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Current Biology

Pre-exposure to stress reduces loss of community and genetic diversity following severe environmental disturbance

Highlights

- Species sorting during pre-exposure increased community resistance to stress
- Pre-exposure helped maintain genetic diversity across eight acidophiles
- *Acidiphilium rubrum* dominated after severe acidification regardless of pre-exposure
- Severe acidification selected different SNPs in pre-exposed versus naive *A. rubrum*

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In brief

Xu et al. show that pre-exposure to acid stress can protect communities from loss of species and genetic diversity caused by future severe acidification. Their study demonstrates the importance of simultaneous species sorting and evolutionary adaptation in the response of complex natural communities to environmental change.

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Pre-exposure to stress reduces loss of community and genetic diversity following severe environmental disturbance

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SUMMARY

Environmental stress caused by anthropogenic impacts is increasing worldwide. Understanding the ecological and evolutionary consequences for biodiversity will be crucial for our ability to respond effectively. Historical exposure to environmental stress is expected to select for resistant species, shifting community composition toward more stress-tolerant taxa. Concurrent with this species sorting process, genotypes within resistant taxa that have the highest relative fitness under severe stress are expected to increase in frequency, leading to evolutionary adaptation. However, empirical demonstrations of these dual ecological and evolutionary processes in natural communities are rare. Here, we provide evidence for simultaneous species sorting and evolutionary adaptation across multiple species within a natural freshwater bacterial community. Using a two-phase stressor experimental design (acidification pre-exposure followed by severe acidification) in aquatic mesocosms, we show that pre-exposed communities were more resistant than naive communities to taxonomic loss when faced with severe acid stress. However, after sustained severe acidification, taxonomic richness of both pre-exposed and naive communities eventually converged. All communities experiencing severe acidification became dominated by an acidophilic bacterium, *Acidiphilium rubrum*, but this species retained greater genetic diversity and followed distinct evolutionary trajectories in pre-exposed relative to naive communities. These patterns were shared across other acidophilic species, providing repeated evidence for the impact of pre-exposure on evolutionary outcomes despite the convergence of community profiles. Our results underscore the need to consider both ecological and evolutionary processes to accurately predict the responses of natural communities to environmental change.

INTRODUCTION

Any environmental event that reduces individual fitness is defined as a stress, and such stress is common in ecological communities.[1] Environmental stress is expected to intensify worldwide due to increasing anthropogenic impacts from multiple stressors.[2] Severe stress can induce widespread mortality, causing significant biodiversity decline and large shifts in community composition and functioning.[3] Community responses to stress are characterized by resistance (the capacity of a system to withstand disturbance) and resilience (the ability to recover after disturbance).[4,5] If community resistance and resilience are insufficient and stress is not alleviated, the biodiversity of a community may collapse. Elucidating the ecological and evolutionary mechanisms preventing or permitting community persistence and recovery after severe environmental stress is crucial for understanding biodiversity change and improving its management and conservation.

Pre-exposure to stress can mediate the response of complex communities via phenotypic plasticity, species sorting, and evolutionary adaptation.[6] Phenotypic plasticity may enable individual organisms to exhibit induced tolerance after exposure to sublethal levels of stress.[7] Historical stress may select against susceptible species during pre-exposure, shifting community

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composition toward more stress-tolerant taxa. This species sorting process can maintain essential ecological interactions and functioning and prevent community collapse when confronted with levels of stress that would otherwise be lethal in the absence of pre-exposure.[8,9] While phenotypic plasticity and species sorting are well-known outcomes of pre-exposure to stress,[10,11] much less is known about the role of evolution in this process, especially in complex natural communities. Experimental and observational evo-evolutionary studies have demonstrated the influence of local community context on evolutionary trajectories and outcomes.[12–15] A recent set of mathematical models and laboratory experiments with simple communities demonstrated that stress pre-exposure significantly shapes bacterial community stability and adaptation.[16] Historical exposure to environmental stress is expected to select for genotypes with higher relative fitness under severe stress, provided that adaptive alleles are present as standing variation within a species or appear via mutation and have sufficiently large selection coefficients to counter the effects of drift.[17–19] If an increase in frequency of adaptive genotypes rescues average population fitness, this can ameliorate, prevent, or reverse population decline in response to stress,[20,21] thereby allowing greater mutational input to maintain levels of genetic diversity.[22,23] In an analogy to the way that vaccination with a sublethal dose of a virus can prepare an individual's immune system for a potentially deadly future infection, pre-exposure to low or moderate levels of stressors can protect ecological communities from future severe stress. Pre-exposure has been demonstrated to mitigate the impact of severe environmental stress on community structure in a variety of systems,[24–27] even when the specific stressors are different in each exposure period,[28] and the effects can extend to community and ecosystem functioning as well.[29–31]

Acidification is well-known to be a major environmental stressor for aquatic ecosystems, and its detrimental effects on biodiversity have been a considerable challenge for management and conservation.[31–35] Several whole-ecosystem studies have been conducted on the acidification of freshwater lakes, revealing declines in species diversity and disruptions to primary production and nutrient cycling.[36–38] Even though freshwater acidification continues to be a relevant issue to ecological and human health,[39,40] understanding of the evolutionary processes underlying community responses in aquatic ecosystems remains limited. This holds for microbial communities despite their capacity for rapid evolution.[41,42] It is difficult to disentangle evolutionary adaptation from ecological species sorting because their effects can be indistinguishable using standard community profiling techniques such as 16S or 18S rRNA gene metabarcoding. As such, investigating the relative contributions of ecological and evolutionary responses of natural communities requires careful experimentation and monitoring of taxonomic and genetic diversity dynamics over time.

To address this gap, we experimentally manipulated aquatic mesocosms containing diverse microbial communities derived from a natural unpolluted lake to test the impact of acidification pre-exposure on community-wide taxonomic composition and intraspecific genetic diversity after severe acidification. In addition, we tested the role of dispersal rate in moderating these effects. Under environmental stress, immigration via dispersal can buffer against demographic decline and help maintain standing genetic and taxonomic variation, but the introduction of maladapted types can also decrease average fitness depending on dispersal rates.[8,27,43] We used 16S metabarcoding to profile microbial communities and metagenomic shotgun sequencing to investigate the potential role of evolutionary adaptation within individual species during community response. We hypothesized that (1) pre-exposure to moderate acidification will select for acidophiles, thereby producing a more resistant community during severe and sustained acidification than naive communities whose first experience with acidification occurs at severe levels; (2) dispersal will mitigate loss in taxonomic diversity; and (3) species that survive severe acidification in pre-exposed communities will evolve along distinct trajectories compared with those in naive communities, leading to genetic differentiation and greater resistance to genetic diversity loss. Our experiment provides evidence for simultaneous species sorting and evolutionary adaptation across multiple species within a natural community. More broadly, this study generates novel insight into the ecological and evolutionary dynamics of complex microbial communities as they respond to severe environmental stress.

RESULTS

Changes in community diversity following severe acidification

We first tested the hypothesis that the ecological response (species sorting) to acid stress would differ depending on whether communities experienced pre-exposure to a milder acid stress. Briefly, the experiment consisted of two phases (Figure 1). In phase I, pre-exposed mesocosms (red) were subjected to weekly acidification treatments of pH 4 while naive mesocosms (blue) naturally fluctuated around pH 8.5 for approximately 7 weeks. In phase II, all mesocosms were subjected to a press acidification treatment of pH 3 for approximately 8 weeks apart from four additional control mesocosms (green). Alpha diversity as measured by the Shannon index differed significantly between pre-exposed and naive communities, indicating the effect of pre-exposure on amplicon sequence variant (ASV) richness and evenness (Figure 2A). The experiment began with all mesocosms at approximately a Shannon index of 5.6, but pre-exposure caused a significant decrease by the end of the pre-exposure treatment (sample 2) ($H' = 18.64$, $Q < 0.05$, Benjamini-Hochberg Q-value based on p values from Kruskal-Wallis tests) (Figure S1A). Although both pre-exposed and naive communities decreased in diversity 1 week after severe acidification, naive communities experienced a significantly greater decline, resulting in lower Shannon values than pre-exposed communities (sample 3) ($H' = 22.91$, $Q < 0.05$). Pre-exposed communities continued to decrease in diversity during the 8 weeks of severe acidification while naive communities recovered slightly such that all treated communities converged on a Shannon index of 1.8 regardless of pre-exposure (sample 4) ($H' = 1.37$, $Q > 0.05$). The diversity of control communities remained unchanged throughout the experiment (sample 2−sample 3: $H' = 2.08$, $Q > 0.05$; sample 3−sample 4: $H' = 0.33$, $Q > 0.05$). The observed recovery in Shannon diversity of naive communities by the end of the experiment coincided with a small but significant increase in the number of observed

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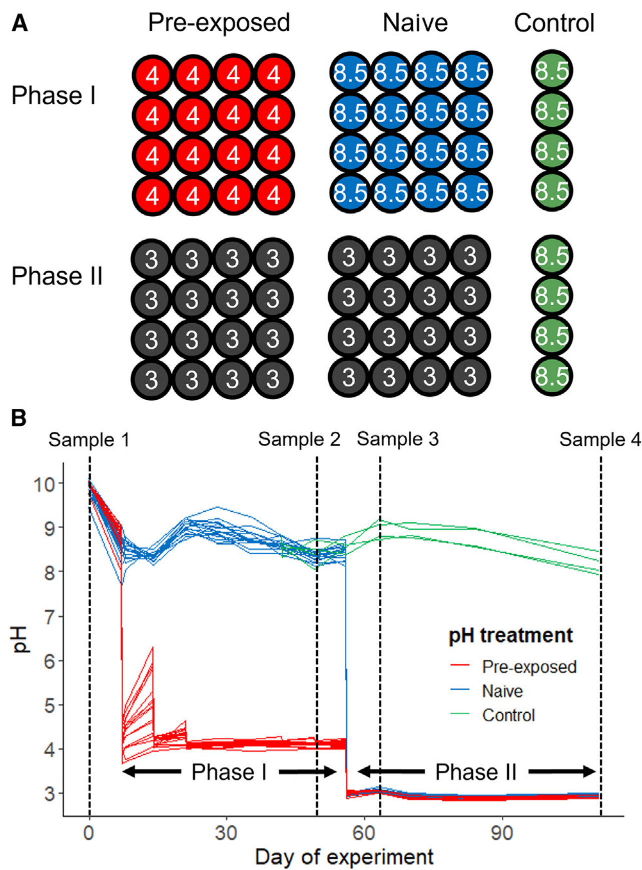

Figure 1. Experimental design and pre-exposure treatments

(A) Schematic representation of the subset of mesocosms (circles) from the two-phase experiment included in this study. Colors indicate pre-exposed (red), naive (blue), and control (green) mesocosms, and numbers indicate pH. Half of pre-exposed and naive mesocosms were under a global dispersal regime in phase I while the other half were isolated (dispersal regimes not shown).

(B) Measured pH of each mesocosm throughout the experiment. Each line represents an individual mesocosm, colors indicate pH treatments, and arrows indicate the duration of each experimental phase. Pre-exposed mesocosms were acidified to pH 4 at the beginning of phase I on June 14/day 7, and, in phase II, all mesocosms were acidified to pH 3 on August 2/day 56 until the end of the experiment. Black dashed lines mark the four time points during the experiment when samples were taken (June 7/day 0, July 26/day 49, August 9/day 63, and September 25/day 111), referred to as samples 1, 2, 3, and 4.

ASVs (sample 3−sample 4: $H' = 17.95$, $Q < 0.05$) whereas no significant change was observed in pre-exposed communities (sample 3−sample 4: $H' = 4.11$, $Q > 0.05$) (Figure S1B). The pairwise change in diversity of pre-exposed communities was significantly negative between all time points, whereas in naive communities it was only significantly negative immediately after severe acidification (sample 2−sample 3), and by the end of the experiment, it was significantly positive (sample 3−sample 4), indicating recovery (Table S1; Figure S2). Pairwise change in the Shannon index was significantly different between pre-exposed and naive communities across all time points (Table S2). Dispersal did not significantly affect Shannon values throughout the experiment (Table S3). We also assessed the effects of pre-exposure on community dynamics with a community stability analysis, using the temporal mean divided by the temporal standard deviation of aggregate abundances.[44] The results indicated that pre-exposure slightly increased stability after severe acidification in accordance with Shannon diversity, but this difference was statistically insignificant (Table S4).

Distinct ecological dynamics of dominant acidophilic bacteria in pre-exposed versus naive communities

Beyond changes to alpha diversity, we also investigated how the ecological effects of acidification pre-exposure influenced community composition. While dispersal did not significantly affect Bray-Curtis dissimilarity ($F = 0.545$, $p = 0.831$), pre-exposure was a significant factor ($F = 8.54$, $p = 0.001$). Community composition of mesocosms shifted drastically due to acidification in phases I and II (Figure 2B). All communities began with a large diversity of bacteria, primarily from the Bacteroidota and Proteobacteria phyla (representing 93.3% of bacterial ASVs at the start of the experiment). Pre-exposed communities became dominated by the family Acetobacteraceae and genera *Mucilaginibacter* and *Granulicella* by the end of phase I (sample 2). By contrast, naive communities remained highly diverse, with an increase in the genus *Polynucleobacter* and the family Sporichthyaceae, which were also present at relatively high frequencies in control communities. Immediately after severe acidification in phase II (sample 3), communities within pre-exposed mesocosms continued to shift, with an increase in *Acidocella* and *Acidosoma* and the disappearance of most minor genera. By sharp contrast, the composition of naive communities collapsed to a single genus, *Acidocella*, which overwhelmingly dominated (Figure 2B). Although pre-exposed and naive communities were distinct in the initial period after severe acidification, by the end of the experiment (sample 4), regardless of pre-exposure and dispersal, all communities converged and became dominated by *Acidiphilium*, followed by Acetobacteraceae, *Granulicella*, and *Acidocella*. In comparison, control communities not acidified in phase II showed no clear change in community composition over time (Figure 2B).

Divergence followed by convergence in the evolutionary trajectories of *Acidiphilium rubrum* in pre-exposed and naive communities

Next, we tested the hypothesis that following severe acidification, the evolutionary trajectories of the dominant species, *Acidiphilium rubrum*, would differ depending on whether it had experienced pre-exposure. Metagenomic shotgun sequencing produced a final contig database of approximately 3.1 million contigs totaling approximately 20.8 Gb, which were binned into 81 metagenome-assembled genomes (MAGs) that included *A. rubrum* and *Acidocella* also present in the 16S rRNA gene sequencing data (supplemental information). To characterize the impact of pre-exposure on the genetic diversity within *A. rubrum*, we mapped reads to the *A. rubrum* reference genome (NCBI RefSeq assembly GCF_900156265.1, strain ATCC 35905) to call single nucleotide polymorphisms (SNPs) for population genetic analyses. At the start of the experiment, *A. rubrum* across communities were genetically similar (pre-exposed $F_{ST} = 0.02$, naive $F_{ST} = 0.04$), but by the end of phase I (sample 2), they had diverged significantly both from starting populations (pre-exposed $F_{ST} = 0.92$, naive $F_{ST} = 0.66$) as well as between pre-exposed and naive communities ($F_{ST} = 0.71$) (Figure 3). After

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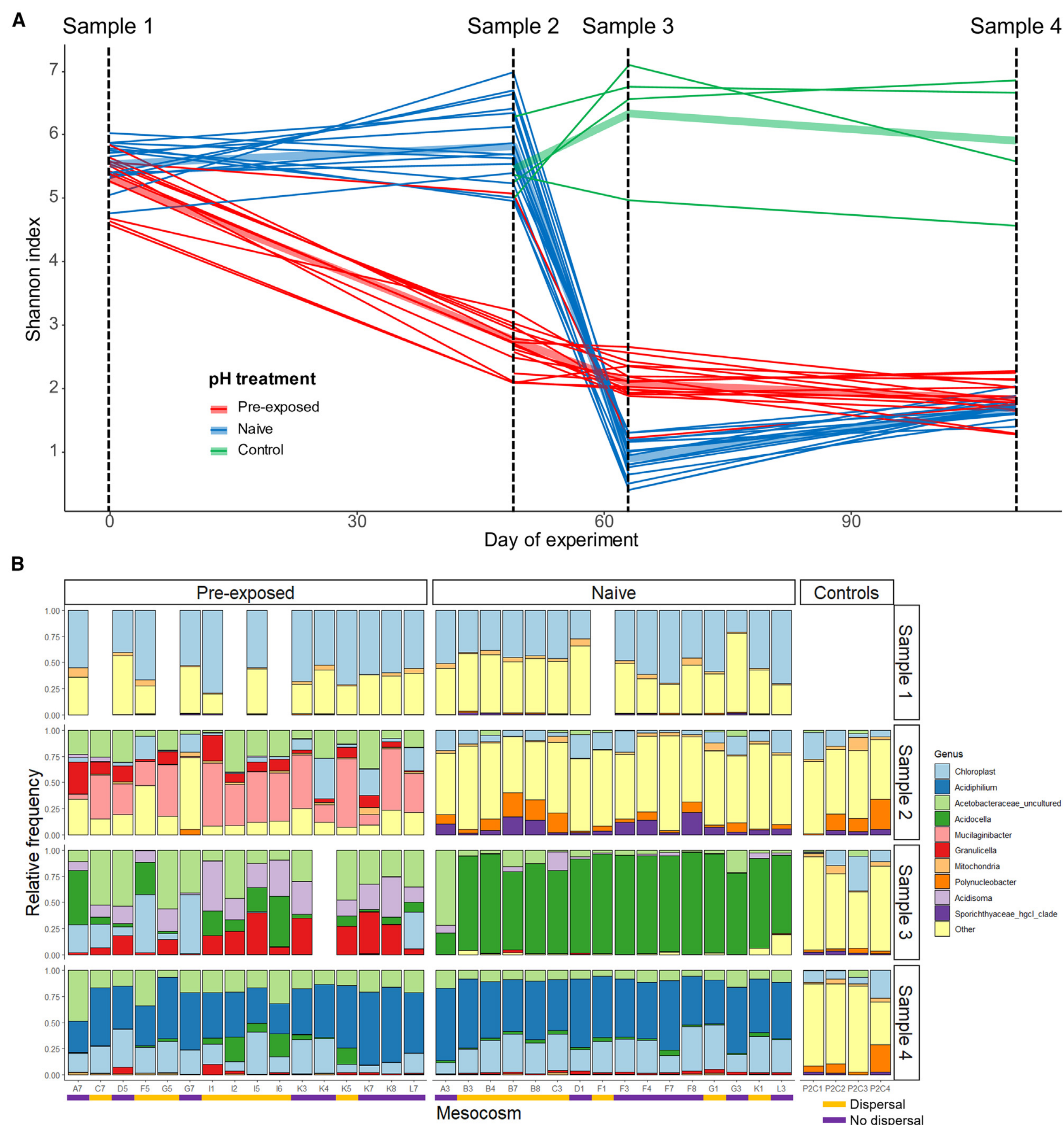

Figure 2. Pre-exposure caused significant changes in alpha diversity and community composition

(A) Shannon index values for pre-exposed (red), naive (blue), and control (green) communities over time. Black dashed lines mark the four time points during the experiment when samples were taken (June 7/day 0, July 26/day 49, August 9/day 63, and September 25/day 111), referred to as samples 1, 2, 3, and 4.

(B) Genus-level taxonomic composition of pre-exposed, naive, and control communities. The top ten genera are colored individually, and all others are grouped together in yellow. Phase I dispersal treatment is indicated by orange (dispersal) and purple (no dispersal) lines.

See also Figures S1 and S2 and Tables S1–S4.

severe acidification (sample 3), *A. rubrum* across pre-exposed and naive communities began to converge (F_{ST} = 0.45), resulting in similar levels of genetic differentiation among all communities by the end of the experiment regardless of pre-exposure (sample 4) (F_{ST} = 0.19–0.21) (Figure 3).

Parallel evolution and the maintenance of genetic diversity in pre-exposed acidophilic bacteria

To assess changes in the genetic diversity of the species remaining at the end of the experiment, we mapped reads to a custom genome database created by combining assembled MAGs with

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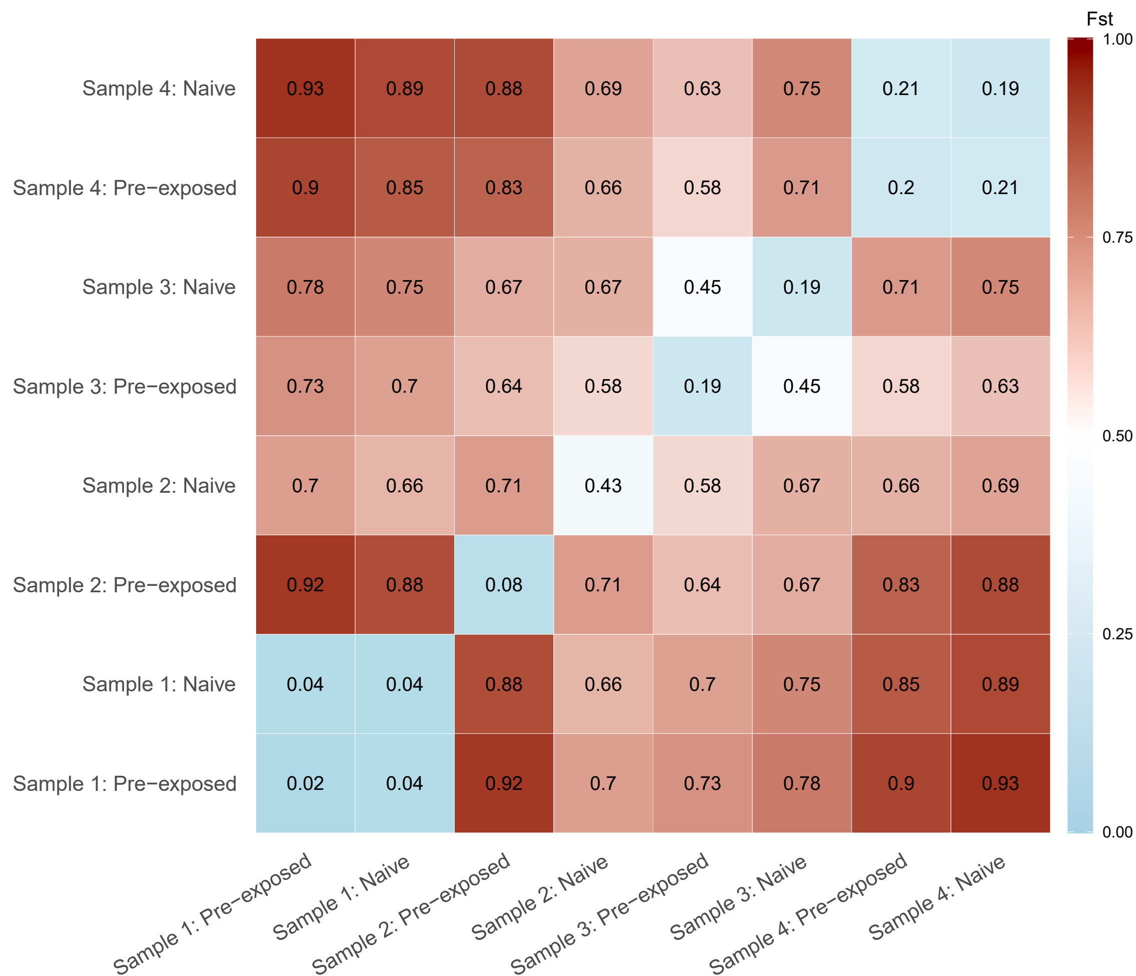

Figure 3. Average pairwise fixation index of SNPs within *Acidiphilium rubrum*

F_{ST} values are listed chronologically from left to right and from bottom to top (sample 1−sample 4), where values range from 0 (light blue) to 1 (dark red).

reference genomes of *Acidiphilium*, *Acidocella*, and *Granulicella* species (supplemental information). We measured how major alleles in these species shifted over time by calculating genome-wide polarized major allele frequency change (subtracting the frequency of each major allele by the frequency of that same allele at the next time point). Despite large heterogeneity in read mapping across different species, changes in SNP allele frequencies showed similar patterns within *Acidiphilium* and *Acidocella* (Figure 4). For pre-exposed communities, all five *Acidiphilium* and all three *Acidocella* species exhibited significantly greater change in allele frequencies 1 week after severe acidification (sample 2−sample 3) than the subsequent 8 weeks of sustained severe acidification (sample 3−sample 4). Additionally, except for *A. iwatense* and *A*. sp. *KAb 2–4*, species in pre-exposed communities exhibited less change in allele frequencies than naive communities in those 8 weeks (sample 3−sample 4).

Because *A. rubrum* was by far the most dominant species at the end of the experiment (Figures S3 and S4), we further assessed its evolutionary trajectory and parallelism. We hypothesized that pre-exposed *A. rubrum* populations would maintain greater genetic diversity during severe acidification, as reflected by lower average nucleotide identity (ANI) and higher nucleotide diversity (π). In support of this hypothesis, mean pairwise ANI of *A. rubrum* was significantly lower in pre-exposed than naive communities at both time points after severe acidification (sample 3: Z = 2.93, $p < 0.05$; sample 4: Z = 2.82, $p < 0.01$, Holm-Bonferroni adjusted p values from Dunn's test) (Figure 5A), and π was also significantly higher in pre-exposed than naive communities (sample 3: Z = −4.24, $p < 0.001$; sample 4: Z = −3.19, $p < 0.001$) (Figure 5B). Additionally, we found evidence of parallel genetic adaptation to acidification among *A. rubrum* populations. Of all SNPs present after severe acidification (present at both sample 3 and sample 4), 36 SNPs were shared among all pre-exposed communities, 31 SNPs were shared among all naive communities, and only 12 SNPs were shared across populations in pre-exposed and naive communities (Figure 6A; Table S5). Permutation tests indicated that the number of shared SNPs among the pre-exposed and naive communities was significantly higher than neutral expectations when SNPs were randomized across mesocosms (pre-exposed: $p < 0.05$; naive: $p < 0.05$, n = 10,000) (Figure 6B).

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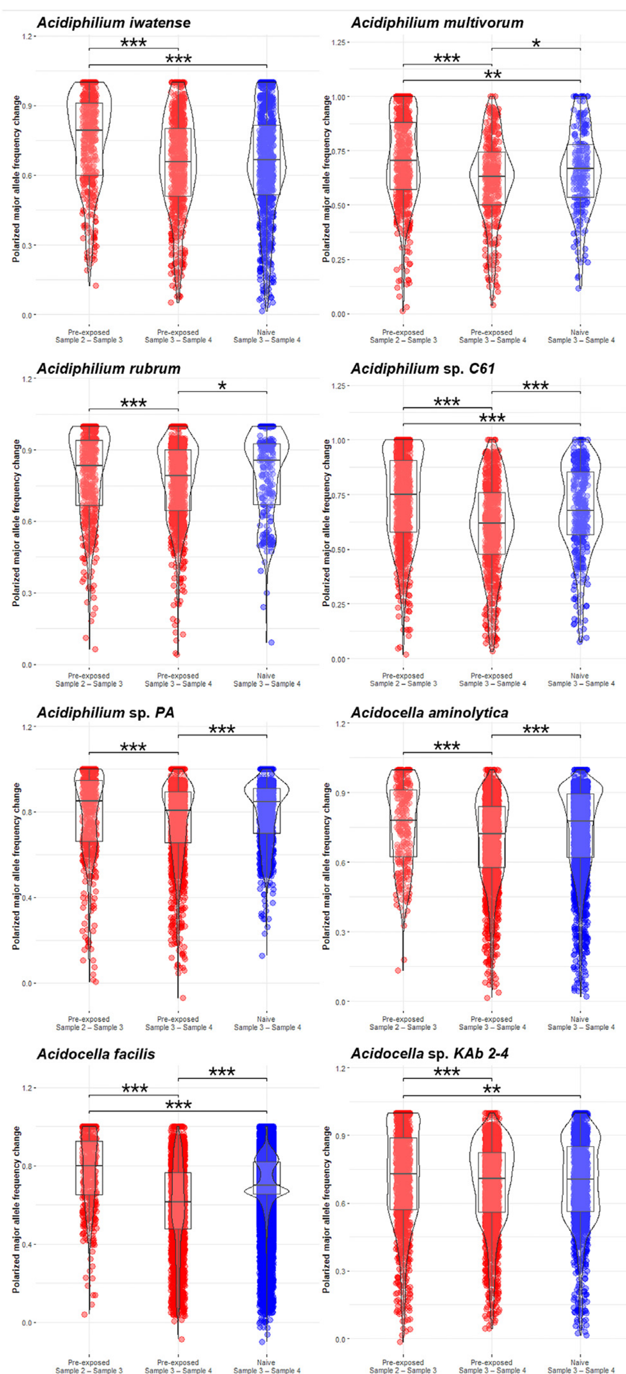

Figure 4. Allele frequency change of single nucleotide polymorphisms polarized to major alleles across species

We calculated allele frequency change by subtracting the frequency of the major allele by the frequency of that same allele in a subsequent time point. Colors indicate pre-exposed (red) and naive (blue) communities. Asterisks indicate statistical significance based on Holm-Bonferroni adjusted p values from Dunn's test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). See also Figure S5.

In summary, two major evolutionary patterns emerged: (1) in pre-exposed populations, the greatest genetic change occurs immediately after severe acidification, and (2) following long-term severe acidification, genetic change was significantly less in pre-exposed than in naive populations. These patterns are consistent with pre-exposure to moderate acid stress causing parallel directional selection for acid-tolerant alleles, resulting in more resistant communities that experience less dramatic population bottlenecks when confronted with severe acidification, thereby retaining greater genetic diversity.

DISCUSSION

Environmental stress can negatively impact community diversity and ecosystem functioning, but pre-exposure to sublethal levels of stress could mediate their persistence and recovery. Investigating the contributions of species sorting and evolutionary adaptation to this process deepens our understanding of the underlying mechanisms of community recovery during sustained stress. Here, we provide evidence that pre-exposure to moderate acidification altered the response of aquatic microbial communities during severe acidification, both ecologically through changes in community composition and evolutionarily via genome-wide shifts of genetic variation across multiple species.

Species sorting reshapes community diversity under stress

Pre-exposure to acidification had profound effects on bacterial communities through species sorting, as indicated by significantly lower Shannon index and markedly different relative abundances of genera. Although pre-exposure was initially detrimental to alpha diversity, pre-exposed communities exhibited relatively greater resistance to severe acidification. Shannon diversity not only decreased significantly less than naive communities but also exhibited significantly higher absolute values immediately after severe acidification (sample 3).

This initial relative increase of community resistance in pre-exposed communities against the immediate effects of severe acidification (between sample 2 and sample 3) may be due in part to species sorting that favored acidophiles previously at very low frequencies at the start of the experiment, such as the family Acetobacteraceae, which contains several acidophilic genera, including *Acidiphillium*, *Acidisoma*, and *Acidocella*.[45] Pre-exposure also selected for *Granulicella*, a genus of acidophiles within the family Acidobacteriaceae,[46] and *Mucilaginibacter*, a diverse genus within the family Sphingobacteriaceae that contains species previously isolated from acidic forest soils[47] and documented to grow in acidic conditions as low as pH 2.[48] By contrast, these taxonomic groups were not detected in naive communities at the end of phase I (sample 2) due to either low abundance or absence. Instead, there was an increase in the genus *Polynucleobacter*, a ubiquitous and diverse freshwater bacterioplankton that can tolerate a wide range of environmental conditions,[49–52] and the family Sporichthyaceae, which contains four named species of motile facultative anaerobes with aerial hyphae isolated from soil, lake sediment, and human skin.[53–57] Both *Polynucleobacter* and Sporichthyaceae were also observed in control communities at the end of pre-exposure (sample 2), so their presence likely indicates seasonal effects or selection by the mesocosm environment.

Immediately after severe acidification, alpha diversity within naive communities crashed and became overwhelmingly

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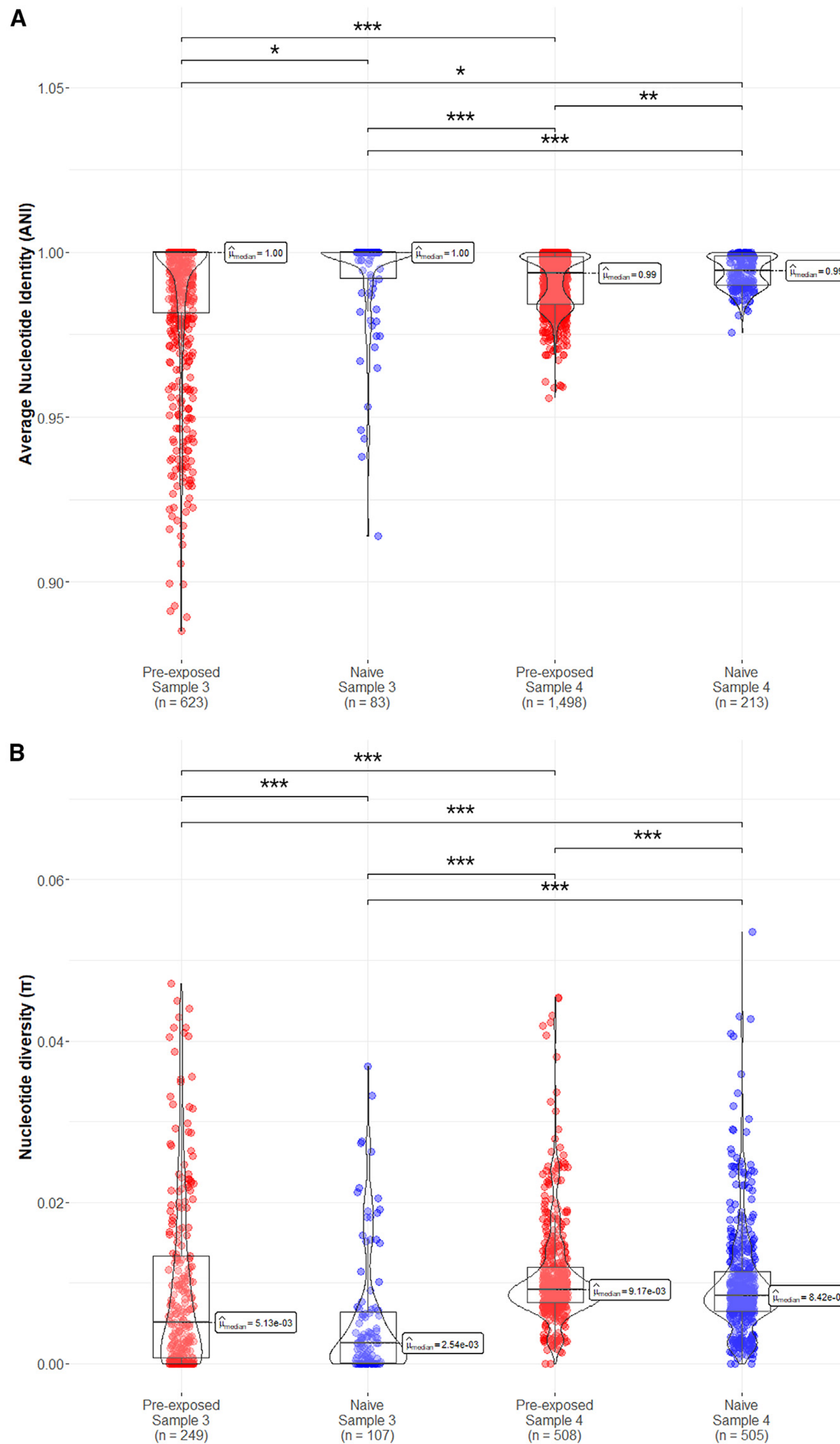

Figure 5. Effects of pre-exposure on evolution of *Acidiphilium rubrum*

(A) Pairwise average nucleotide identity (ANI) and (B) nucleotide diversity (π) of *A. rubrum* genomes within pre-exposed (red) and naive (blue) communities after 1 week (sample 3) and 8 weeks (sample 4) of severe acidification. The number of scaffolds is in parentheses. Asterisks indicate statistical significance based on Holm-Bonferroni adjusted p values from Dunn's test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

dominated by *Acidocella* with minor contributions from Acetobacteraceae, *Acidisoma*, and *Granulicella* in some but not all mesocosms. Notably, Acetobacteraceae and *Granulicella* were among the selected taxa in pre-exposed communities at the end of pre-exposure (sample 2). Contrary to our hypothesis, pre-exposure did not improve long-term community resistance, with both Shannon indices and community composition ultimately converging across pre-exposed and naive communities by the end of the experiment, perhaps because responses of bacterial communities to stress are rapid enough to occur over a few weeks even in communities with no history of exposure to a stressor. However, despite this convergence, pre-exposure did cause a significantly different species sorting trajectory immediately after severe acidification (sample 3), thus providing supporting evidence that pre-exposure to a weaker dose of a stressor could allow more time for both plastic and evolutionary responses by resistant organisms.

Shannon diversity consistently decreased in pre-exposed mesocosms throughout the experiment, albeit at lower rates between each successive time point. By contrast, alpha diversity and richness (observed number of ASVs) in naive communities did recover significantly under severe acidification (sample 3−sample 4) to eventually reach the same levels as pre-exposed communities by the end of the experiment. Although the 16S rRNA analysis included mesocosms under dispersal, we did not observe any significant effects of dispersal on alpha diversity or richness at any time point. Furthermore, there was no additional input from the source lake to mesocosms after the start of the experiment, so the increase in the number of observed ASVs during recovery is unlikely to be attributable to migration. Thus, seemingly novel ASVs at the end of the experiment, such as those assigned to the genus *Acidiphilium*, were most likely previously present but at undetectably low absolute or relative abundances and would have required sufficient positive selection to overcome loss through ecological drift. Our findings are consistent with a previous study on the same source lake that successfully recovered non-obligate acidophilic bacteria capable of surviving at pH 2, which suggests that such acidophiles are ever present in ordinary freshwater, even from

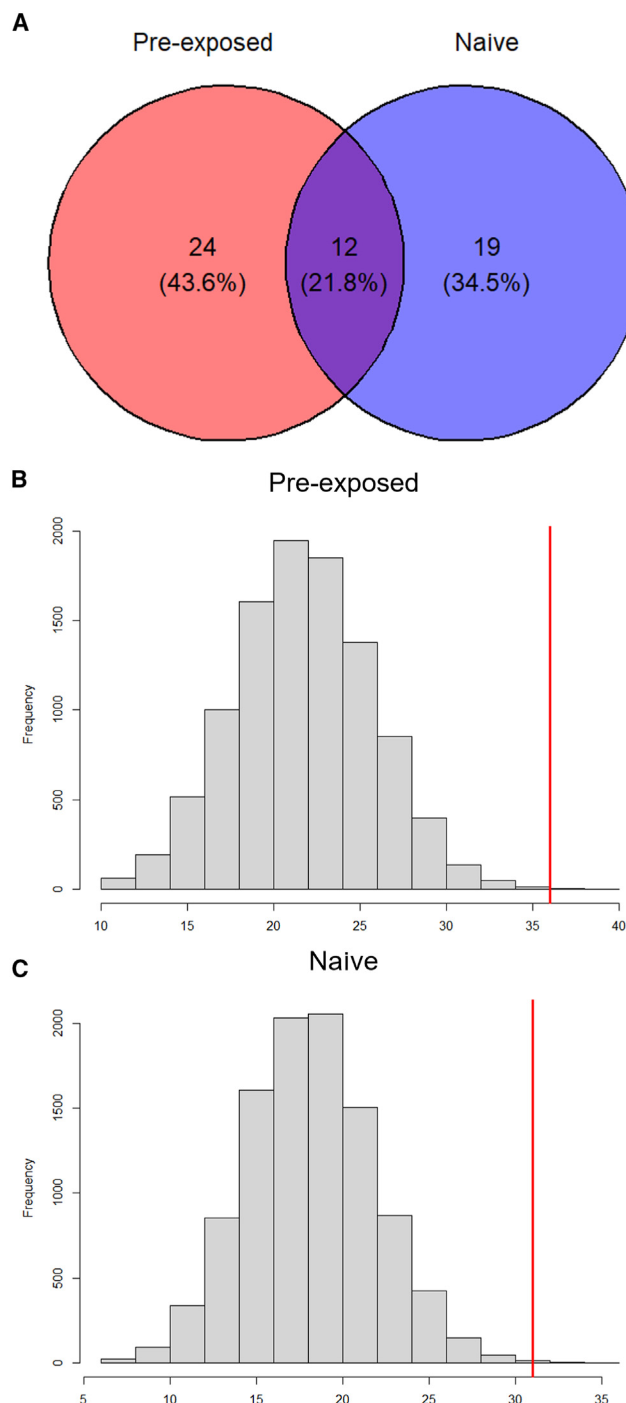

Figure 6. Shared single nucleotide polymorphisms between and among *Acidiphilium rubrum* populations

(A) The number of shared SNPs between and among pre-exposed and naive communities consistently present after severe acidification (at both sample 3 and sample 4).

(B and C) Permutations of mesocosms over SNPs indicate that the number of shared SNPs within (B) pre-exposed and (C) naive communities is significantly greater than neutral expectations (pre-exposed: $p < 0.05$; naive: $p < 0.05$, N = 10,000). Red lines indicate the observed number of shared SNPs. See also Table S5.

protected water sources.[58] Recovery of alpha diversity in naive communities under severe acidification (sample 3−sample 4) was therefore driven by a combination of population growth of initially undetected acidophiles and increases in taxonomic evenness.

All communities besides controls converged to a single profile composed of mostly *Acidiphilium* at the end of the experiment. *Acidiphilium* (meaning "acid lover") is a genus of gram-negative, motile, flagellated, photosynthetic, straight rod Proteobacteria containing eight named species.[59–61] *Acidiphilium* are known to be mesophilic and obligately acidophilic, growing between pH 2.0–5.9 but not above 6.1.[61] Here, we show that while *Acidiphilium* may not grow well under neutral pH conditions, it does persist at low levels in natural lake freshwater of approximately pH 8.5 and can rapidly increase if environmental conditions become sufficiently acidified. Putative acidophiles selected through species sorting in pre-exposed communities (Acetobacteraceae and *Granulicella*) also persisted at the end of the experiment and at slightly greater frequencies than in naive communities, supporting the hypothesis that pre-exposure has long-term effects on species composition.

Evolutionary responses to acidification

We identified and tracked SNPs across time in nine reference genomes (five *Acidiphilium* species, three *Acidocella* species, and *Granulicella* sp. *5B5*) and a single MAG annotated as an unknown Rickettsiales bacterium. Significant changes in genome-wide allele frequencies, including SNPs where the major allele was completely replaced, were observed in all genomes between at least two time points, demonstrating the role of evolutionary processes in community responses to acidification. Although we cannot definitively conclude that adaptation played a causal role in community responses (i.e., evolutionary and ecological responses could be occurring at least partially independently[62]), we did observe significant evolutionary change within successful acidophiles, which may have contributed to their persistence. Because these genomes were not detected in control communities, we were unable to characterize and directly compare SNPs from populations that did not experience severe acidification. However, of the six other MAGs that mapped reads from control communities, genome-wide allele frequency changes were significantly lower, as expected in the absence of a stressor (Figure S5).

We tracked SNPs in pre-exposed communities at the onset of severe acidification (sample 2−sample 3) as well as in both pre-exposed and naive communities throughout the 8 weeks of severe acidification (sample 3−sample 4) across all detected species of *Acidiphilium* and *Acidocella*. These eight acidophilic species independently exhibited strikingly similar patterns of genome-wide allele frequency changes (Figure 4). In pre-exposed communities, significantly greater change was observed 1 week after severe acidification (sample 2−sample 3) than the following 8 weeks until the end of the experiment (sample 3−sample 4). In those 8 weeks of severe acidification, six of the eight acidophilic species (excluding *A. iwatense* and *A*. sp. *KAb 2–4*) exhibited significantly lower change in genome-wide allele frequencies in pre-exposed communities than naive communities. Importantly, this shows that the magnitude of evolutionary response is largest upon initial exposure despite the stressor being applied at a lower level. This

is consistent with these evolutionary changes representing the beginning of an “adaptive walk” toward a new fitness optimum, when theory predicts changes will be the greatest.[63] Thus, pre-exposure served to initiate adaptation earlier, with the response diminished upon subsequent exposure to greater levels of stress. This may be viewed as analogous to the response to vaccination, where exposure to a weak dose of virus permits an immune response that reduces the impact of exposure to greater viral loads in the future.

The dominance of *Acidiphilium rubrum*

16S rRNA and MAG analysis indicated that by the end of the experiment, all communities, regardless of pre-exposure, were dominated by a single genus, *Acidiphilium* (with minimal contributions by *Acidocella*). Competitive metagenomic read mapping of *Acidiphilium* reference genomes revealed that *Acidiphilium rubrum* was largely responsible for this dominance, followed by *Acidiphilium* sp. *PA* (Figure S4). *A. rubrum* is a highly acidophilic purple bacterium that can be isolated from acid mine drainage sites of pH 2–3.[64–66] The ascendancy of *A. rubrum* over other *Acidiphilium* and *Acidocella* species suggests that *A. rubrum* has a selective advantage over other acidophiles at such low pH, but the mechanisms of pH homeostasis are not well understood in acidophiles.[67] The genetic divergence of *A. rubrum* caused by acidification pre-exposure and subsequent convergence after severe acidification indicates independent evolutionary trajectories that eventually reached the same destination (Figure 3). However, further population genetic analysis via pairwise ANI and π reveals that pre-exposure allowed *A. rubrum* to retain significantly greater genetic diversity at both time points after severe acidification, potentially because pre-exposure induced directional selection, producing more acid-tolerant populations that underwent less severe bottlenecks when confronted by severe acidification (Figure 5). This is consistent with previous theoretical and empirical findings that mercury pre-exposure retained greater microbial biomass and better-maintained community stability following a high-level mercury pulse perturbation.[16] While we were unable to determine the genetic basis for acid resistance in *A. rubrum*, we did identify a number of genes containing parallel SNPs, including 16S and 23S rRNA (Table S5). This may indicate that ribosomes were targets of selection under severe acidification, as they are in extremophiles adapted to extreme heat.[68,69] However, these parallel SNPs remained polymorphic at the end of the experiment (sample 4), indicating that sweeps were incomplete. In pre-exposed mesocosms, genome-wide diversity was maintained, and parallel SNPs were concentrated in the 16S-23S rRNA operon, consistent with a gene-specific sweep.[70] In summary, the high number of parallel SNP changes suggests that severe acidification caused strong selection, and the distribution of shared SNPs suggests that distinct SNPs were targeted in pre-exposed and naive communities (Figure 6). While it may be counterintuitive that selection due to pre-exposure can prevent loss of genetic diversity, our study indicates that pre-exposed communities may suffer less dramatic population bottlenecks when confronted with stronger selection, which can ultimately help maintain genetic diversity.

In this study, we show that pre-exposure to environmental stress can help biological communities maintain taxonomic and genetic diversity against future severe stress. Despite the convergence of community composition between pre-exposed and naive communities, and despite the loss of many taxa in all acidified communities, we demonstrate that species sorting due to pre-exposure generated greater community resistance and mitigated declines in taxonomic diversity. Additionally, we show that pre-exposure caused distinct evolutionary processes that resulted in reduced genome-wide change following severe acidification and greater levels of genetic diversity in the species that dominated acidified environments. Thus, we provide evidence for the dual roles of species sorting and evolutionary adaptation in community responses to severe stress, as well as the importance of pre-exposure for adaptation.[10,17,71–74]

RESOURCE AVAILABILITY

Lead contact

Requests for further information and resources should be directed to and will be fulfilled by the lead contact, Dr. Rowan D.H. Barrett (rowan.barrett@mcgill.ca).

Materials availability

This study did not generate new, unique reagents.

Data and code availability

- Amplicon and metagenomic sequencing data have been deposited at NCBI Sequence Read Archive (SRA) as BioProjects SRA: PRJNA 1168875 and SRA: PRJNA1171820, respectively, and are publicly available as of the date of publication.
- All original code has been deposited at Zenodo and is publicly available at https://doi.org/10.5281/zenodo.13381651 as of the date of publication.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

All authors designed research; C.C.Y.X., V.F., and N.B.C. performed research; C.C.Y.X. analyzed data; R.D.H.B. supervised the project; B.E.B., G.B., M.E.C., G.F.F., A.G., B.J.S., and R.D.H.B. provided funding; and C.C.Y.X. and R.D.H.B. wrote the paper with assistance from all co-authors.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Critical commercial assays		
Multiparameter Sonde	Yellow Spring Instruments	N/A
PES membrane filter, 0.22 μm pore size	Millapore	Cat#GPWP04700
DNeasy PowerWater Kit	QIAGEN	Cat#14900-100-NF
RNase A, DNase and protease-free (10 mg/mL)	Thermo Scientific	Cat#EN0531
GenElute-LPA	Sigma-Aldrich	Cat#56575-1ML
Deposited data		
16S rRNA amplicon sequencing data	This paper	BioProject: PRJNA1168875
Whole-genome shotgun metagenomic sequencing data	This paper	BioProject: PRJNA1171820
SILVA 138 SSU database	Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH; Yilmaz et al.[75]	https://www.arb-silva.de/no_cache/download/archive/release_138/
Genome Taxonomy Database (GTDB)	Australian Centre for Ecogenomics; Parks et al.[76]	https://gtdb.ecogenomic.org/
Annotated genomes of *Acidiphilium*, *Acidocella*, and *Granulicella*	NCBI RefSeq; O'Leary et al.[77]	https://www.ncbi.nlm.nih.gov/datasets/genome/?taxon=50709,522,940557&annotated_only=true&refseq_annotation=true; RRID:SCR_003496
Oligonucleotides		
U515_F forward primer: 5'- ACACGACGCTCTTCCGATCTYRYRGTGCCAGCMGCCGCGGTAA-3'	Preheim et al.[78]	N/A
E786_R reverse primer: 5'- CGGCATTCCTGCTGAACCGCTCTTCCGATCTGGACTACHVGGGTWTCTAAT-3'	Preheim et al.[78]	N/A
Software and algorithms		
QIIME2	Bolyen et al.[79]	https://qiime2.org/; RRID:SCR_021258
cutadapt	Martin[80]	https://cutadapt.readthedocs.io/en/stable/; RRID:SCR_011841
DADA2	Callahan et al.[81]	https://benjjneb.github.io/dada2/; RRID:SCR_023519
FastTree 2	Price et al.[82]	http://www.microbesonline.org/fasttree/; RRID:SCR_015501
Qiita	Gonzalez et al.[83]	https://github.com/qiita-spots/qiita
redbiom	McDonald et al.[84]	https://github.com/biocore/redbiom; RRID:SCR_017285
scikit-learn	Pedregosa et al.[85]	http://scikit-learn.org/; RRID:SCR_002577
anvi'o	Eren et al.[86]	https://anvio.org/; RRID:SCR_021802
illumina-utils	Eren et al.[87]	https://github.com/merenlab/illumina-utils
MEGAHIT	Li et al.[88]	https://github.com/voutcn/megahit; RRID:SCR_018551
Bowtie 2	Langmead et al.[89]	https://bowtie-bio.sourceforge.net/bowtie2/index.shtml; RRID:SCR_016368
SAMtools	Danecek et al.[90]	https://www.htslib.org/; RRID:SCR_002105
Prodigal	Hyatt et al.[91]	https://github.com/hyattpd/Prodigal; RRID:SCR_011936
CONCOCT	Alneberg et al.[92]	https://github.com/BinPro/CONCOCT
DAS Tool	Sieber et al.[93]	https://github.com/cmks/DAS_Tool

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Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
MetaBAT 2	Kang et al.[94]	https://bitbucket.org/berkeleylab/metabat/src/master/; RRID:SCR_019134
DIAMOND	Buchfink et al.[95]	https://github.com/bbuchfink/diamond; RRID:SCR_016071
InStrain	Olm et al.[96]	https://instrain.readthedocs.io/en/latest/
CheckM	Parks et al.[97]	https://ecogenomics.github.io/CheckM/; RRID:SCR_016646
Mash	Ondov et al.[98]	https://github.com/marbl/Mash; RRID:SCR_019135
dRep	Olm et al.[99]	https://github.com/MrOlm/drep
FastANI	Jain et al.[100]	https://github.com/ParBLiSS/FastANI; RRID:SCR_021091
codyn	Hallett et al.[44]	https://cran.r-project.org/web/packages/codyn/
Other		
Illumina MiSeq System	McGill Genome Centre	RRID:SCR_016379
Illumina NovaSeq 6000 Sequencing System	McGill Genome Centre	RRID:SCR_016387

METHOD DETAILS

Study site

We conducted the experiment at the Large Experimental Array of Ponds (LEAP) facility located at the Gault Nature Reserve in Mont-Saint-Hilaire, Quebec, Canada. We filled replicate 1,000 L mesocosms on May 23/24, 2017, by sourcing water from the nearby Lake Hertel (45°32’ N, 73°09’ W), which is protected under UNESCO as part of the Mont Saint Hilaire Biosphere Reserve. The naturally mesotrophic lake has a maximum depth of 8.2 m and a natural pH of 7.5–9.5.[101,102] We used a cloth with approximately 1 mm mesh size to filter water from Lake Hertel before it entered the mesocosms, which prevented introduction of fish and large invertebrates leaving a community of naturally co-occurring zooplankton, phytoplankton, bacteria, and viruses.

Experimental design

We designed a biphasic experiment to test the isolated and interacting effects of several levels of acidification pre-exposure and dispersal regimes on community response to severe acidification, which has been described in a previous study.[26] Here, we focused on only the 16 mesocosms pre-exposed to the strongest acidification treatment of pH 4 as well as the 16 naïve mesocosms that were left untreated and remained at their natural acidity of approximately pH 8.5 (Figure 1A). In phase I of the experiment, we maintained pre-exposure to pH 4 through weekly pulse titration with 10N H_2SO_4 for seven weeks, from June 14 – August 2. Pre-exposed mesocosms exhibited a sharp decrease in pH buffering capacity with each acidification treatment in the first weeks of phase I (Figure 1B). Half of pre-exposed and naïve mesocosms were also under a global dispersal regime where we mixed 1% of water from each metacommunity of four mesocosms in a pool and then redistributed it on a weekly basis allowing for migration within metacommunities. No additional water was added to any mesocosm after the experiment commenced. We initiated phase II on August 2 when all mesocosms were acidified to a stable pH of 3 and dispersal regimes were terminated. No mesocosms exhibited pH buffering capacity in phase II. Phase II lasted for approximately eight weeks until the end of the experiment on September 25. We also established four isolated control mesocosms subjected to neither phase I nor phase II treatments.

Sample collection

We monitored mesocosms weekly for water pH (Figure 1B) using multiparameter sondes (Yellow Spring Instruments, Ohio). We used integrated water samplers made from 2.5 cm diameter PVC tubing to sample water biweekly from the top 35 cm of the water column at five random locations within each mesocosm until a total of 2 L of water was collected. We subsequently stored water samples in dark, triple-washed Nalgene bottles at 4°C before filtration later that same day. We used independent samplers and dark bottles for each mesocosm to minimize cross-mesocosm contamination. For each sample, we filtered 500 mL of water at an on-site lab using 0.22 μm pore size, 47 mm diameter hydrophilic polyethersulfone (PES) membrane filters (Millapore). We then transported filters to the laboratory on dry ice and stored them at -80 °C prior to DNA extraction.

DNA extractions

We extracted DNA from samples collected across four time points: 1. At the beginning of the experiment one week prior to pre-exposure treatment (June 7/day 0), 2. At the end of phase I after six weeks of pre-exposure (July 26/day 49), 3. At the beginning of phase II one week after severe acidification (August 9/day 63), and 4. At the end of the experiment after approximately eight weeks of severe

acidification (September 25/day 111) respectively referred to as Sample 1, Sample 2, Sample 3, and Sample 4 (Figure 1B). In total, there were 128 samples (16 mesocosms X 2 treatments X 4 time points) and 12 controls (4 control mesocosms X 3 time points excluding Sample 1). We extracted and purified total genomic DNA from half filter papers using the DNeasy PowerWater kit (QIAGEN) following QIAGEN guidelines including a 5-minute vortex of the filter with beads and an additional incubation of 30 minutes at 37°C with 1 μL Rnase (Thermo Scientific) after cell lysis and before the first supernatant transfer to remove RNA contamination.[103]

16S rRNA gene sequencing

We profiled bacterial community composition using 16S rRNA amplicon sequencing. Specifically, we used the primers U515_F (5'-ACACGACGCTCTTCCGATCTYRYRGTGCCAGCMGCCGCGGTAA-3') and E786_R (5'- CGGCATTCCTGCTGAACCGCTCTTCC GATCTGGACTACHVGGGTWTCTAAT-3') to target an approximately 200 bp amplicon of the V4 region of the 16S rRNA gene as described previously.[78] We treated samples that initially failed to PCR amplify with sodium acetate and then ethanol precipitated with GenElute-LPA linear polyacrylamide (Sigma-Aldrich) to increase DNA concentration.[104] Genomic DNA quality control, sequencing library preparation, two-step PCR,[103] and amplicon sequencing via Illumina MiSeq v2 PE250 was conducted at the McGill Genome Centre. An average of 28,387 (range: 0–85,039) 16S rRNA reads were produced per sample. A total of 134 (95.71%) samples were retained for subsequent analysis after removing six samples that each produced less than 5 reads.

Amplicon sequence analysis

We processed raw 16S rRNA amplicon sequences using the QIIME2 bioinformatics pipeline.[79] We first removed primer sequences using cutadapt followed by identification of ASVs using DADA2.[80,81] We aligned ASVs using MAFFT and constructed phylogenetic trees using FastTree 2 based on Jukes-Cantor distances.[82,105] We created a custom reference database by using the U515_F/E786_R primers to *in silico* extract the target 16S rRNA V4 region from the SILVA 138 database.[75]

We generated taxonomic weights according to occurrence records in freshwater habitats using redbiom and Qiita by limiting sample type to "fresh water" or "freshwater" and context to "Deblur_2021.09-Illumina-16S-V4-90nt-dd6875"[83,84,106]. We used a total of 6,206 V4 16S rRNA sequences (6,003 "fresh water" and 203 "freshwater") to weight taxonomic assignment towards those found previously in freshwater environments. We then assigned taxonomies to ASVs with a naïve Bayes classifier trained using scikit-learn on the extracted SILVA 138 database that was modulated by the freshwater taxonomic weights.[85] We accepted taxonomic assignments if classification confidence was at least 0.7.[107]

Metagenomic shotgun sequencing

We selected samples from all isolated (*i.e.,* no dispersal in phase I) pre-exposed and naïve mesocosms except for one mesocosm from each phase I treatment for further metagenomic analysis along with control mesocosms. In total, we subjected 68 samples across the four time points to deep sequencing at an average of 220 million reads per sample. We focused sequencing on phase I samples (Sample 1 and Sample 2 at ~330 million reads/sample) compared to phase II samples (Sample 3 and Sample 4 at ~110 million reads/sample) to maximize the probability of detecting and quantifying genetic diversity within dominant phase II species that were initially at low abundances in phase I. Quality control, library prep, and sequencing on Illumina NovaSeq 6000 PE150 were conducted at the McGill Genome Centre.

Metagenomic analysis

We processed and analyzed metagenomic sequences within the anvi'o framework.[86] We first removed Illumina TruSeq LT adaptors with cutadapt and quality filtered reads using illumina-utils.[87] We used MEGAHIT to co-assemble reads from the same mesocosm across the four time points.[88] We merged all contigs and removed those less than 2,500 bp. We then mapped reads from each sample to contigs using Bowtie 2 and SAMtools.[89,90] We identified prokaryotic genes in the contigs using Prodigal.[91] We used hidden Markov models for collections of 71 bacteria, 76 archaea, and 83 protist single-copy core genes (SCGs) to identify and recover them from contigs.[108–110] The final contig database consisted of about 3.1 million contigs totaling approximately 20.8 Gb, and 2.1 million annotated genes from an estimated 2,328 bacterial and 64 eukaryotic genomes.

We clustered contigs into bins using CONCOCT and MetaBAT 2, which we then dereplicated and aggregated into 81 metagenome-assembled genomes (MAGs) using DAS Tool.[92–94] We estimated completeness and redundancy of MAGs based on SCG collections. We taxonomically classified 22 MAGs based on 22 unique bacterial SCGs from the Genome Taxonomy Database (GTDB) using DIAMOND (Table S6).[95,76] The MAG assigned to *A. rubrum* constituted 97%–98% of all mapped reads at the end of the experiment (Sample 4) across both pre-exposed and naïve communities. We included *A. rubrum* along with high-quality MAGs (completeness >90% and redundancy <10%) for further analysis. For *A. rubrum*, we used a reference genome approach to identify single nucleotide polymorphisms (SNPs) occurring in at least two samples and calculated pairwise fixation index (F_{ST}) within anvi'o.[111,112]

Three genera (*Acidiphilium*, *Acidocella*, and *Granulicella*) dominated phase II (Sample 3 and Sample 4) communities based on 16S amplicon and MAG results. We further assessed population microdiversity of species within these genera using InStrain.[96] We obtained 44 reference genomes from 17 species within *Acidiphilium*, *Acidocella*, and *Granulicella* from NCBI RefSeq and merged them with MAGs to create a custom genome database. We dereplicated this database to 29 reference genomes and 29 MAGs using dRep, CheckM, Mash, and FastANI with a Mash sketch size of 10,000 and a minimum overlap between genomes of 0.3.[77,97–100] We used Prodigal to profile genes for each genome, and we competitively mapped reads against reference genomes and MAGs using Bowtie2 and SAMtools.[89–91] Reads from pre-exposed and naïve communities mapped to five *Acidiphilium* species (*A. iwatense*,

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A. multivorum, *A. rubrum*, *A.* sp. *C61*, *A.* sp. *PA*), three *Acidocella* species (*A. aminolytica*, *A. facillis*, *A.* sp. *KAb 2-4*), *Granulicella* sp. *5B5*, and a single MAG assigned to an unnamed species within the order Rickettsiales (family SXRF01, genus *RFOF01*) (Figures S3 and S4). Reads from control communities mapped only to other MAGs. We called SNPs using a minimum coverage threshold of 5 and a minimum SNP frequency of 0.05 using InStrain.[96]

QUANTIFICATION AND STATISTICAL ANALYSIS

Amplicon sequence analysis

We assessed alpha diversity using the natural logarithm Shannon index computed after we rarified ASVs of each sample to a depth of 1,178 based on saturation of rarefaction curves and maximization of the number of included samples[113] (Figure S6). We compared Shannon values between pre-exposed and naïve communities at each time point using Kruskal-Wallis tests.[114] We also assessed longitudinal differences in Shannon diversity using Wilcoxon signed-rank and Mann-Whitney U tests and statistical significance via Benjamini & Hochberg corrected q-values.[115–118] We also assessed community stability using a community stability metric calculated as the temporal mean divided by the temporal standard deviation of aggregate abundances across all four time points.[44] We assessed the effects of pre-exposure and dispersal on beta diversity via PERMANOVA of Bray-Curtis dissimilarity with 999 permutations using the rarified ASVs.[119]

Metagenomic analysis

We used InStrain to calculate scaffold-level metrics including ANI and π.[96,120,121] We calculated allele frequency change of polarized major alleles through subtracting the frequency of the major allele at each SNP by the frequency of that same allele in a subsequent time point. We longitudinally compared pairwise ANI and π as well as allele frequency changes between pre-exposed and naïve communities using Dunn's test and assessed statistical significance via Holm-Bonferroni adjusted p-values.[122,123] For the most dominant species identified at the end of the experiment (*A. rubrum*), we used permutation tests randomizing the mesocosm of each SNP to assess the significance of the number of shared SNPs between populations in pre-exposed and naïve communities. We only considered SNPs consistently present after severe acidification (at both Sample 3 and Sample 4) as a conservative approximation for adaptation.