect (Extended Data Figure 8). Similarly, the effect was removed when all species were pre-exposed to streptomycin, without Shannon diversity reduction even at high disturbance level. Species richness alone displayed a gradual drop at increasing streptomycin level with the extinction of susceptible species (Extended Data Figure 9; Supplementary Table 8). Similar to its effect on Shannon diversity, in the presence of streptomycin, pre-exposure of all species in the community maintained species richness.

Pre-exposure decreased impact of disturbance on community

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

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

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

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

Dispersal spread patch features to metacommunity

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

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

Pre-exposure removed dispersal-resilience relationship

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

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

Discussion

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

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

resilience in uncertain. It could, for instance, be caused by decreased recovery time for the undisturbed patch between dispersal events at high dispersal rate. As we only collected end-point data, future studies including sampling over time are needed to test this hypothesis. Second, we found that pre-exposure of the dominant *Aeromonas* species to disturbance altered the abundance of also the other community members, protecting the community from change with disturbance, but the reason for this should be addressed by future studies. A focal species could alter community-wide species composition through competition for shared resources, altering the resource landscape for the other species or through species interactions such as producing useful or harmful metabolites[33-35]. Stress conditions may also change the nature of species interactions.[36,15,37,38]

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

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

Fifth, we observed a strong capacity of pre-exposure, particularly for the most abundant and stress susceptible community members, to decrease the effect of the disturbance, also cancelling the spread of disturbance across the metacommunity at high dispersal rate (Figure 5A). This demonstrates the potential of rapid evolution to improve ecological resilience, 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 community[13]. 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 pulses[16]. 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 IC_{50} 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

may facilitate metacommunity resilience. Our previous study suggests that one-way immigration from an undisturbed (*i.e.*, source) to a disturbed patch is ideal for resilience, but this may not always be achievable[16]. However, our findings in this study suggest that with high levels of dispersal, there is a risk of spreading eco-evolutionary effects of disturbances across metacommunities (see also [21]). Nevertheless, when there is high potential for evolutionary change in response to the disturbance, or if pre-exposed populations of abundant taxa are introduced into a community, rapid trait evolution can be harnessed to protect from the disturbance at both local (patch) and global (metacommunity) levels.

Exploiting these features of rapid evolution and dispersal could be an effective tool for promoting compositional resilience in species communities facing environmental change. Nevertheless, this approach is also accompanied by risks. Reduced within-species diversity[16] or pleiotropic effects of resistance mutations[44,45] in an evolved species may cause community-wide changes in composition (as observed here) and function, or affect the viability and resilience of the evolved species. Exploiting evolution and connectivity may still be considered worthwhile, as it is increasingly acknowledged that control interventions to steer ecology and evolution virtually always carry associated costs due to the complexity of biological systems[4,9]. An optimal control strategy for a given eco-evolutionary system, including one seeking to improve resilience, is one that strikes a balance between the importance of achieving a particular target and the importance of minimizing associated costs.

Methods

Synthetic bacterial community and experimental evolution

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

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

Serial passage experiment and measurements

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

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

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

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

Sequencing and bioinformatics

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

TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG–3', PCR1_Reverse (55 bp): 5'–GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC–3' in a limited-cycle PCR reaction, then attaches Nextera XT barcodes using a dual-index arrangement. The libraries were then pooled and sequenced on an Illumina MiSeq using paired 300-bp reads and MiSeq v3 reagents at the Finnish Institute of Molecular Medicine (FIMM). Library indices were subsequently demultiplexed using bcl2fastq v2.2. Paired-end 16S rRNA amplicon reads were then quality trimmed, merged, filtered, and mapped to a reference of the 16S rRNA gene from the 23 species as previously described[46]. Prior to data analysis, species counts were normalized by species-specific 16S rRNA gene copy number.

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

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

The competitive mapping process generated a set of read pairs unique to the expected species from each experimentally evolved population. Evolved species with <25× coverage of the target genome (HAMBI 97, 105, 262, 1988, and 3237; Table S16) were excluded from downstream analysis. Taxonomically verified read pairs were mapped to closed reference genomes for each species[47] using BWA-mem v0.7.17[48]. Alignment files were preprocessed with GATK v4.4 following best practices.[49] Mutect2 from GATK v4.4[50] was used to call genomic variants using default parameters, and mutect calls were filtered to exclude spurious calls using FilterMutectCalls with the --microbial-mode option. Filtered variants were annotated using SnpEff v4.3.[51] Gene calls were from Prokka v1.14.6.[52] Functional annotations of genes were derived from the Prokka internal database and the eggNOG 6.0 database[53] using eggNOG-mapper v2.1.10.[53]

Inference of IC_{50} values

Using a dose-response curve analysis, half maximal inhibitory streptomycin concentrations (IC_{50}) were estimated for the ancestral forms of each bacterial species, the pre-exposed populations of the three abundant species used in the pre-exposure treatment (*Citrobacter koseri* HAMBI 1287, *Aeromonas caviae* HAMBI 1972, and *Pseudomonas chlororaphis* HAMBI 1977), and for clones randomly picked from the final time point in the experiment. Clones of ancestral/evolved forms of each species and from the experiment end point (i.e., day 46) were picked from agar plates and precultured for 24 hours in PPY medium, then inoculated at a density of 0.01 OD_{600} into 200 µL R2A medium at streptomycin concentrations ranging from 0, 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024, 2048, 4096, and 8192 µg mL^{-1}. Cultures were grown for 48 hours at 25 °C in 96 well-plates with shaking (1,000 rpm), and culture density was assessed at 48 hours using optical density (OD_{600}). For the strains used to initate the experiment, four replicate dose-response experiments were performed for each ancestral species (four clones per species) and 64 for each population of the three pre-exposed species. For the experimental end point communities, eight clones were isolated and tested from each of the 180 communities (1,440 clones in total).

Dose-response curves were fit to the resulting blank-corrected optical density data from each species or clone following Sebaugh[54] but using a four-parameter log-logistic function of the form

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

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

Downstream data analyses

All downstream analyses were performed in the R v4.2.3 environment[55]. The t-distributed stochastic neighbor embedding (t-SNE) map for Figure 3B and Extended Data Figure 2 was created using the Rtsne package[56] with the options perplexity = 20 and theta = 0.5. Permutational analysis of variance (PERMANOVA)[57] as implemented in the adonis function in the vegan package[58] was used to test whether the antibiotic level, community mixing rate or pre-exposure treatment affected community composition. The method tests the probability that the observed distances between groups could arise by chance by comparing them with random permutations of the raw data.[59] The influence of the experimental treatments on IC_{50} and KL divergence values relative to the pre-exposure history specific baseline (streptomycin- and mixing-free condition) was investigated using linear regression models.

Data availability

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

Code availability

All code needed to reproduce the downstream analyses and figures are available in Zenodo: https://doi.org/10.5281/zenodo.14015860.[60]

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Author Contributions Statement

Design of serial passage experiment: TH, SH. Performing serial passage experiment: EA. Design of data analysis: JC, SH, VM. Performing data analysis: JC, SH. Interpreting results: JC, TH, SH, VM. JC wrote the first manuscript draft, with contributions from all authors. All authors approve the final version of the manuscript.

Competing Interests Statement

The authors state that they have no competing interests.

Figure legends

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

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

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

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

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

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

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