Stabilizing role of seed banks and the maintenance of bacterial diversity

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ABSTRACT

Coexisting species often exhibit negative frequency dependence due to mechanisms that promote population growth and persistence when rare. These stabilizing mechanisms can maintain diversity through interspecific niche differences, but also through life-history strategies like dormancy that buffer populations in fluctuating environments. However, there are few tests demonstrating how seed banks contribute to long-term community dynamics and the maintenance of diversity. Using a multi-year, high-frequency time series of bacterial community data from a north temperate lake, we documented patterns consistent with stabilizing coexistence. Bacterial taxa exhibited differential responses to seasonal environmental conditions, while seed bank dynamics helped maintain diversity over winter. Strong negative frequency dependence in rare, but metabolically active, taxa suggested a role for biotic interactions in promoting coexistence. Together, our results provide field-based evidence that niche differences and seed banks contribute to recurring community dynamics and the long-term maintenance of diversity in nature.

INTRODUCTION

The maintenance of biodiversity is important for regulating species interactions, stabilizing ecosystem functions, and promoting resilience in response to perturbations. Diversity is maintained by many processes, including niche differentiation in resource use (Tilman 1982; Gudelj *et al.* 2010; Johnson *et al.* 2012), defensive abilities (Leibold 1996; Thingstad *et al.* 2014; Cadier *et al.* 2019), and abiotic constraints (Holt 2009). These stabilizing niche differences among species contribute to the maintenance of diversity by causing species to limit their own growth more than the growth of other species, thereby preventing competitive exclusion and allowing populations to recover from low abundances (Chesson 2000; Chase & Leibold 2003; Adler *et al.* 2007). Some stabilizing mechanisms of coexistence rely on environmental fluctuations and can further increase the number of species in a community (Chesson 1994; Chesson & Huntly 1997; Descamps-Julien & Gonzalez 2005). For example, in seasonal environments, species that are favored at different times of the year may be able to coexist in the community if they can survive through periods of unfavorable environmental conditions (Pake & Venable 1996). Given the near ubiquity of environmental variability, a central and unresolved question is how stabilizing mechanisms promote the maintenance of diversity across the wide range of taxonomic groups and ecosystems that exist in nature.

Stabilization from niche differences should generate negative frequency-dependence (NFD) in population growth. The implication of NFD is that rare populations grow faster than common populations (Chesson 2000; Adler *et al.* 2007). NFD may arise from mechanisms that promote coexistence in relatively constant environments, such as trade-offs in resource acquisition and allocation, but also in temporally variable environments. In fluctuating environments, the storage effect is a coexistence mechanism that reflects the ability of species to

grow well during favorable conditions while minimizing losses during unfavorable conditions by "storing" individuals in long-lived life stages (Warner & Chesson 1985; Chesson 2000). The storage effect requires that taxa differ in their responses to environmental conditions, that intraspecific limitation peaks during favorable conditions, and that population growth is buffered in suboptimal environments (Pake & Venable 1996; Cáceres 1997; Angert *et al.* 2009). The storage effect may be particularly important in communities where species experience periods of extremely slow growth (Gray *et al.* 2019) or engage in various forms of dormancy, which are common among plants and animals, but also microorganisms (Lennon & Jones 2011).

Support for stabilizing coexistence has largely come from plant and animal communities (Cáceres 1997; Angert *et al.* 2009; Yenni *et al.* 2017), while evidence for its role in complex microbial systems is less common (Zhang *et al.* 2010). In microbial communities, which contain a disproportionately large number of rare taxa (Sogin *et al.* 2006; Lynch & Neufeld 2015; Shade *et al.* 2018), populations may vary widely in their stability and long-term contributions to diversity. Although rare taxa are prone to extinction (Lande 1993), some persist for longer periods of time (Alonso-Sáez *et al.* 2015; Lynch & Neufeld 2015; Newton & Shade 2016). In fluctuating environments, many of these rare taxa can quickly respond to favorable conditions (Shade *et al.* 2014; Linz *et al.* 2017; Nyirabuhoro *et al.* 2020), suggesting that temporally variable opportunities for growth may be important for population persistence. While niche differences among bacterial taxa are well documented (Lennon *et al.* 2012; Evans *et al.* 2014; Meier *et al.* 2017), the long-term implications of these differences and their contribution to the maintenance of biodiversity in nature remain understudied.

A leading hypothesis for the long-term maintenance of microbial diversity has been coexistence mediated by dormant seed banks (Jones & Lennon 2010; Lennon & Jones 2011;

Mestre & Höfer 2020; Sorensen & Shade 2020). Dormancy can buffer microbial populations in different ways (Rittershaus *et al.* 2013). For example, some species form physical resting structures that protect individuals from harsh abiotic stress (Setlow 2006; de Rezende *et al.* 2013). Other species may reduce mortality associated with resource limitations by shifting energetic demands from growth to maintenance energy levels (Lennon & Jones 2011; Hoehler & Jørgensen 2013; Lever *et al.* 2015). Dormancy may even protect against top-down pressure from grazers (which may be unable to digest or extract energy from starved cells or endospores) and phage (which cannot replicate due to inactive host machinery) (Pernthaler 2005; Klobutcher *et al.* 2006; Bautista *et al.* 2015; Kearney *et al.* 2018). Consequently, dormant bacteria may exhibit reduced mortality in the environment (Hoehler & Jørgensen 2013), thereby accumulating into seed banks until favorable conditions return (Wörmer *et al.* 2019). Much insight has been gained from short-term microbial studies and analogies with plant and zooplankton communities, but evidence from long-term field studied demonstrating how the temporal dynamics of microbial seed banks help maintain diversity in fluctuating environments is lacking.

In this study, we tracked bacterioplankton dynamics over time in a north temperate lake using high-resolution molecular data to infer ecological processes that maintain microbial diversity. Bacterial communities in fluctuating aquatic environments often exhibit recurrent, seasonal community patterns (Shade *et al.* 2007; Gilbert *et al.* 2012; Fuhrman *et al.* 2015; Ward *et al.* 2017), but the potential mechanisms that contribute to cyclical dynamics and maintain diversity in nature are poorly resolved. We characterized how persistent (and putatively coexisting) taxa respond to environmental fluctuations and used null models to assess whether stabilizing biotic interactions (e.g., self-limitation, as evidenced by strong NFD) help maintain rare, but metabolically active, taxa in the community (Yenni *et al.* 2017; Rovere & Fox 2019).

Specifically, we compared patterns of NFD and population dynamics in the active and total portions of the community (inferred by 16S rRNA transcripts and genes, respectively) to quantify the importance of slow growth or dormancy strategies for the maintenance of diversity. Our results provide empirical evidence that stabilizing biotic interactions and seed bank dynamics underlie seasonal community dynamics and play key roles in maintaining bacterial diversity in natural ecosystems.

METHODS

Study site and sampling: University Lake is a 3.2 ha meso-eutrophic reservoir located in the Indiana University Research and Teaching Preserve, Bloomington, Indiana, USA (39°11' N, 86°30' W). The surrounding watershed is dominated by oak, beech, and maple forests. Three streams drain into University Lake, which has an estimated volume of 150,000 m^3 and a maximum depth of 10 m. From April 2013 to September 2015, we took weekly water samples (1 L) from the epilimnion using a 1 m depth-integrated sampler for microbial biomass, and measured environmental variables commonly associated with aquatic microbial community dynamics: total phosphorus (TP), total nitrogen (TN), and dissolved organic carbon (DOC). Microbial biomass was filtered on 0.2 µm Supor filters (Pall, Port Washington, NY, USA) and frozen at -80 °C. We quantified TP using the ammonium molybdate method (Wetzel & Likens 2000) and TN with the second derivative method after persulfate digestion (Crumpton *et al.* 1992). DOC was quantified on 0.7 µm filtrates using nondispersive infrared (NDIR) detection on a Shimadzu TOC-V (Kyoto, Japan). We also quantified water transparency with a Secchi disk and used a Quanta Hydrolab (OTT, Kempton, Germany) water sonde to measure temperature, conductivity, dissolved oxygen, salinity, and pH of the samples.

Bacterial community structure: We characterized the structure of the bacterial community using high-throughput 16S rRNA sequencing. We extracted total nucleic acids from biomass retained on 0.2 µm filters using the MoBio PowerWater RNA extraction kit and the DNA elution accessory kit. Because sequences obtained from DNA can come from metabolically active or inactive (e.g., slow growing or dormant) individuals, this sample represents the "total" community. In contrast, RNA is a more ephemeral molecule that is essential for synthesizing proteins; therefore, it is often used to characterize the metabolically "active" subset of the community (Molin & Givskov 1999; Steiner *et al.* 2019; Locey *et al.* 2020). After extracting nucleic acids, we used DNase (Invitrogen) to remove DNA from the RNA extractions and then synthesized cDNA with SuperScript III First Strand Synthesis kit and random hexamer primers (Invitrogen). To amplify the 16S rRNA gene (DNA) and transcripts (cDNA), we used barcoded V4 primers (515F and 806R) designed for the Illumina MiSeq platform (Caporaso *et al.* 2012). We then purified the PCR products with AMPure XP, quantified DNA concentrations using PicoGreen, and pooled samples at 10 ng per sample. The resulting libraries were sequenced on an Illumina MiSeq at the Indiana University Center for Genomic and Bioinformatics Sequencing Facility using 250 × 250 bp paired-end reads (Reagent Kit v2). Sequences were subsequently processed using the software package mothur (version 1.41.1) (Schloss *et al.* 2009). We assembled contigs, removed low quality sequences (minimum score of 35), aligned sequences to the SILVA Database (version 132) (Quast *et al.* 2013), removed chimeras using the VSEARCH algorithm (Rognes *et al.* 2016), and created 97% similar operational taxonomic units (OTUs) using the OptiClust algorithm (Westcott & Schloss 2017), and classified sequences with the RDP taxonomy (Cole *et al.* 2009). To account for variation in

154 sequencing depth, subsequent analyses were performed on rarefied abundance data subsampled
155 to the fewest number of reads in the time series (N = 5979 per sample) using R (version 3.6.0) (R
156 Core Team 2020).
157
158 *Differential responses to environment:* We evaluated whether niche partitioning occurred
159 along a suite of environmental variables. First, we performed a principal component analysis
160 (PCA) on Hellinger-transformed abundances to visualize seasonal patterns of compositional
161 trajectories. Next, we identified environmental drivers of community dynamics using redundancy
162 analysis (RDA). We then looked more closely at the persistent, and potentially coexisting, subset
163 of taxa in the community (OTUs present in ≥80% of the DNA-based samples, Table S1). To
164 determine whether environmental fluctuations facilitated temporal niche partitioning, we
165 identified the week of the year when each persistent OTU (n = 82) experienced its maximum
166 average growth rate (see calculations in *Stabilizing niche differences* below). For each of the
167 persistent OTUs, we compared the environmental conditions at the time of the year when they
168 experienced maximum growth (as an index of optimal conditions along an annual environmental
169 cycle). Specifically, we performed a principal component analysis (PCA) on the environmental
170 variables including temperature, specific conductivity, transparency, pH, TP, TN, and DOC (all
171 standardized to mean = 0, standard deviation = 1). We plotted each time point along the first two
172 PC axes, along with the PC loadings of the environmental variables, and labeled each point with
173 the name of the OTU that exhibited maximum growth at that particular time point (Fig. S1).
174
175 *Stabilizing niche differences:* We then inferred whether niche differences contributed to
176 patterns of negative frequency dependence (NFD) in the community. In particular, we examined

177 (1) whether growth exhibited negative frequency dependence overall, which is indicative of
178 stabilization in the community, and (2) whether rare taxa experienced stronger negative
179 frequency dependence than common taxa (Yenni *et al.* 2012, 2017; Rovere & Fox 2019). Such
180 patterns of NFD shed light on coexistence for three reasons. First, taxa are more likely to be
181 common in the community when they are environmentally favored relative to other taxa in the
182 community. Second, taxa that experience strong intraspecific limitation during environmentally
183 favorable periods should grow faster when rare than when common, thereby generating NFD.
184 Third, differences among taxa in the strength of self-limitation during favored growth periods
185 should lead to different average relative abundances in the community, implying that rare yet
186 persistent taxa may be strongly stabilized (Yenni *et al.* 2012, 2017; Rovere & Fox 2019).
187 Patterns of NFD often arise from density-dependent processes, such as nutrient limitation, but
188 density dependence will only generate NFD if species limit their own growth more strongly than
189 they limit the growth of other species (Adler *et al.* 2007). However, metabolically inactive
190 individuals are unlikely to engage in the biotic interactions that generate NFD. Therefore, we
191 focused on NFD in the metabolically active (i.e., RNA-based) portion of the community
192 comprised of the persistent taxa (Table S1).
193 We then calculated NFD for each OTU by comparing rates of change in relative
194 abundance between weekly samples. We inferred the strength of NFD for a given OTU as the
195 magnitude of the negative slope of the relationship between an OTU's relative abundance and its
196 per capita growth rate at each time step (t) across the time series. We calculated the relative
197 abundance ($x_{t,s}$), of each OTU (s) as its abundance ($N_{t,s}$) in the community of s OTUs relative
198 to the total abundance of all s OTUs ($\sum_s N_{t,s}$) at a given time step (t), such that $x_{t,s} = \frac{N_{t,s}}{\sum_s N_{t,s}}$.
199 From this, we then calculated the natural log of the per capita growth rate of each OTU as $y_{t,s} =$

200 $\log_e\left(\frac{N_{t+1,s}}{N_{t,s}}\right)$. To estimate the strength of NFD for each OTU, we fit simple linear regressions
201 ($y_s = \beta_{0,s} + \beta_{1,s}x_s + \epsilon_s$), where the equilibrium frequency of an OTU (f) is the x-intercept, $f =$
202 $-\frac{\beta_{0,s}}{\beta_{1,s}}$, and the degree of NFD is the slope, NFD $= \beta_{1,s}$. In the end, f describes whether an OTU
203 is common or rare, and negative slopes with greater magnitudes indicate stronger negative
204 frequency dependence.
205
206 *Stabilization of rare bacterial taxa:* We then inferred whether rare taxa exhibited stronger
207 NFD than expected by chance. Stabilization of rare taxa would be supported if OTUs with lower
208 equilibrium frequencies (smaller values of f) had more negative slopes (larger $|\beta_{1,s}|$) indicating
209 stronger self-limitation. We inferred the overall relationship between rarity and strength of NFD
210 from the covariance between the log(NFD) and log(f): a more negative covariance would
211 indicate that rarer taxa were more strongly stabilized than common taxa. To account for the fact
212 that the expectation of this covariance is already negative, and to control for spurious statistical
213 correlations in the temporal data due to other factors, we implemented a null model approach
214 (Yenni *et al.* 2017; Rovere & Fox 2019). We shuffled the abundances of each OTU
215 independently, recalculated relative abundances and per capita growth rates, estimated
216 equilibrium frequencies (fs) and negative frequency dependences (NFDs), and calculated the
217 covariance, repeating this procedure 5000 times to generate a null distribution of covariance
218 values ($COV[\log(f), \log(\text{NFD})]$) (Yenni *et al.* 2017). This procedure maintains the potential
219 abundances detected for each OTU but erases the temporal structure of each taxon's growth
220 dynamics, removing signatures of intraspecific limitation as well as any interspecific limitations
221 correlated with the population dynamics of other OTUs. We then compared our observed

222 covariance with the null distribution to infer the strength of asymmetry in NFD (i.e., the degree
223 to which rare OTUs experience disproportionately stronger self-limitation than common OTUs).
224 We quantified divergence from null distributions using standardized effect sizes (SES = mean
225 observed covariance / standard deviation of covariances in the null distribution) and the ratio of
226 observed covariance to the average covariance of the null distribution (Yenni *et al.* 2017). More
227 negative SES values and larger ratios would indicate greater deviations from the null expectation
228 of equal NFD across taxa. We inferred the degree of statistical significance by calculating a p-
229 value as the proportion of null covariance values less than or equal to our observed covariance.
230
231 *Seed bank dynamics:* Given the hypothesis that seed banks are important for the
232 maintenance of bacterial diversity in nature, we analyzed the temporal dynamics of buffered
233 population growth, a key criterion of the storage effect. First, we examined whether the seed
234 bank served as a reservoir of taxonomic diversity by comparing the ratio of total richness to
235 active richness at each time point in the time series, where larger ratios indicate that the total
236 community had higher α-diversity than the active subset of the community. Second, we sought to
237 determine whether seed bank dynamics were more important for the maintenance of rare or
238 common taxa in the community. To do so, we developed a reactivation metric to quantify each
239 OTU's frequency of reactivation from the seed bank. For each OTU, its reactivation score is the
240 number of times an OTU was present (i.e., detected in the DNA pool) but likely in an inactive
241 (i.e., absent from the RNA pool) state at time point t, yet active (present in the RNA pool) at the
242 subsequent time point $t+1$. This represents a transition from the inactive to active state mediated
243 by slow growth or dormancy based on recovery of sequences in the DNA and RNA pools. Thus,
244 OTUs with higher reactivation scores may be more reliant on the seed bank for long-term

245 persistence in the community. We then analyzed the relationship between the average relative
246 abundance of active OTUs (excluding zeroes) and their reactivation score to determine whether
247 seed banking was more important for maintaining rare taxa than common taxa in the
248 bacterioplankton community.

249 We compared observed patterns of reactivation to null models of community dynamics
250 because the probability of resuscitation may not be independent of relative abundance. Such non-
251 independence could be expected, for example, if rarer OTUs were more likely to be inactive than
252 common OTUs. We generated null models of bacterial time series (n = 1000) by randomly
253 redistributing observed counts of each OTU across the time series, keeping total observed counts
254 for each OTU constant to preserve the relationships among common and rare taxa in the
255 community. By redistributing individuals across sampling dates for each taxon independently,
256 we removed population dynamic signatures of intraspecific density dependence as well as
257 interspecific density dependence arising from biotic interactions with other taxa in the
258 community. Thus, our null models represent the range of reactivation scores possible for an OTU
259 of a given mean relative abundance in the community if its dynamics were stochastic. We then
260 compared our observed reactivation scores to the null models to identify whether common or
261 rare OTUs reactivated more or less frequently than expected by chance.

262

263 **RESULTS**

264 *Differential responses to environment:* Bacterial community dynamics were related to
265 environmental variability, with different taxa favored at different times of the year. During the
266 summer months, the community followed a recurrent successional trajectory (Fig. 1A). This
267 trajectory was strongly aligned with seasonal trends in temperature (Fig. 1B). Across longer time

268 scales, inter-annual variation in dissolved oxygen and pH was associated with compositional
269 differences in the active bacterial community during winter months. Within an annual cycle, the
270 persistent OTUs (n = 82) demonstrated temporal partitioning in their maximal growth rates in the
271 active portion of the community (Fig. 2, Table S1), corresponding to different environmental
272 conditions (Fig. S1).
273
274 *Stabilizing biotic interactions:* Persistent taxa exhibited stabilizing NFD, which varied in
275 strength depending on each taxon's mean relative abundance in the community (Fig. S2). In
276 particular, NFD was significantly stronger for rare taxa than common taxa, but only in the active
277 portion of the community ($p = 0.0002$; SES = -4.03, covariance ratio = 1.08), not in the total
278 community ($p = 0.221$; SES = -0.777, covariance ratio = 1.01) (Fig. 3). The p-values reflect the
279 rank of observed NFD compared with null simulations, while SES values take into account the
280 variance in the null distribution. In other words, the total community showed nearly the same
281 degree of stabilization ($|SES| < 2$, covariance ratio ~ 1) as the null communities.
282
283 *Seed bank dynamics:* Our data suggest seed banks of dormant or slow growing
284 individuals contribute to the maintenance of diversity. Over the course of our study, total
285 richness ranged from 1.2–2.0 times higher than the richness of the active portion of the
286 community (Fig. 4). Furthermore, this discrepancy between total and active richness exhibited
287 seasonality, demonstrating a time-varying role for the bacterial seed bank. In particular, the seed
288 bank played a weaker role (i.e., active and total richness were more similar in magnitude) during
289 the summer, while proportionally higher diversity was found in the seed bank over winter, when
290 growing conditions may be less optimal (Fig. 4). In addition, the taxa that exhibited more

291 reactivations from the seed bank were also the taxa that were, on average, consistently rare when
292 active in the community (Fig. 5). In contrast, common taxa exhibited fewer transitions between
293 active and inactive states in the community. Compared to null models of community dynamics,
294 the observed relationship between relative abundance and reactivation actually implies a far
295 stronger role for the seed bank among taxa of low-to-intermediate abundance ranks than
296 expected by chance alone (Fig. S3), suggesting that the seed bank may be an important source of
297 reestablishment in the active community. Thus, our findings support the view that rare taxa in the
298 community benefit from life-history strategies such as slow growth or dormancy that minimize
299 the probability of local extinction.
300

301 **DISCUSSION**

302 Our findings from a multi-year survey support the view that biodiversity was maintained
303 by stabilizing mechanisms, including niche differentiation and seed bank dynamics, that
304 generated negative frequency dependence (NFD) in a natural bacterioplankton community. High
305 resolution sampling revealed recurrent seasonality in community dynamics (Fig. 1), driven by
306 taxon-specific responses to annual environmental fluctuations (Fig. 2, Fig. S2). Our results also
307 showed that the maintenance of diversity may be enhanced by life-history strategies, such as
308 slow growth or dormancy, that buffered rare taxa from local extinction during environmentally
309 unfavorable periods (e.g., winter) and facilitated reestablishment when conditions improved (Fig.
310 4-5). These apparent niche differences and seed bank dynamics contributed to stabilizing biotic
311 interactions (e.g., stronger intra- than interspecific limitation) among rare, but metabolically
312 active, taxa in the community (Fig. 3).
313

314 *Negative frequency dependence in microbial communities:* We found evidence for
315 stabilization through negative frequency dependence (NFD). While documented in some plant
316 and animal assemblages (Harpole & Suding 2007; Yenni *et al.* 2017; Rovere & Fox 2019),
317 observations of NFD in complex microbial communities are uncommon. In particular, our study
318 revealed disproportionately strong NFD for rare taxa, offering an explanation for why some taxa
319 appear to stably persist at low relative abundances in nature (Alonso-Sáez *et al.* 2015; Lindh *et*
320 *al.* 2015), potentially as members of the "rare biosphere" (Sogin *et al.* 2006; Lynch & Neufeld
321 2015; Shade *et al.* 2018). Our approach also allowed us to identify stabilizing mechanisms
322 operating among metabolically active rare taxa that were not detectable from the dynamics of the
323 total bacterial community (Fig. 3). Ignoring this metabolic heterogeneity can obscure inferences
324 of underlying ecological processes (Wisnoski *et al.* 2020), and would have gone otherwise
325 undetected in this study as well.
326 Stronger NFD among rare taxa is also important for coexistence in plant and animal
327 communities, but the magnitude of this effect varies across taxonomic groups (Yenni *et al.* 2017;
328 Rovere & Fox 2019). For example, NFD is less asymmetric for herpetofauna than plant or
329 mammal communities (Yenni *et al.* 2017), possibly due to higher evenness (Rovere & Fox
330 2019). Compared to macro-organismal systems, the degree of NFD asymmetry in our highly
331 uneven bacterial community was moderate (SES = -4.03, covariance ratio = 1.08), suggesting
332 that coexistence among rare taxa may be weak, or that additional factors not captured by this
333 metric are important for maintaining diversity in our study system. However, we provide critical
334 evidence that active bacteria mediate the biotic interactions responsible for generating stronger
335 NFD among rare taxa in the community. Consistent with prior work showing that rare taxa may
336 be disproportionately active in freshwater bacterial communities (Jones & Lennon 2010), our

337 study demonstrates that rare, metabolically active bacteria may also be critical for the long-term
338 maintenance of bacterial diversity.
339
340 *Dynamic microbial seed banks:* Seed bank dynamics are thought to maintain diversity in
341 fluctuating environments. In particular, seed banks provide a demographic buffering effect that
342 satisfies one criterion of the storage effect. Evidence for coexistence via the storage effect comes
343 largely from communities of desert annuals (Pake & Venable 1996; Angert *et al.* 2009),
344 grasslands (Adler *et al.* 2006), tropical trees (Usinowicz *et al.* 2012), zooplankton (Cáceres
345 1997), and marine fish (Secor 2007). In most bacterial studies, the role of seed banks for
346 coexistence has been inferred from short-term observations, but here we provide temporal
347 evidence that bacterial seed banks may be important for community dynamics and the
348 maintenance of diversity over longer, multi-annual time scales. In the temperate climate of our
349 study lake, different taxa showed maximum growth rates at different times of the year,
350 coinciding with seasonal transitions in environmental conditions (Fig. 2), and contrasting active
351 and total community dynamics suggested buffered population dynamics (Figs. 3-5). Bacterial
352 taxa may also exhibit more fine-grained differences in temporal niches than can be characterized
353 by peak activity, which may be a somewhat conservative estimate of temporal niche differences.
354 Nevertheless, our data provide evidence that two out of the three criteria for a storage effect
355 (differential responses to the environment and buffered population dynamics) may be operating
356 in the community.
357 The third criterion of the storage effect is that there is covariance between environmental
358 conditions and competition. Documenting this pattern, especially in highly diverse communities,
359 can often be a challenge. Our study demonstrated that species experienced greater self-limitation

(consistent with stronger intraspecific than interspecific competition) when they were more common in the active community (and were thus more likely favored by the environment) (Fig. 3, Fig. S2), and diversity was maintained during potentially unfavorable growth environments (Fig. 4), but it is unclear whether environmental fluctuations in this system generate the covariance between environment and competition necessary for a storage effect (Chesson 2000; Miller & Klausmeier 2017). Theoretical models indicate that the storage effect may be more likely to evolve when species' generation times are much shorter than the timescale of environmental fluctuations (Miller & Klausmeier 2017), a scenario that is well aligned with bacterioplankton living in a highly seasonal north temperate lake. In addition, we cannot rule out the potentially strong contribution to NFD by another non-mutually exclusive class of fluctuation-dependent mechanisms. Namely, relative nonlinearity in competition can promote coexistence if species differ in their responses to competition in ways that benefit their competitors (Yuan & Chesson 2015; Letten *et al.* 2018; Hallett *et al.* 2019). While our data cannot provide definitive proof, the documented patterns are consistent with the criteria needed for a storage effect to contribute to the long-term maintenance of bacterial diversity.

Seasonal reoccurrence in bacterial communities: Seed banks may also have more general implications for bacterial community dynamics. The persistence of taxa with temporal niche differences could contribute to the repeatability of summer community dynamics in the active portion of the community (Hellweger *et al.* 2008) by favoring overwinter survival (Fig. 4). For example, we found that the seed bank exhibited seasonality, such that diversity stored in the seed bank was maximized when environmental conditions (e.g., water temperature, resource/consumer densities) were least favorable for bacterial growth (Neuenschwander *et al.* 2015). This pattern is consistent with the notion that dormant seed banks help buffer individuals

383 from harsh conditions. In addition, transitions from inactive to active metabolic states were more
384 frequently detected among taxa that were, on average, rare when active in the community.
385 Analogous to the methodological challenges of finding and identifying dormant individuals in
386 non-microbial seed banks (e.g., plants), detection limits may affect the classification of
387 metabolically active bacteria. Nevertheless, our reactivation metric should capture rapid shifts in
388 the metabolically active portion of the community. Indeed, when compared to null models, our
389 observations indicate that bacteria of rare-to-intermediate abundance ranks exhibited more
390 frequent reactivations than would be expected by chance alone (Fig. S3), providing further
391 evidence that seed banks are likely important sources of recolonization for bacterial communities
392 inhabiting seasonal freshwater environments. Overall, these patterns suggest that recurrent
393 environmental cues regulate active community dynamics by favoring different taxa at different
394 times of the year, and that seed banks are important for maintaining these seasonal community
395 trajectories at multi-annual timescales.

396 *Future directions and conclusions:* Our study provides empirical evidence consistent
397 with the theory that niche differences and seed bank dynamics stabilize bacterial communities
398 and maintain diversity in nature. In the naturally fluctuating lake environment of our study, we
399 demonstrated key differences in the diversity, dynamics, and stabilization between the active and
400 total subsets of the bacterial community, but an ultimate goal is to tighten the mechanistic links
401 between rates of ribosomal RNA transcription and *in situ* growth rates for individual taxa
402 (Newton & Shade 2016; Papp *et al.* 2018) or through other techniques that involve the physical
403 sorting of cells based on metabolic activity prior to sequencing (Couradeau *et al.* 2019; Reichart
404 *et al.* 2020). While our results showed that stabilizing mechanisms generated NFD in the
405 community, an important next step is to quantify the strengths and directions of the multiple

406 fluctuation-independent and -dependent coexistence mechanisms that may be operating in
407 diverse microbial communities (Letten *et al.* 2018; Ellner *et al.* 2019; Hallett *et al.* 2019).
408 A grand challenge at the intersection of microbial and community ecology is to extend
409 the experimental investigations of microbial coexistence in the lab (Zhang *et al.* 2010; Letten *et*
410 *al.* 2018) into systems reflecting the high diversity and complex interaction networks of most
411 natural microbial communities. It will require careful experimentation as well as a clear
412 consideration of the spatial scale of the study (e.g., to account for sampling biases and
413 immigration that deviate from clear alignment with coexistence theory). For example, it may also
414 be important to consider the dispersal of terrestrial bacteria into aquatic ecosystems (Crump *et al.*
415 2012), since immigration could contribute to inferences made about local processes. However,
416 previous work in our study system revealed that most terrestrial bacteria were metabolically
417 inactive, most likely reflecting the abrupt environmental transitions that accompany cross-
418 ecosystem dispersal (Wisnoski *et al.* 2020). Thus, the lack of asymmetric NFD we observed in
419 the total community could also arise in part from allochthonous inputs of inactive bacteria that
420 decouple local population growth of each OTU from its relative abundance in the community.
421 However, by focusing on the dynamics of metabolically active bacteria, our approach was able to
422 uncover the presence of stabilizing biotic interactions that may have otherwise been obscured by
423 metabolic heterogeneity in the total community.
424 In conclusion, we show that stabilizing biotic interactions and the ability to engage in
425 dormancy or slow growth strategies play important roles in maintaining microbial diversity in a
426 natural ecosystem over a multi-year time scale. Our results demonstrate the mechanisms at the
427 community scale that preserve Earth's vast microbial diversity, building on other explanations
428 that emphasize the importance of metabolic diversity (Sala *et al.* 2008), capacity for rapid growth

(Shade *et al.* 2014), and spatial scale (Vos *et al.* 2013). In particular, strong NFD offers a new explanation for why the majority of bacterial taxa persist at low average relative abundances in nature (Lynch & Neufeld 2015). Furthermore, our work builds on inferences about the roles of microbial dormancy (and other persistence strategies) obtained from shorter time scales, and provides temporal evidence that dormancy is an important buffer against local extinction over longer time scales. More generally, our work demonstrates the importance of stabilization in microbial systems, offering new insight into the long-term maintenance of microbial diversity.

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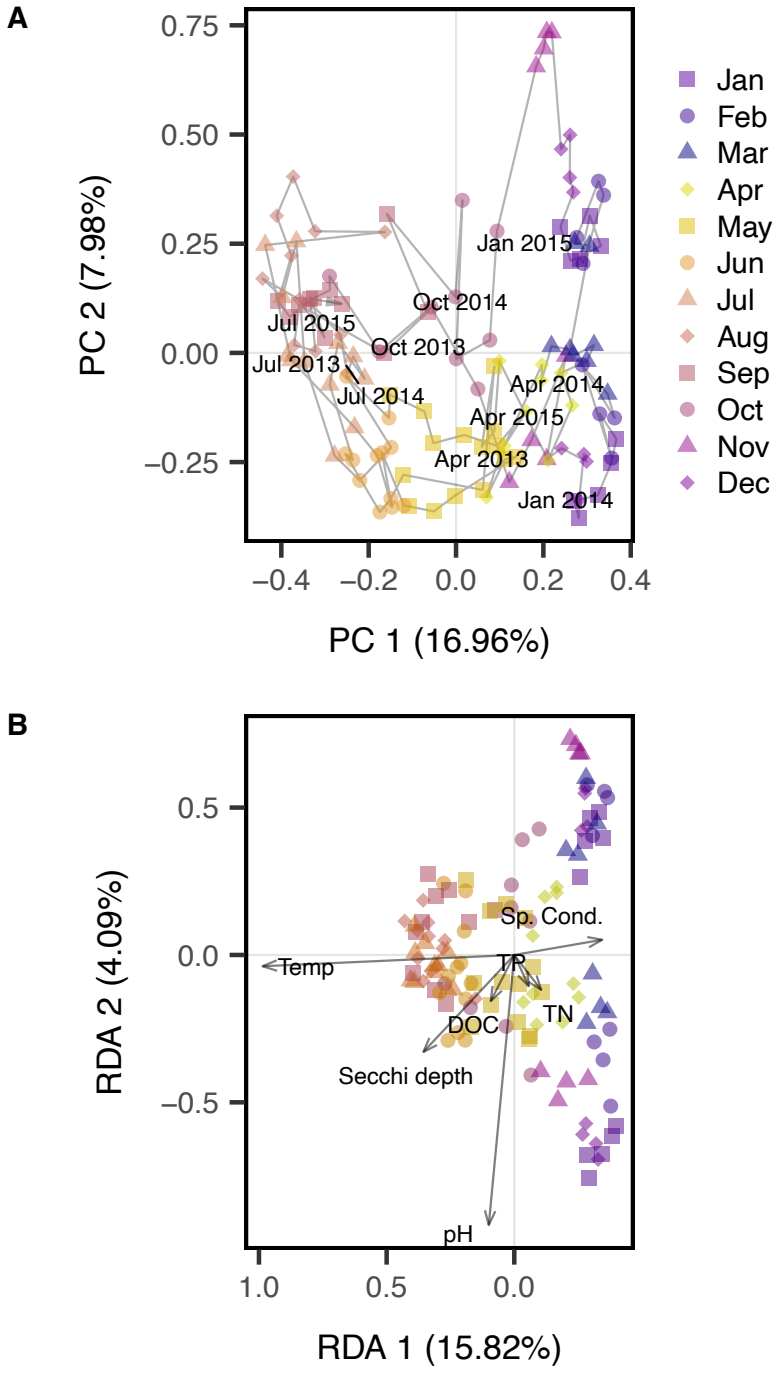
650 # FIGURE LEGENDS

651 **Figure 1.** Seasonal dynamics of the active bacterial community in University Lake. (A) The
652 compositional trajectory of the active community (determined by high-throughput sequencing of
653 16S rRNA transcripts) shows strong seasonality, but the community remains relatively static
654 over winter. The first two axes of the principal component analysis (PCA) depict summer/winter
655 differences (PC1) along the major axis, and slight inter-annual differences in winter composition
656 (cool colors) along the minor axis (PC2). The summer successional trajectory (warmer colors) is
657 highly repeatable across years. (B) Constrained ordination using redundancy analysis (RDA)
658 shows the environmental drivers of community structure, along with strong correlates of
659 individual taxa in the community. This analysis reveals that differences in pH explain variation
660 in winter composition among years.
661
662 **Figure 2.** Temporal partitioning of maximum growth rate among persistent bacterial taxa in
663 University Lake. Lines represent the mean daily growth rate for each taxon over the time series.
664 Points indicate the maximum growth observed for each bacterial taxon (OTU). Overall, the 82
665 persistent OTUs have maximum growth rates at different seasons of the year. Points are color-
666 coded such that warmer colors correspond to spring and summer months and cooler colors
667 correspond to winter months. Colored lined trace out the growth dynamics of three individual
668 taxa with different environmental responses (blue = OTU 1, Betaproteobacteria; yellow = OTU
669 17, Actinobacteria; mauve = OTU 18, Gammaproteobacteria). More taxonomic details can be
670 found in Table S1.
671

672 **Figure 3.** Negative frequency dependence (NFD) among persistent bacterial taxa (n = 82) was
673 significantly stronger for rare than common taxa only in the active portion of the community.
674 The degree of asymmetry in NFD is determined by the covariance between the equilibrium
675 frequency of each OTU and its strength of NFD; negative covariance indicates that rarer taxa
676 exhibit stronger NFD. Compared with expected covariances from a null distribution, the
677 standardized effect size (SES) of the observed covariance in the active portion of the community
678 was -4.03, while the SES of the total community was -0.77. The overall strength of NFD
679 (observed NFD / mean NFD) was 1.08 in the active portion and 1.01 in the total community. The
680 metabolic state on the y-axis indicates whether the NFD comparison is for the active portion of
681 the community (inferred from 16S rRNA transcripts) or the total portion (inferred from 16S
682 rRNA genes, i.e., DNA).
683
684 **Figure 4.** Seasonal importance of the seed bank for bacterial diversity in University Lake.
685 Richness was much higher in the total community, relative to the active community, during the
686 fall and winter months. The active and total communities converged over the summer, indicated
687 by values on the y-axis closer to 1. Warmer colors correspond to spring and summer months,
688 while cooler colors correspond to winter months. Shaded regions correspond to the fall and
689 winter months (October through March).
690
691 **Figure 5.** Rare taxa showed more seed bank transitions than common taxa. For the 82 persistent
692 taxa identified over the time series, OTUs that were (on average) rare in the active portion of the
693 community had a higher number of reactivations from the seed bank, while more common taxa
694 had fewer reactivations. Regression lines are locally estimated scatterplot smoothing (LOESS).

695 **FIGURES**

696 Figure 1



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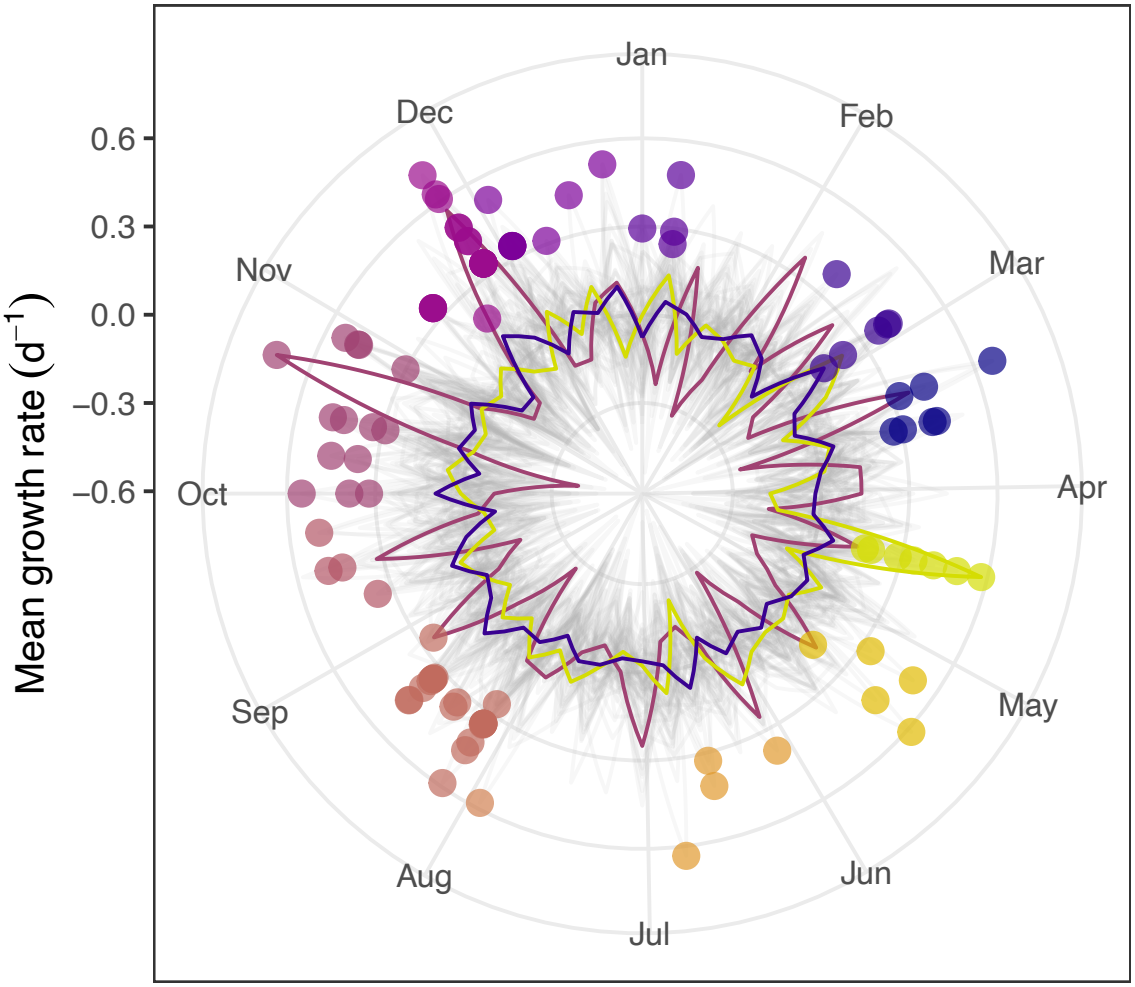
700 Figure 2.



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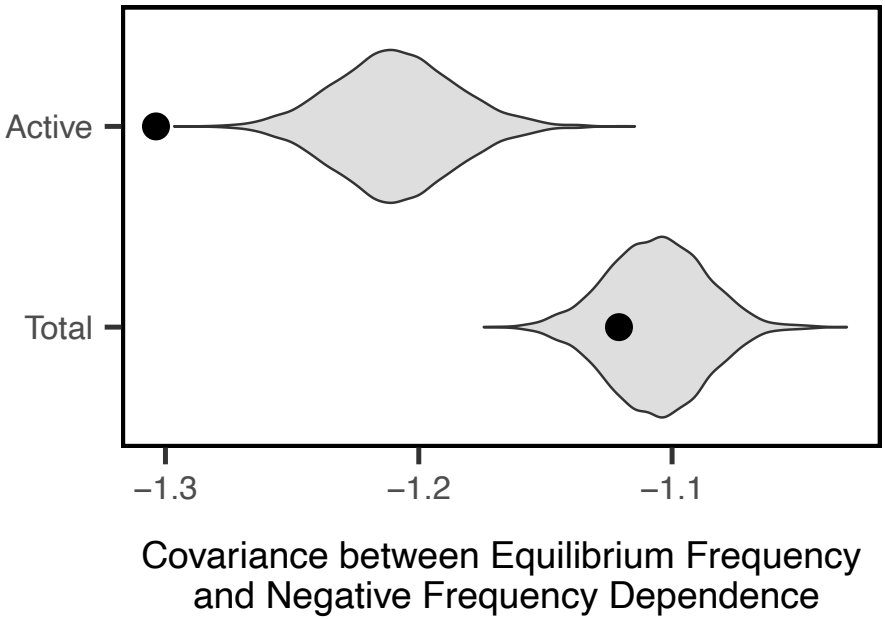
704 Figure 3.



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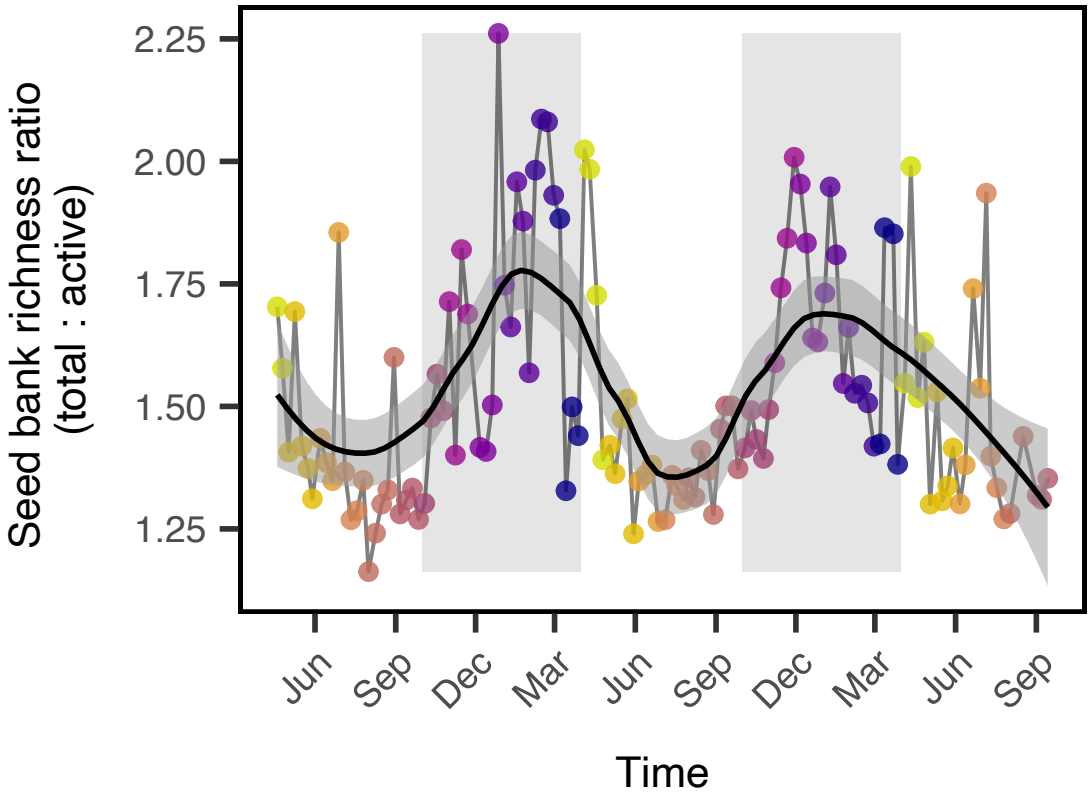
708 Figure 4.



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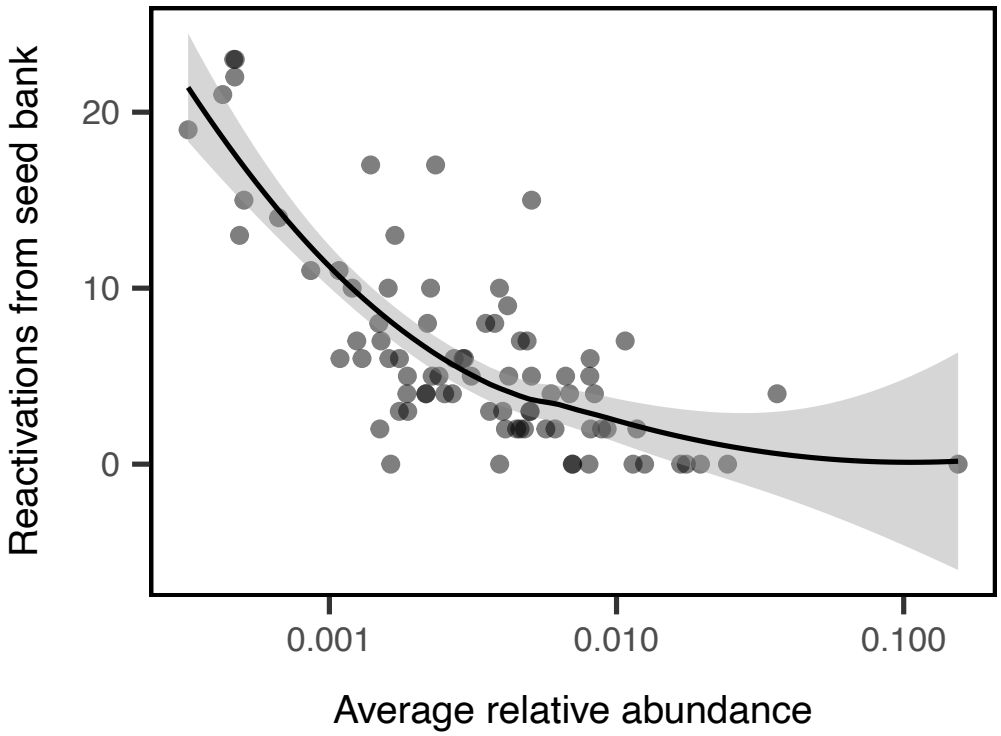
712 Figure 5.



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714

SUPPLEMENTAL INFORMATION

Table S1. Operational taxonomic units (OTUs) that were classified as persistent in the bacterioplankton community based on being detected in ≥80% of the total (i.e., DNA) community samples. The table is sorted by Julian date of max growth.

OTU	Class	Max growth rate (d^{-1})	Date of max growth
Otu00034	Alphaproteobacteria	0.3426	2014-01-17
Otu00045	Betaproteobacteria	0.8527	2014-01-03
Otu00196	Actinobacteria	0.5873	2015-01-09
Otu00019	Cytophagia	0.7346	2014-02-14
Otu00039	Betaproteobacteria	0.7167	2014-02-14
Otu00102	Betaproteobacteria	0.6729	2015-02-28
Otu00105	Alphaproteobacteria	0.8666	2014-02-28
Otu00065	Sphingobacteriia	0.8563	2014-03-21
Otu00292	Alphaproteobacteria	0.6851	2014-03-07
Otu00006	Sphingobacteriia	0.3643	2013-04-25
Otu00012	Betaproteobacteria	0.8784	2014-04-18
Otu00014	Actinobacteria	0.4906	2015-04-26
Otu00016	Actinobacteria	0.7836	2014-04-18
Otu00017	Actinobacteria	1.0200	2015-04-04
Otu00021	Gammaproteobacteria	1.0449	2014-04-18
Otu00033	Alphaproteobacteria	0.5306	2014-04-25
Otu00048	Verrucomicrobiae	0.7426	2015-04-11

Otu00049	Actinobacteria	0.6090	2014-04-04
Otu00055	Flavobacteriia	0.7950	2014-04-18
Otu00058	Armatimonadia	0.8153	2015-04-11
Otu00148	Bacteria sp.	0.7646	2013-04-25
Otu00172	Gammaproteobacteria	0.6729	2015-04-11
Otu00219	Betaproteobacteria	0.7503	2014-04-18
Otu00002	Actinobacteria	0.3190	2015-05-03
Otu00008	Actinobacteria	0.3637	2013-05-09
Otu00022	Opitutae	0.8003	2015-05-03
Otu00031	Cytophagia	0.6593	2014-05-09
Otu00051	Flavobacteriia	0.9187	2013-05-09
Otu00062	Flavobacteriia	0.7836	2015-05-23
Otu00064	Alphaproteobacteria	0.6964	2013-05-29
Otu00113	Bacteroidetes sp.	0.7950	2013-05-09
Otu00116	Betaproteobacteria	0.8373	2014-05-09
Otu00151	Betaproteobacteria	0.7259	2013-05-17
Otu00183	Bacteria sp.	0.6090	2015-05-03
Otu00200	Bacteria sp.	0.6851	2015-05-23
Otu00083	Flavobacteriia	1.0031	2015-06-06
Otu00087	Betaproteobacteria	0.5873	2014-06-05
Otu00098	Betaproteobacteria	0.8104	2013-06-14
Otu00123	Sphingobacteriia	0.7259	2014-06-20
Otu00194	Deltaproteobacteria	0.9277	2014-06-13

Otu00294	Alphaproteobacteria	0.6964	2013-06-21
Otu00004	Actinobacteria	0.3500	2015-07-11
Otu00009	Gammaproteobacteria	1.3329	2013-07-26
Otu00011	Betaproteobacteria	0.9841	2015-07-18
Otu00192	Bacteria sp.	0.4350	2013-07-26
Otu00195	Actinobacteria	0.6277	2014-07-18
Otu00020	Betaproteobacteria	0.4150	2013-08-01
Otu00026	Betaproteobacteria	0.6277	2013-08-01
Otu00029	Actinobacteria	0.6593	2013-08-23
Otu00036	Alphaproteobacteria	0.7576	2013-08-16
Otu00037	Actinobacteria	0.7646	2014-08-29
Otu00052	Alphaproteobacteria	0.6444	2013-08-09
Otu00076	Actinobacteria	0.6444	2014-08-08
Otu00112	Alphaproteobacteria	0.5873	2014-08-23
Otu00226	Opitutae	0.6729	2015-08-02
Otu00250	Actinobacteria	0.5306	2013-08-23
Otu00010	Proteobacteria sp.	0.6277	2014-09-26
Otu00024	Bacteroidetes sp.	0.7259	2014-09-19
Otu00056	Cytophagia	0.7711	2013-09-06
Otu00067	Betaproteobacteria	0.5306	2015-09-02
Otu00177	Proteobacteria sp.	0.5306	2013-09-20
Otu00005	Sphingobacteriia	0.5294	2014-10-04
Otu00015	Actinobacteria	0.6277	2014-10-17

Otu00018	Gammaproteobacteria	0.9516	2014-10-11
Otu00060	Betaproteobacteria	1.0031	2013-10-25
Otu00066	Betaproteobacteria	0.8153	2013-10-25
Otu00082	Bacteroidetes sp.	0.8003	2014-10-04
Otu00154	Alphaproteobacteria	0.7070	2014-10-04
Otu00158	Gammaproteobacteria	0.9141	2013-10-04
Otu00001	Betaproteobacteria	0.2860	2013-11-15
Otu00007	Betaproteobacteria	0.4577	2013-11-15
Otu00038	Actinobacteria	0.7346	2014-11-29
Otu00047	Betaproteobacteria	0.6729	2013-11-15
Otu00073	Betaproteobacteria	0.4906	2013-11-22
Otu00077	Flavobacteriia	0.7774	2013-11-15
Otu00109	Actinobacteria	0.6729	2013-11-15
Otu00118	Actinobacteria	0.6090	2013-11-22
Otu00129	Alphaproteobacteria	0.4906	2013-11-15
Otu00198	Betaproteobacteria	0.9210	2013-11-15
Otu00208	Betaproteobacteria	0.6964	2013-11-22
Otu00217	Proteobacteria sp.	0.7167	2013-11-22

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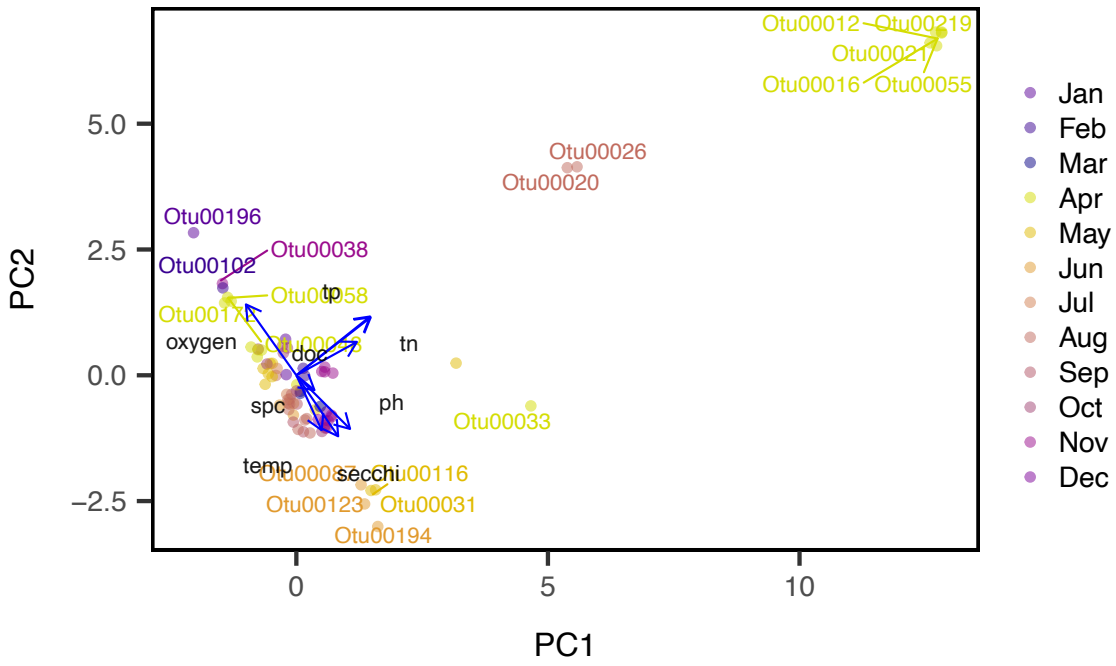

722

723 **Figure S1**. Temporal partitioning of maximum growth rates corresponds to environmental
724 conditions. Here, we depict a principal component analysis of the environmental conditions at
725 each date. OTU labels indicate the individual taxa that exhibit maximum growth at the
726 corresponding time of the year. Vectors indicate the loadings of environmental variables along
727 the two PC axes. Warmer colors correspond to spring and summer months, cooler colors
728 correspond to winter months.

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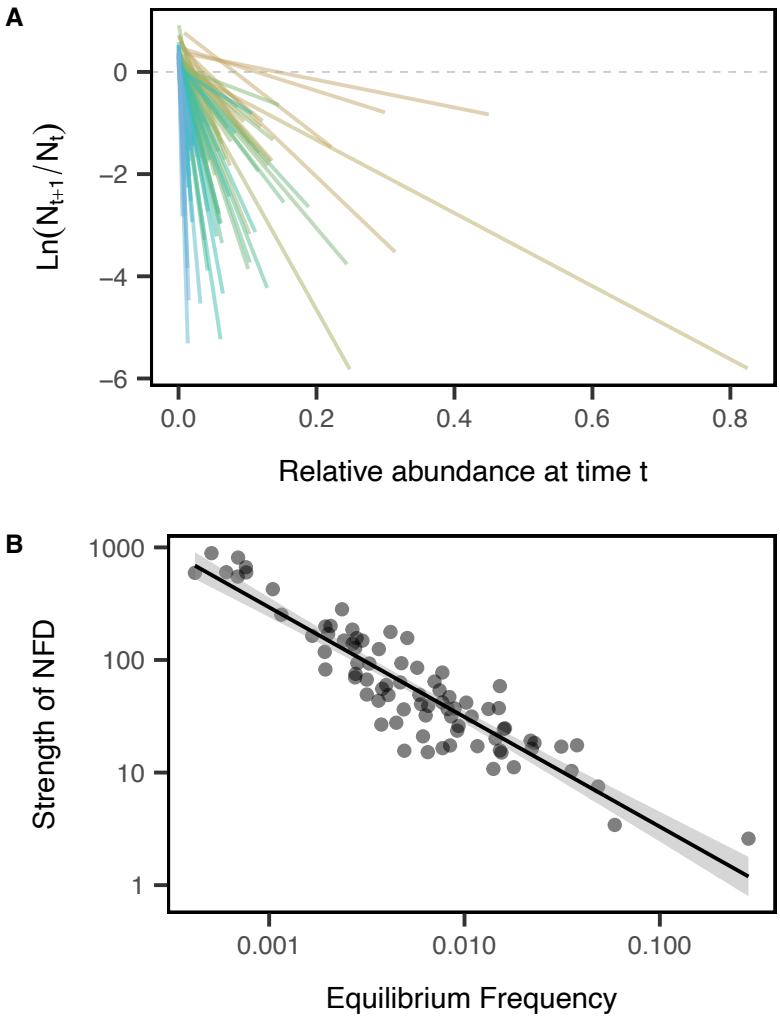

730

731 **Figure S2.** Negative frequency dependence (NFD) in the active portion of the community for the
732 82 persistent bacterial taxa. (A) Relationship between the rate of change of an OTU and its
733 relative abundance. Depicted in this graph are simple linear regression fits for the 82 taxa
734 individually, data points not shown to reduce clutter. Negative relationships indicate NFD
735 growth and variation in slopes indicates variation in the strength of NFD. (B) Rare taxa (lower
736 equilibrium frequencies) exhibit stronger NFD, while common taxa (higher equilibrium
737 frequency) have weaker NFD.

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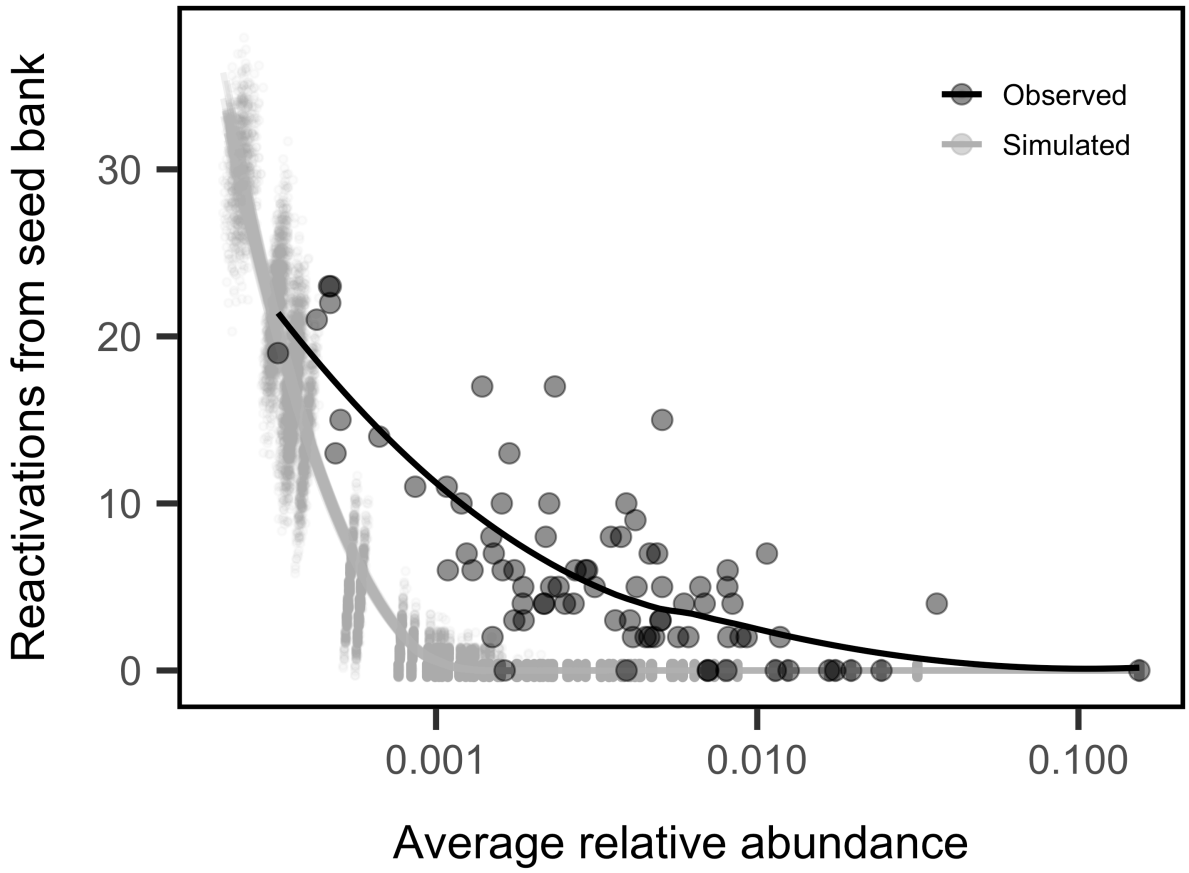

739

740 **Figure S3**. Observed reactivations from the seed bank are more frequent than expected by
741 chance alone. Null model simulations (n = 1000) describe the range of expectations expected by
742 chance for the relationship between relative abundance and reactivation rate (gray points). For
743 observed data (black points) and the null simulations, we fit local regression lines with locally
744 estimated scatterplot smoothing (LOESS).