

Disturbance pre-exposure of key species improves resilience of entire communities and metacommunities

Johannes Cairns

`johannes.cairns@helsinki.fi`

University of Helsinki <https://orcid.org/0000-0003-1329-2025>

Shane Hogle

University of Turku <https://orcid.org/0000-0003-2595-3863>

Elizaveta Alitupa

University of Turku

Ville Mustonen

University of Helsinki <https://orcid.org/0000-0002-7270-1792>

Teppo Hiltunen

University of Turku <https://orcid.org/0000-0001-7206-2399>

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1 Disturbance pre-exposure of key species improves resilience of entire
2 communities and metacommunities

3 Johannes Cairns^{1,2}, Shane Hogle¹, Elizaveta Alitupa¹, Ville Mustonen^{2,3} & Teppo Hiltunen^{1*}

4
5 Author information

6
7 ¹ Department of Biology, University of Turku, Turku, Finland

8 ² Department of Computer Science, Organismal and Evolutionary Biology Research
9 Programme, University of Helsinki, Helsinki, Finland

10 ³ Institute of Biotechnology, University of Helsinki, Helsinki, Finland

11
12 * Corresponding author: teppo.hiltunen@utu.fi

Abstract

Understanding when communities are resilient, that is, able to resist and recover from change when facing disturbances, is critical for solving key global challenges. These include the effects of medication on the gut microbiota and global warming on biodiversity. There is still scant evidence from controlled experiments on factors affecting resilience. Among those hypothesized to affect community response are prior disturbance exposure, priming communities to cope with future disturbance, and species flow between disturbed and undisturbed patches in a metacommunity. The rate of species flow may also be important for whether connectivity protects from or spreads the disturbance effect across a metacommunity. Here we set up an *in vitro* system to test these questions, manipulating the pre-exposure history of a 23-species model bacterial community and serially passaging each community type in three patches exposed to no, low or high disturbance level (antibiotic concentration) at no, low or high rate of community mixing (connectivity). As expected, pre-exposure, causing evolution of resistance traits, protected species from change in abundance. The more abundant the pre-exposed species had been in the undisturbed community, the less the entire community changed, with protective effects on other species potentially contributing to this effect. In the absence of pre-exposure, increasing rates of community mixing caused an increasing spread of the disturbance across the metacommunity. By protecting communities from the disturbance, pre-exposure cancelled this effect of community mixing rate, keeping the metacommunity close to the undisturbed state. Our findings demonstrate that pre-exposure is an important modifier of ecological resilience in a metacommunity setting.

Introduction

Ecological disturbances are events directly or indirectly causing ecosystem change.¹ They vary considerably in magnitude, frequency and extent, with durations ranging from discrete, short-term pulse disturbances to long-term or continuous press disturbances.^{2,3} Currently, numerous ecosystems on Earth are experiencing unprecedented anthropogenic ecological disturbances owing to large-scale 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, many being long-term in nature.

For there to be any hope to mitigate unwanted effects of ecological disturbances for sustainable agriculture and healthcare, maintaining ecosystem services and nature conservation, a critical first step is to develop a mechanistic understanding of disturbance response.⁴ In particular, this means understanding the conditions where ecological disturbances compromise the structure or function, that is, the resilience of ecosystems. There exist two frameworks on resilience that stress different aspects. The engineering resilience framework is focused on the return of a system to its pre-disturbance state, and can be partitioned into two phases: 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.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 (i.e., regime shift) occurs in the state of an ecosystem (i.e., reaching a tipping point) as this can impair ecosystem functioning or even result in community collapse.⁷⁻⁹

Prior, primarily observational research has identified several factors hypothesized to impact resilience in species communities. Here we focus on two key factors. The first is past exposure to a disturbance by a community. Past disturbances can prime communities to better cope with future disturbances through several 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 have been shown 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.¹⁰⁻¹⁴ 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 associated mutations.¹⁵ 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).

Migration is another critical factor affecting resilience. Species communities rarely exist in isolation but rather are typically nested within patches in a metacommunity with varying levels of connectivity, here denoting the magnitude of species flux between connected patches¹⁶. In general, migration is a powerful factor determining diversity, increasing local patch (alpha) and decreasing metacommunity (beta) diversity.^{13,17} On the one hand, when occurring from an undisturbed to a disturbed patch, migration can enable resilience which would otherwise be compromised, a process entitled source-sink dynamics.^{15,18} On the other hand, migration from a disturbed to an undisturbed patch can introduce features of the disturbed community to the undisturbed environment, including ecological (altered species composition) and evolutionary (altered within-species genetic composition) components.^{19,20} However, there are very few studies examining the net effect of these processes across a metacommunity. A recent study examined these processes for morphotypes of a single bacterial species cultured in patches with different antibiotic levels subjected to low-level migration (i.e., dispersal).¹³ Consistent with metacommunity theory, the study found that migration was associated with higher local and lower metacommunity diversity (due to homogenizing effect across patches). Another recent study found a positive relationship between connectivity and resilience (improved resilience with higher connectivity) at lower stress levels, which was inverted into a negative relationship (decreased resilience with higher connectivity) at higher stress levels.²¹ This suggests that both the level of the disturbance and the level of connectivity can be critical for resilience at the metacommunity level.

To test the effect of pre-exposure and connectivity level on ecological resilience, we performed a full-factorial serial passage experiment for the 23-species model bacterial community with eight pre-exposure histories: communities containing (1) only naïve species, (2–7) a disturbance-adapted population of one of the six most abundant species, or (8) disturbance-adapted 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 (concentrations of the antibiotic 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). We collected ecological and phenotypic data for species frequencies and antibiotic resistance (quantified by the half maximal inhibitory concentration, IC_{50} , for streptomycin) for communities at the end-point of the serial transfer experiment, allowing us to test the conditions driving or preventing system change (Figure 1B).

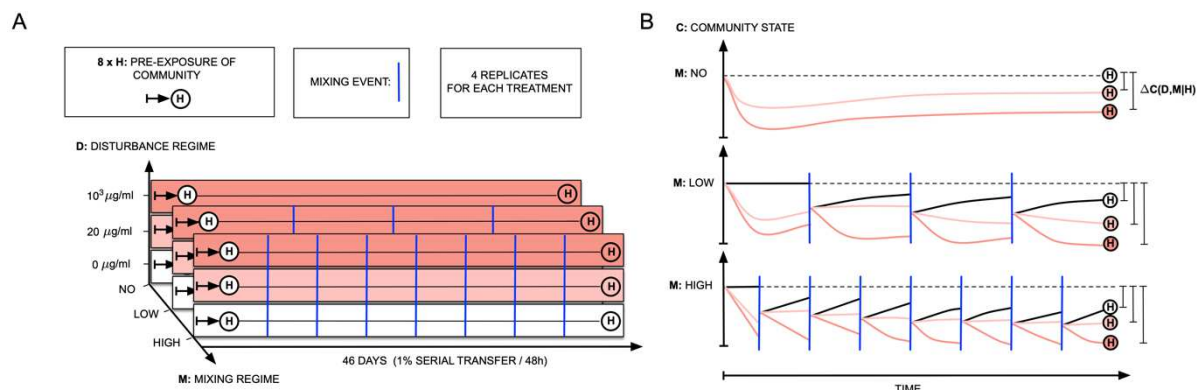


Figure 1 | Design of experiment to test effect of pre-exposure and community mixing rate on disturbance response in multispecies community. (A) The experimental system consisted of a 23-species bacterial model community where evolutionary history was modified by pre-exposing each species to the disturbance, the antibiotic streptomycin, and constructing communities with no pre-exposed species, one among six of the most abundant species being pre-exposed, or all 23 species being pre-exposed. These communities were each subjected to a regime of three rates of community mixing (no, low or high) between three patches experiencing different levels of disturbance (*i.e.*, patch harshness: no, low or high antibiotic level). Each unique treatment combination was replicated four times, making up a total of 288 communities (eight evolutionary histories, 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. (B) To test the study questions, high-throughput sequencing was used to quantify community composition 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 from the no-disturbance, no-mixing baseline within each pre-exposure history as a proxy for ecological resilience.

Results

Pre-exposure to disturbance was accompanied by trait evolution

The 23 species in the model community were heterogeneous in terms of intrinsic susceptibility to the model disturbance, the aminoglycoside antimicrobial streptomycin (Extended Data Figure 1). Pre-exposure of six of the most abundant community members to progressively increasing levels of the antimicrobial led to a marked increase in disturbance resistance level for most species. Expectedly, the evolutionary response depended on intrinsic susceptibility such that the magnitude of the susceptibility difference between ancestral and evolved forms was greatest for susceptible species (HAMBI 6, 1972, and 1977) compared to species with some intrinsic resistance (HAMBI 105, 1287, and 1896).

Whole-genome sequence data for pre-exposed populations of the 23 species supported trait evolutionary change in response to the disturbance (Extended Data Figures 2 & 3). The most common recurrent target of nonsynonymous mutations that reached fixation or high prevalence among the species was the gene *rpsL*, encoding for the streptomycin binding site in the small subunit of the bacterial ribosome, a known target of high-level streptomycin resistance mutations (Extended Data Figure 3)^{22,23}. Nonsynonymous mutations in *rpsL* were observed in 18/23 species, including four of the six most abundant species. Moreover, recurrent mutations were observed in *rsmG* previously associated with low-level streptomycin resistance²⁴, as well as in various genes associated with bacterial stress response (e.g. *dnaK*, *groL*, *mutB*, *uvrA*, and *uvrB*; Extended Data Figure 2)²⁵. Two abundant species, HAMBI 1287 and HAMBI 1896, lacked mutations in *rpsL*, consistent with them being already intrinsically highly resistant and therefore lacking the selection pressure to evolve *de novo* resistance (Extended Data Figure 1). Among these, the pre-exposed population of HAMBI 1287 had no SNPs or small indels, indicating potential lack of evolutionary change owing to intrinsic resistance, although structural variation cannot be ruled out. This is consistent with the number of potential aminoglycoside efflux pumps in the genome. The pre-exposed population of HAMBI 1896 had a nonsynonymous mutation in the gene *nrdE* encoding ribonucleoside-diphosphate reductase subunit alpha, which makes deoxyribonucleotide precursors for DNA synthesis. The known molecular mode of action for streptomycin is ribosome interference, but there are no mutations in HAMBI 1896 genes involved with protein synthesis.

Stronger disturbance led to stronger system change

Regardless of pre-exposure history, as a general feature, in the absence of community mixing, stronger disturbance level led to a stronger change in community composition relative to the disturbance-free condition (PERMANOVA model: disturbance level $r^2 = 0.09$, $P = 0.01$, pairwise comparisons all $P < 0.03$; Figure 2A top row, Extended Data Figures 4, 5A top row & 6; Supplementary Table S1). Expectedly, this was caused by favoring species or genotypes with high-level streptomycin (*i.e.*, disturbance) resistance, particularly at high disturbance level, with the model disturbance streptomycin explaining the vast majority (73.0 %) of variation in the half-maximal inhibitory concentration values of eight (8) clones isolated at random from each experimental end-point community (Figure 2B; Extended Data Figure 5B; Supplementary Tables S2–S5).

When the relative abundance of the species in the different experimental treatments is compared to their relative abundance in the absence of disturbance and community mixing (within each pre-exposure history), an increasing depth and number of negative peaks can be observed from low to high disturbance level (Figure 2B top row; Extended Data Figure 5A top row). These negative species responses are accompanied by a few increasing positive peaks demonstrating that some species gained in community fraction and even in absolute abundance as the biomass in the community remained relatively constant (Extended Data Figure 7). Therefore, an increasing number of species responded negatively to increasing disturbance level, while a few species, with a favorable combination of competitive fitness and disturbance resistance, benefited and took up the freed resources.

Intriguingly, the successively stronger community response is not reflected by a typical metric used to capture diversity, the Shannon index, which accounts for both species evenness and species richness (Extended Data Figure 8). Shannon diversity increases for the low disturbance level because a dominant species (HAMBI 1972), which is highly susceptible to the disturbance (Extended Data Figure 1), is lost, increasing species evenness. Moreover, metacommunity theory posits that migration increases local patch (alpha) diversity, but because of this effect in the low-disturbance community, here we see that increasing migration levels only increase alpha diversity for the disturbance-free and high-disturbance communities (Extended Data Figure 8). This case example illustrates that conventional simplified metrics on the disturbance response of communities can lose important information.

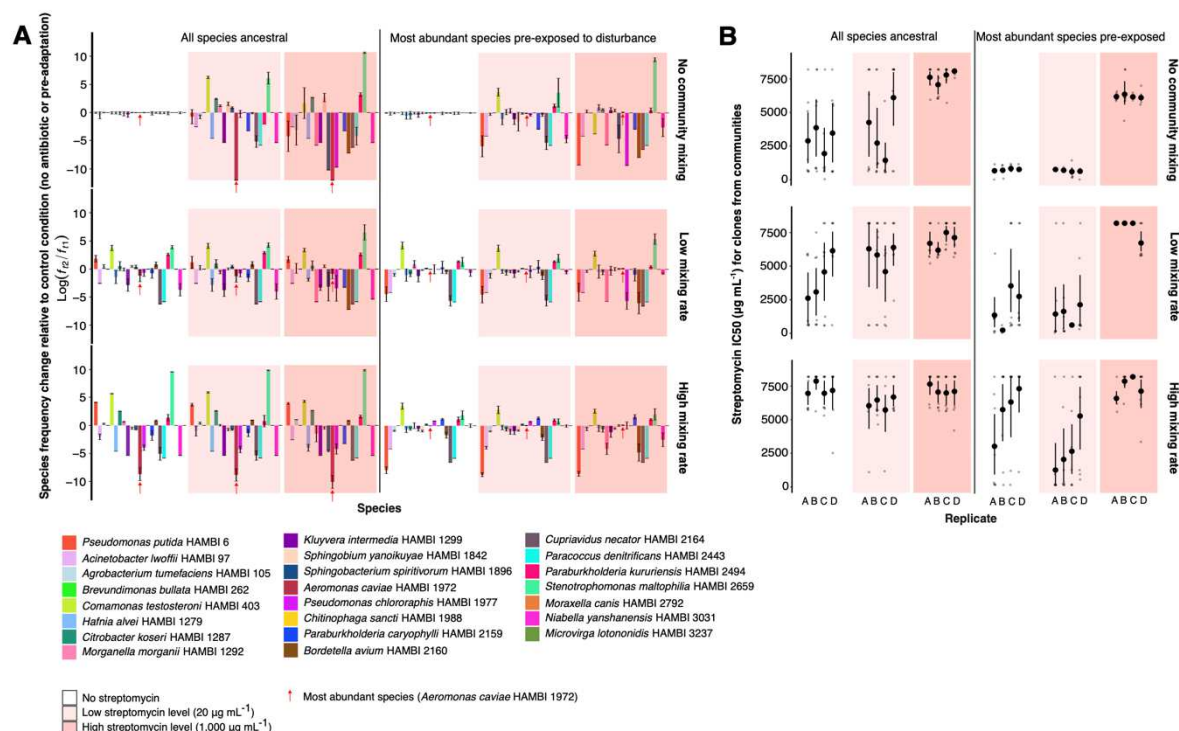


Figure 2 | Effect of disturbance, community mixing and pre-exposure of single most abundant community member (*Aeromonas caviae* HAMBI 1972) on community composition and disturbance resistance. (A) Frequency of each species relative to the frequency of the same species in the evolutionary history specific control condition with no antibiotic or community mixing (i.e., upper left-hand white corner) at the end-point of 46-day serial passage experiment (mean with s.e.m. for 4 replicates for each unique treatment combination). Subcolumns show data for the three increasing disturbance levels (no, low or high antibiotic 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; (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. To facilitate comparison of HAMBI 1972 frequency between treatments, it is indicated by a red arrow across the treatments. A model bacterial community consisting of 23 gram-negative species was employed in the experiment. **(B)** Half maximal inhibitory concentrations (IC_{50}) for the model disturbance streptomycin in the same treatments as in panel A are indicated for eight clones isolated from each population at the experimental end-point (mean with bootstrapped 95 % confidence intervals). The data is considered to represent the IC_{50} values of dominant community members as the species identity of the clones was not determined. Within each pre-exposure treatment, the three antibiotic disturbance patches mixed at low or high rate have a shared history and can be identified by the replicate letter code in the x-axis.

Pre-exposure dampened disturbance response affecting also other than pre-exposed species

Pre-exposure history affected the community response such that the pre-exposed species had higher abundance at increasing levels of disturbance compared to their abundance without pre-exposure (Figure 2A red arrows; Extended Data 5A red asterisks; PERMANOVA model: pre-exposure treatment $r^2 = 0.28$, $P = 0.01$; Supplementary Table S1). An exception is one dominant, intrinsically resistant species (*Citrobacter koseri* HAMBI 1287) whose abundance remained unchanged at the different disturbance levels. This translated into a decrease in community divergence from the disturbance-free baseline (and hence, decrease in beta diversity among the three patches) as a function of the abundance of the pre-exposed species in the disturbance-free patch (Figure 3; Pearson correlation for log10-transformed values $r(69) = -0.84$, *i.e.*, $r^2 = 0.72$, $P < 0.001$). This is, in part, expected as a computational consequence of dissimilarity estimation, since the more abundant a species is in a community, the more its change inflates community dissimilarity metrics. Therefore, when evolution due to pre-exposure prevents this change, the effect on total community dissimilarity is greater for initially more abundant species. Expectedly, pre-exposure had the strongest effect when all species were pre-exposed, with a large fraction of species exhibiting a decreased negative response at the low or high disturbance levels, strongly mitigating the community consequences of the disturbance (Extended Data Figure 5A).

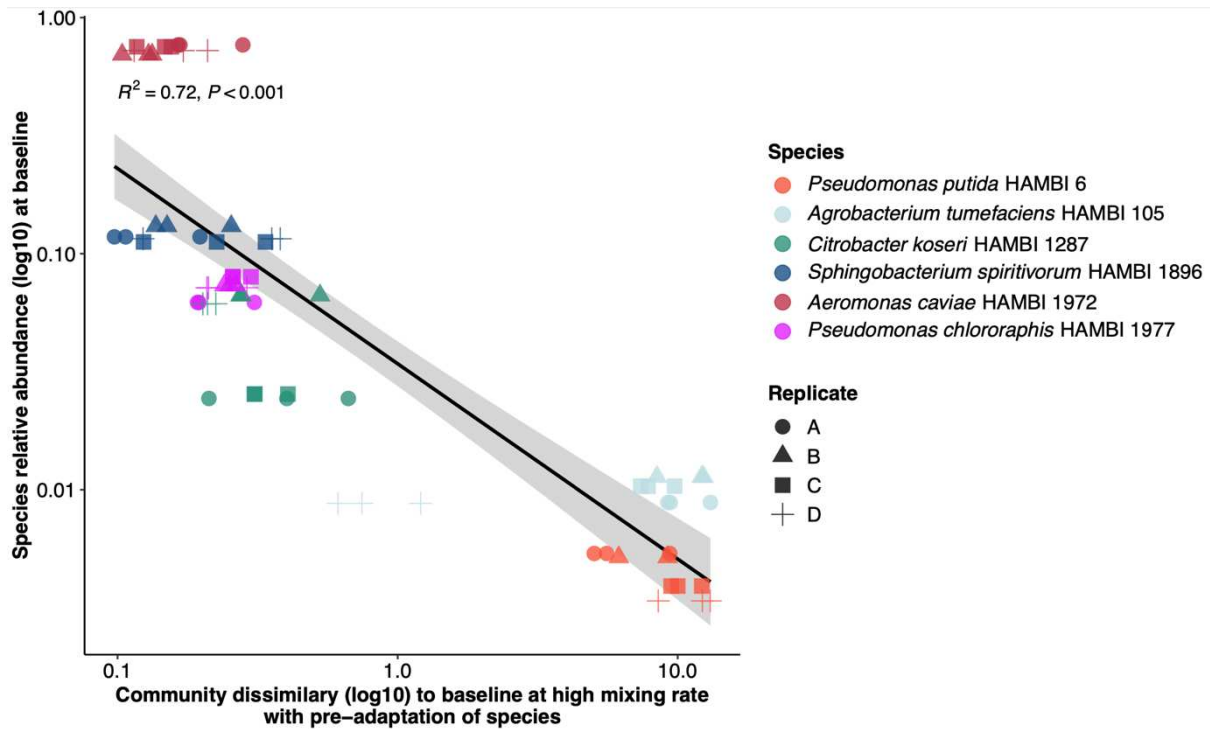


Figure 3 | Association between focal species abundance in undisturbed community and the magnitude of community change with its pre-exposure to disturbance at high community mixing rate. A regression line (mean $\pm$ s.e.m.) has been drawn on a scatter plot of focal species abundance in the undisturbed community in the absence of pre-exposure versus the degree to which the entire community diverges (KL divergence) from the disturbance- and mixing-free baseline at high mixing rate when the same species has been pre-exposed to be disturbance-resistant. The data is shown separately for the four (4) replicates per treatment combination, based divergence values for the three antibiotic patches at high mixing rate (hence three identical symbols for each species-replicate pair). The undisturbed community replicates and pre-exposure treatment replicates are independent. The two sets of replicates have been coupled here instead of relating pre-exposure treatment values to undisturbed community value means to avoid artificially reducing noise in the data.

Pre-exposure of one species also resulted in considerable fold changes in the abundance of several other species in the community (Figures 2A top row & 4; Extended Data Figure 5A top row). For instance, with pre-exposure of the most dominant community member, *Aeromonas caviae* HAMBI 1972, which would otherwise have been highly susceptible to the disturbance and evolved high resistance level during pre-exposure (Extended Data Figures 1–3), there were changes in the abundance of multiple other species in the presence of the disturbance compared to the absence of pre-exposure. These include considerably decreased decline in *Hafnia alvei* HAMBI 1279 and *Kluyvera intermedia* HAMBI 1299 at low disturbance level and in *Sphingobacterium spiritivorum* HAMBI 1896 at high disturbance level, as well as over 2-fold changes in abundance (increase or decrease) in several other species (Figure 2A top row; Figure 4). Since the other species responded differentially depending on the pre-exposed species and there are multiple potential underlying mechanisms (see Discussion), a more detailed investigation of the causes behind this finding lies beyond the scope of this study. Nevertheless,

this finding suggests that the pre-exposure of a member species is a form of biotic disturbance to a community. At least for some of the pre-exposed species, effects on the abundance of other species may also have contributed to the dampened community change in the presence of disturbance.

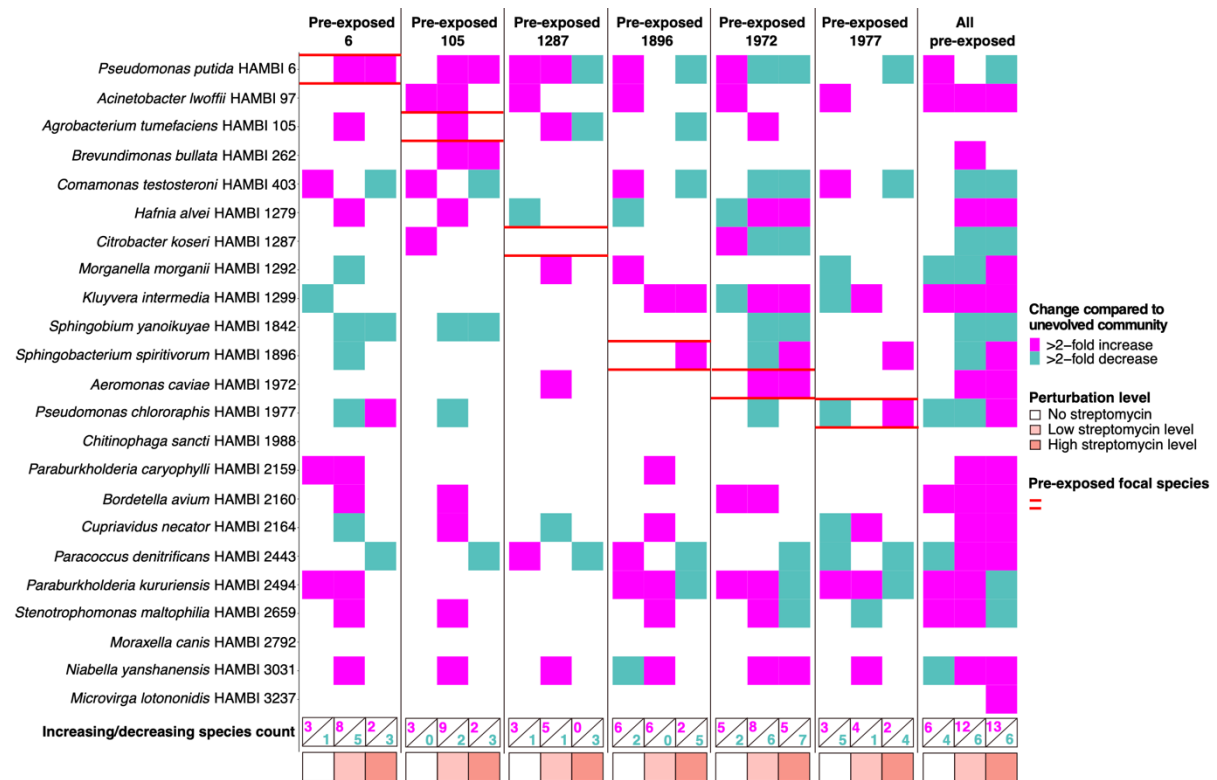


Figure 4 | Effect of pre-exposure on abundance of pre-exposed and other species in the absence of community mixing. Only above or below 2-fold differences compared to the pre-exposure-free condition are indicated. The data is based on mean values of 4 replicates per unique treatment combination. Columns show data for the three increasing disturbance levels (deepening shades of red) in eight pre-exposure treatments, separated by black vertical lines from left to right as follows: (1) ancestral populations used for all species; (2–7) a population used for one of the six most abundant species that had been pre-exposed to be highly resistant to the disturbance (HAMBI Culture Collection code indicated above the columns); and (8) pre-exposed resistant populations used for all species in the community. An artificial bacterial community consisting of 23 gram-negative species was employed. The baseline has been set separately for the three antibiotic disturbance levels. Therefore, the pre-exposure-free treatment would be completely white and is not included in the figure. The total count of species with decreased (blue) or increased (bright red) community fraction is shown in the last row.

Community mixing homogenized effects among patches

Expectedly, community mixing introduced features of the disturbed patches to the undisturbed patch, and vice versa, such that each patch assumed an intermediate community composition reflecting the compositions of the two other patches (Figure 2AB middle and bottom rows; Extended Data Figure 5AB middle and bottom rows & 6; PERMANOVA model: community mixing rate $r^2 = 0.03$, $P = 0.01$; community mixing rate $\times$ streptomycin level $r^2 = 0.13$, $P = 0.01$; community mixing rate $\times$ pre-exposure treatment $r^2 = 0.18$, $P = 0.01$; pairwise comparisons significant at $P < 0.05$ except for low vs. high mixing rate; Supplementary Table S1).

Therefore, consistent with metacommunity theory, migration led to decreased metacommunity (beta) diversity (Figure 5 left vs. middle and right columns; ANOVA for linear model on Kullback-Leibler divergence of communities from pre-exposure treatment specific baseline: community mixing rate $F_{2,213} = 88.5$, $P < 0.001$; community mixing rate $\times$ streptomycin level $F_{4,213} = 53.1$, $P < 0.001$; community mixing rate $\times$ pre-exposure treatment $F_{2,213} = 18.0$, $P < 0.001$; Tukey HSD for all pairwise comparisons on community mixing rate $P < 0.01$; Supplementary Table S6). Moreover, the level of variance between the individual replicates decreased from low to high community mixing rate, demonstrating a stronger homogenizing effect by a higher mixing rate (Figure 5 middle vs. right column). This is also seen in the associated traits of eight (8) clones isolated at random from the communities at the experimental end-point, such that the level of variation in IC_{50} values explained by the model disturbance streptomycin decreases from 73.0 % at no mixing through 20.8 % at low mixing rate to 6.5 % at high mixing rate (Supplementary Tables S2–S6).

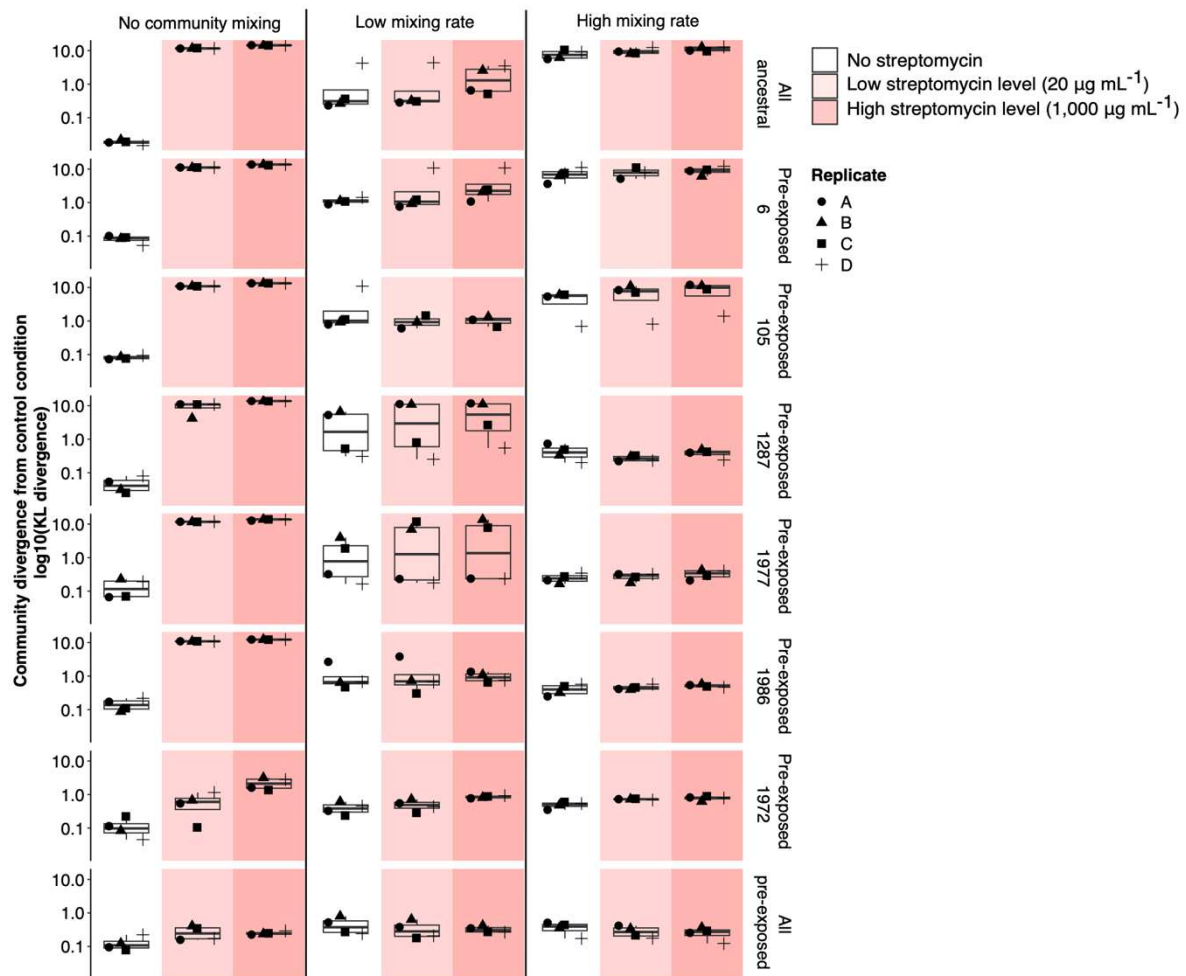


Figure 5 | Effect of disturbance, community mixing and pre-exposure on community divergence from evolutionary history specific control condition. The data is depicted as Kullback–Leibler (KL) divergence of species relative abundance vectors (i.e., community composition) compared to the pre-exposure treatment specific control condition (i.e., leftmost column; $N = 4$ replicates per unique treatment combination). KL divergence values are depicted for three patches with different disturbance regimes (no, low or high antibiotic level) at no, low or high community mixing rates (columns separated by black bars). Subcolumns show data for the three increasing disturbance levels (deepening shades of red) in eight pre-exposure treatments (rows) as follows: (1) ancestral populations used for all species; (2–7) a population used for one of the six most abundant species that had been pre-exposed to be highly resistant to the disturbance, in order of increasing species abundance in the undisturbed community (HAMBI Culture Collection code indicated in row label); and (8) pre-exposed populations used for all species. A bacterial model community consisting of 23 gram-negative species was employed. The control condition values differ from zero since they show divergence values for each individual replicate from the mean value of all four replicates. Hence, higher control condition values in the evolved treatments compared to the all ancestral treatment indicate higher variance between replicates. The box plot depicts the interquartile range (IQR) from the first to the third quartile with the median indicated by a line across the box and the whiskers extending from the hinges to no further than $1.5 \times \text{IQR}$. Within each pre-exposure treatment, the three antibiotic disturbance patches mixed at low or high rate have a shared history and can be identified by the replicate letter code indicated by shape. For instance, replicates D for each antibiotic level at high community mixing rate in the “Evolved 105” evolutionary treatment represent three patches that were mixed regularly and therefore resemble each other more than the other replicates in the same antibiotic level.

Negative relationship between mixing rate and resilience was cancelled by pre-exposure of high-abundance species

In the absence of pre-exposure, mixing rate was negatively associated with ecological resilience, such that the metacommunity was compositionally closer to the undisturbed, no-mixing community at low mixing rate while diverging more from it at high mixing rate (Figure 5 top row; PERMANOVA model: community mixing rate $\times$ streptomycin level $r^2 = 0.13$, $P = 0.01$; ANOVA for linear model on KL divergence: community mixing rate $\times$ streptomycin level $F_{4,213} = 53.1$, $P < 0.001$; Supplementary Tables S1 & S6). However, with pre-exposure of abundant community members, with the exception of the two least abundant species among them (*Pseudomonas putida* HAMBI 6 and *Agrobacterium tumefaciens* HAMBI 105), this relationship was cancelled (Figure 5 rows below top row; PERMANOVA model: streptomycin level $\times$ community mixing rate $\times$ pre-exposure treatment $r^2 = 0.05$, $P = 0.02$; ANOVA for linear model on KL divergence: streptomycin level $\times$ community mixing rate $\times$ pre-exposure treatment $F_{28,213} = 2.83$, $P < 0.001$; Supplementary Tables S1 & S6). This shows that a decreased community effect of the disturbance resulting from pre-exposure of abundant species can dramatically alter the connectivity-resilience relationship.

Discussion

Here we tested predictions concerning the effect of pre-exposure to disturbance and community mixing (modeling migration and connectivity) on the disturbance response in a multispecies community. We used a model system consisting of a 23-species microbial community cultured in three patches exposed to different antimicrobial levels with three rates of community mixing. In line with predictions based on theory and previous empirical evidence, community mixing homogenized traits and species composition across the patches, reducing metacommunity (beta) diversity, while increasing local (alpha) diversity for most patches²¹. Moreover, pre-exposure of abundant community members to the disturbance, causing associated trait and genomic evolution, decreased the effect of the disturbance on the individual patches and thereby also on the metacommunity.

In the absence of pre-exposure, we found a strong negative relationship between community mixing rate and ecological resilience. High mixing rate led to percolation of the disturbance effect across the metacommunity. As predicted, this is a potential outcome of decreased recovery time for the undisturbed patch, leading to a decreasing proportion of the undisturbed community in the metacommunity over time with the accumulation of mixing events. As we only collected end-point data, future studies including sampling over time are needed to test this hypothesis. Our results have implications for extending existing community coalescence theory²⁶ to a dynamic temporal setting. A recent study found that the connectivity-resilience relationship can also be inverted by stress level²¹. Together these findings highlight that the relationship between migration and ecological resilience is highly dependent on key system parameters.

We found signals that pre-exposure not only caused evolutionary trait change in the target (*i.e.*, pre-exposed) species, decreasing the effect of the disturbance at the local (patch) and global (metacommunity) levels, but that it also resulted in altered abundance among several other species. This shows that pre-exposure of individual community members represents a form of large-scale biotic disturbance itself. The reasons for this remain unclear and represent an interesting avenue of future research. One obvious case where stress adaptation of a focal species could alter community-wide species composition is in the presence of species interactions. For instance, one species may produce metabolites that another species can use as a resource (positive interaction) or metabolic byproducts that are harmful to another species

(negative)²⁷⁻²⁹. Species interactions can also change sign under stress.³⁰ For instance, under benign conditions, species interactions may primarily be competitive, but in the presence of a stressor one species may protect others (e.g., the production of antibiotic-degrading enzymes^{14,31} or biofilm protecting against exposure to the antibiotic³²) creating new positive interactions. With the evolution of an abundant stress-susceptible species, these interactions can change for multiple reasons, either directly when evolution changes the strength or sign of an interaction between species or indirectly when altered resource availability affects the abundance of other non-interacting species. Altered species interactions will subsequently result in compositional changes in the community.

We observed a strong capacity of pre-exposure, particularly for highly abundant and stress susceptible community members, to decrease the effect of the disturbance, also cancelling the spread of disturbance across the metacommunity at high community mixing rate. This demonstrates the potential of rapid evolution to improve ecological resilience, including in a metacommunity setting. Notably, however, we designed our experimental setup to quantify the maximum potential of rapid evolution by pre-exposing the species to be highly stress resistant. Moreover, we pre-exposed the species by exposing them to increasing levels of stress. The capacity for rapidly evolvable species to evolve *de novo* stress resistance is likely to be constrained during sudden exposure to high-level disturbance and when nested in a multispecies community¹². In a multispecies community, susceptible species can rapidly become outcompeted by intrinsically resistant species and have lower population sizes (potentially decreasing evolvability) compared to when cultured alone. The reason for our setup was specifically that we had previously failed to observe a clear effect of rapid evolution on community dynamics when communities consisting of initially unevolved species were exposed to antibiotic pulses¹⁵. Similar to that study, *de novo* evolution of streptomycin resistance is also likely to have occurred in multiple species during our study, although we lack the phenotypic and genomic data to test this. However, due to our previous findings, we consider it unlikely that *de novo* evolution would be important for the dynamics in this system. Nevertheless, to better establish the role of rapid evolution on the disturbance response and resilience of communities, further studies are needed to identify the conditions where rapid evolution occurring when species are embedded in multispecies communities is strong enough to alter community dynamics.

Our findings are relevant for designing successful control interventions to improve ecological resilience in natural communities. When there is absence of pre-exposure or low potential for rapid evolutionary change, low levels of connectivity 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 is ideal for resilience, but this may not always be achievable¹⁵. However, our findings in this study suggest that with high levels of connectivity, there is a risk of spreading the disturbance effect across the metacommunity (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 evolution and connectivity 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 diversity¹⁵ or pleiotropic effects of resistance mutations^{33,34} 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 S7). 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. In the first step, monocultures of each species were grown in sub-minimal inhibitory concentrations (sub-MICs) of streptomycin

(MICs from ³⁵) 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 S7), and 1 mL of the previous culture was frozen in 30 % glycerol. In the second 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 eight evolutionary histories: only ancestral forms of the 23 species, 22 ancestral species plus one experimentally evolved population from one of six species (HAMBI 6, 105, 1287, 1896, 1972, or 1977), and experimentally evolved populations of all 23 species. These bacterial mixtures were assembled by first reviving the ancestral form and the evolved 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 eight evolutionary histories 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. 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 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

treatment was the same as the low-mixing treatment, but mixing occurred every six days (three transfers).

Experimental microcosms were maintained for 23 serial transfers (96 deep well plates; 1,500 μ L 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 patch mixing treatments, 780 μ L 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. 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 μ L 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 described³⁶. 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). Reads were mapped to reference genomes for each species using BWA-mem v0.7.17³⁷. Genomic variants from bacterial clones were called using Octopus v0.7.4-2³⁸ using ‘individual’ mode and ‘polyclone’ mode as described previously³⁹. Variants were annotated using SnpEff v4.3⁴⁰, and genomic annotations were from Prokka v1.14.6⁴¹.

Inference of IC₅₀ values

Using a dose-response curve analysis, half maximal inhibitory streptomycin concentrations (IC₅₀) were estimated for the ancestral forms of each bacterial species, the evolved populations of six species (HAMBI 6, 105, 1287, 1896, 1972, 1977), and for clones randomly picked from the final time point in the experiment. Clones of ancestral/evolved forms of each species and from experiment endpoints were picked from agar plates and precultured for 24 hours in PPY medium, then inoculated at a density of 0.01 OD₆₀₀ 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 (OD₆₀₀). Four replicate dose-response experiments were performed for each ancestral species (four clones per species), 16 for each population of the six evolved species, and eight from each replicate of the evolution/streptomycin/mixing treatment combinations (2,304 clones in total).

Dose-response curves were fit to the resulting blank-corrected optical density data from each species or clone following Sebaugh⁴² but using a four-parameter log-logistic function of the form

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

where c is the lower asymptote, d is the upper asymptote, b is the slope at the inflection point, and e is the IC₅₀ 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. IC₅₀ values were set to the maximum tested streptomycin concentration when optical density was always greater than 0.2 OD₆₀₀ 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 environment⁴³. The t-distributed stochastic neighbor embedding (t-SNE) map for Extended Data Figure 3 was created using the Rtsne package⁴⁴ with the options perplexity = 20 and theta = 0.5. Permutational analysis of variance (PERMANOVA)⁴⁵ as implemented in the adonis function in the vegan package⁴⁶ 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.⁴⁷ The influence of the experimental treatments on IC₅₀ and KL divergence values relative to the pre-exposure history specific baseline (streptomycin- and mixing-free condition) was investigated using linear regression models in base R.

Data availability

Raw sequence data (fastq files) has been deposited in the NCBI Sequence Read Archive (SRA) under the accession PRJNAX. All code and pre-processed data needed to reproduce the downstream analyses and figures will be made available upon publication via GitHub: <https://github.com/X> (permanent doi: <https://doi.org/XzenodoX>). Accession codes and links to be added.

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Author contributions

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

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 was modified by pre-exposing each species to the disturbance, the antibiotic streptomycin, and constructing communities with no pre-exposed species, one among six of the most abundant species being pre-exposed, or all 23 species being pre-exposed. These communities were each subjected to a regime of three rates of community mixing (no, low or high) between three patches experiencing different levels of disturbance (*i.e.*, patch harshness: no, low or high antibiotic level). Each unique treatment combination was replicated four times, making up a total of 288 communities (eight evolutionary histories, 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. (B) To test the study questions, high-throughput sequencing was used to quantify community composition 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 from the no-disturbance, no-mixing baseline within each pre-exposure history as a proxy for ecological resilience.

Figure 2 | 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) Frequency of each species relative to the frequency of the same species in the evolutionary history specific control condition with no antibiotic or community mixing (*i.e.*, upper left-hand white corner) at the end-point of 46-day serial passage experiment (mean with s.e.m. for 4 replicates for each unique treatment combination). Subcolumns show data for the three increasing disturbance levels (no, low or high antibiotic 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; (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. To facilitate comparison of HAMBI 1972 frequency between treatments, it is indicated by a red arrow across the treatments. A model bacterial community consisting of 23 gram-negative species was employed in the experiment. (B) Half maximal inhibitory concentrations (IC₅₀) for the model disturbance streptomycin in the same treatments as in panel A are indicated for eight clones isolated from each population at the experimental end-point (mean with bootstrapped 95 % confidence intervals). The data is considered to represent the IC₅₀ values of dominant community members as the species identity of the clones was not determined. Within each pre-exposure treatment, the three antibiotic disturbance patches mixed at low or high rate have a shared history and can be identified by the replicate letter code in the x-axis.

Figure 3 | Association between focal species abundance in undisturbed community and the magnitude of community change with its pre-exposure to disturbance at high community mixing rate. A regression line (mean $\pm$ s.e.m.) has been drawn on a scatter plot of focal species abundance in the undisturbed community in the absence of pre-exposure versus the degree to which the entire community diverges (KL divergence) from the disturbance- and mixing-free baseline at high mixing rate when the same species has been pre-exposed to be disturbance-resistant. The data is shown separately for the four (4) replicates per treatment combination, based divergence values for the three antibiotic patches at high mixing rate (hence three identical symbols for each species-replicate pair). The undisturbed community replicates and pre-exposure treatment replicates are independent. The two sets of replicates have been coupled here instead of relating pre-exposure treatment values to undisturbed community value means to avoid artificially reducing noise in the data.

Figure 4 | Effect of pre-exposure on abundance of pre-exposed and other species in the absence of community mixing. Only above or below 2-fold differences compared to the pre-exposure-free condition are indicated. The data is based on mean values of 4 replicates per unique treatment combination. Columns show data for the three increasing disturbance levels (deepening shades of red) in eight pre-exposure treatments, separated by black vertical lines from left to right as follows: (1) ancestral populations used for all species; (2–7) a population used for one of the six most abundant species that had been pre-exposed to be highly resistant to the disturbance (HAMBI Culture Collection code indicated above the columns); and (8) pre-exposed resistant populations used for all species in the community. An artificial bacterial community consisting of 23 gram-negative species was employed. The baseline has been set separately for the three antibiotic disturbance levels.

Therefore, the pre-exposure-free treatment would be completely white and is not included in the figure. The total count of species with decreased (blue) or increased (bright red) community fraction is shown in the last row.

Figure 5 | Effect of disturbance, community mixing and pre-exposure on community divergence from evolutionary history specific control condition. The data is depicted as Kullback–Leibler (KL) divergence of species relative abundance vectors (i.e., community composition) compared to the pre-exposure treatment specific control condition (i.e., leftmost column; $N = 4$ replicates per unique treatment combination). KL divergence values are depicted for three patches with different disturbance regimes (no, low or high antibiotic level) at no, low or high community mixing rates (columns separated by black bars). Subcolumns show data for the three increasing disturbance levels (deepening shades of red) in eight pre-exposure treatments (rows) as follows: (1) ancestral populations used for all species; (2–7) a population used for one of the six most abundant species that had been pre-exposed to be highly resistant to the disturbance, in order of increasing species abundance in the undisturbed community (HAMBI Culture Collection code indicated in row label); and (8) pre-exposed populations used for all species. A bacterial model community consisting of 23 gram-negative species was employed. The control condition values differ from zero since they show divergence values for each individual replicate from the mean value of all four replicates. Hence, higher control condition values in the evolved treatments compared to the all ancestral treatment indicate higher variance between replicates. The box plot depicts the interquartile range (IQR) from the first to the third quartile with the median indicated by a line across the box and the whiskers extending from the hinges to no further than $1.5 \times \text{IQR}$. Within each pre-exposure treatment, the three antibiotic disturbance patches mixed at low or high rate have a shared history and can be identified by the replicate letter code indicated by shape. For instance, replicates D for each antibiotic level at high community mixing rate in the “Evolved 105” evolutionary treatment represent three patches that were mixed regularly and therefore resemble each other more than the other replicates in the same antibiotic level.

Supplementary Files

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