

Emergence of alternative stable states in microbial communities in a fluctuating environment

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1 Abstract

2 The effect of environmental fluctuations is a major question in ecology. While it is widely
3 accepted that fluctuations and other types of disturbances can increase biodiversity, we have only a
4 limited understanding of the circumstances in which other types of outcomes can occur in a
5 fluctuating environment. Here we explore this question with laboratory microcosms, using
6 cocultures of two bacterial species, *P. putida* and *P. veronii*. At low dilution rates we observe
7 competitive exclusion of *P. veronii*, whereas at high dilution rates we observe competitive
8 exclusion of *P. putida*. When the dilution rate alternates between high and low, we do not observe
9 coexistence between the species, but rather alternative stable states, in which only one species
10 survives and initial species' fractions determine the identity of the surviving species. The Lotka-
11 Volterra model with a fluctuating mortality rate predicts that this outcome is independent of the
12 timing of the fluctuations, and that the time-averaged mortality would also lead to alternative stable
13 states, a prediction that we confirm experimentally. Other pairs of species can coexist in a
14 fluctuating environment, and again consistent with the model we observe coexistence in the time-
15 averaged dilution rate. We find a similar time-averaging result holds in a three-species
16 community, highlighting that simple linear models can in some cases provide powerful insight into
17 how communities will respond to environmental fluctuations.

18 Introduction

19 In nature, environmental conditions vary over time, and this variation can have significant
20 impacts on the structure and function of ecological communities. Examples of the impacts of
21 environmental variability on community composition include daily cycles of light and
22 temperature that allow nocturnal and diurnal organisms to coexist, and seasonal variation that
23 causes reproducible succession patterns in communities of plants¹, freshwater² and marine
24 microbes³. Community function can also be strongly influenced by varying environmental
25 conditions. For example, a single rain event can cause up to 10% of annual carbon dioxide
26 production of a forest⁴, due in part to enhanced microbial activity in rewetted dry soil⁵. Varying
27 environmental conditions may even cause ecosystems to abruptly and irreversibly change states,
28 such as lakes that shift from clear to turbid due to human-induced eutrophication⁶ and reefs that
29 transform from kelp forests to seaweed turfs due to heat waves⁷. Given the inevitability of
30 temporal variability in nature, an improved understanding of how this variability affects ecological
31 communities is essential for understanding natural ecosystems.

32 Both theoretical⁸⁻¹² and empirical¹³⁻¹⁷ studies have shown that disturbances can stabilize
33 communities and enhance diversity. For example, temporal fluctuations of light¹⁸ and
34 temperature¹⁹ have been shown to lead to stable coexistence of microbes. One possible mechanism
35 for this effect is that different species are favored at different times, such that no species can ever
36 dominate the system, and the result might be coexistence²⁰. For example, species A may exclude
37 species B in one environment, whereas B excludes A in another environment. Fluctuating between
38 the two environments might lead to coexistence of the two species. If true, such an effect could
39 propagate to complex communities with more than two species, leading to more coexistence in the
40 fluctuating environment than in the constant ones. Another possible explanation for coexistence in
41 a fluctuating environment is time-averaging: species A and B might coexist when fluctuating
42 between two environments because they coexist in the constant average environment^{21,22}. The
43 former theory predicts that fluctuations are necessary for coexistence, while the latter suggests that
44 coexistence depends upon the average environment, with or without fluctuations.

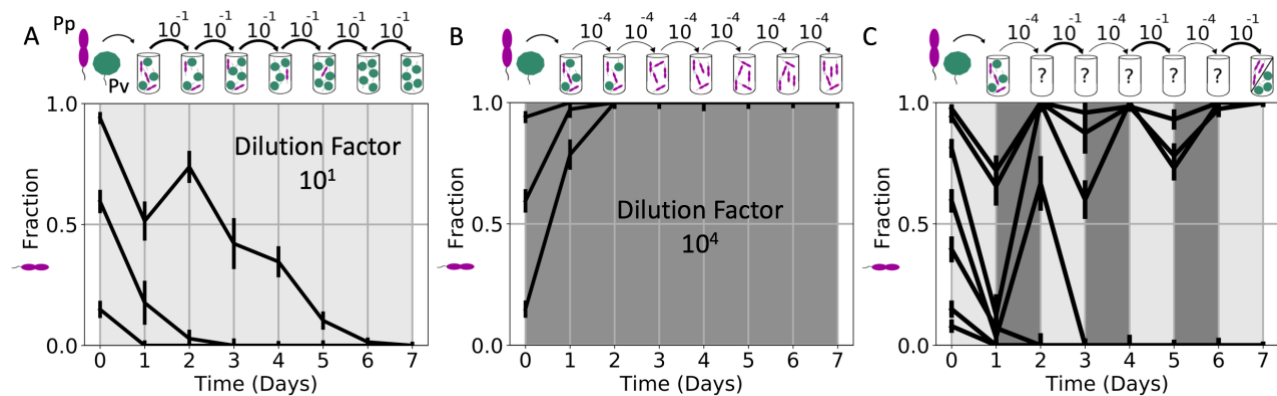
45 Compared to a wealth of studies of fluctuation-induced coexistence, less is known about
46 whether perturbations may lead to outcomes other than increased diversity. For example,
47 alternative stable states have been observed in fluctuating environments, such as in gut microbiota
48 communities subject to laxative treatments²³, intertidal biofilms perturbed by climatic events²⁴, and
49 regions of forest and barrens disturbed by frequent fires²⁵. In these cases, however, it is often
50 difficult to distinguish between alternative states that are simultaneously stable and different states
51 that are stable in different environments. There is some theoretical support for fluctuation-induced
52 alternative stable states in particular systems^{26,27}, but these predictions are challenging to test in the
53 field due to the difficulty of controlling all ecological factors. The proposition that environmental
54 fluctuations may have more complicated effects on ecosystems than simply affecting diversity is
55 therefore in need of systematic study and demonstration.

56 In this paper, we make use of highly controllable microbial microcosms to explore the
57 effects of temporal fluctuations on communities. We grow bacterial species in liquid culture with
58 daily dilution, and implement environmental fluctuations by alternating the amount of the dilution.
59 The growth-dilution process imposes a tunable death rate on the system, where the dilution factor
60 determines the fraction of cells discarded each day. In a two-species coculture, we observe that
61 fluctuating dilution factors leads to bistability, or two alternative stable states that depend on the
62 initial abundances of each species. To explain this result, we use a simple phenomenological
63 model: the Lotka-Volterra competition model adapted to incorporate a fluctuating global mortality
64 rate. This model with a fluctuating mortality rate predicts that the bistability is independent of the
65 timing of the fluctuations, and that the time-averaged mortality would also lead to alternative stable
66 states, a prediction that we confirm experimentally. The model predicts that fluctuating mortality

67 can result not only in bistability, but also in stable coexistence, depending on the strength of
68 interspecies inhibition, which we confirm experimentally. More broadly, the model predicts that
69 an environment with a fluctuating death rate equilibrates to the same outcome as the time-averaged
70 added mortality rate. We test this prediction both in two-species cocultures and in a more complex
71 community with three species, where a fluctuating death rate and a constant death rate lead to the
72 same qualitative outcome. These results suggest that fluctuations can in some cases have
73 predictable consequences on community structure.

74 Results

75 To explore the effect of a fluctuating environment on an experimentally tractable microbial
76 community, we performed coculture experiments with fluctuating as well as constant dilution
77 factors. The cultures were allowed to grow for 24 hours and then diluted by transferring a small
78 amount of culture into fresh growth media. Because the total experimental volume remains
79 constant, the amount of culture added from the previous day determines the dilution factor. We
80 began by coculturing *Pseudomonas veronii* (*Pv*) and *Pseudomonas putida* (*Pp*). At a low dilution
81 factor ($10\times$), *Pv* competitively excludes *Pp*, as the fraction of *Pp* goes to zero from all initial
82 fractions (Fig. 1A). At a high dilution factor ($10^4\times$), the outcome is reversed, with *Pp* excluding *Pv*
83 as its fraction goes to one from all initial fractions (Fig. 1B). We refer to both of these outcomes as
84 competitive exclusion because the final state does not depend on starting conditions; all starting
85 fractions move toward one stable state, which is either zero or one.



86

Figure 1: Experimental observation of alternative stable states in a fluctuating environment. **A:** When a coculture of Pp (purple) and Pv (green) was diluted by a factor of 10 each day (1/10 of the previous day's culture transferred to fresh media, keeping the volume constant), the slow-growing Pv dominated, sending the fraction of fast grower Pp to zero from several starting fractions. **B:** When the same culture was subject to a much higher dilution factor, 10^4 , fast grower Pp dominated. **C:** Surprisingly, fluctuating between the low and high dilution factors shown in A and B resulted in alternative stable states. Either Pp or Pv can dominate, depending on their relative initial abundances. In all plots, we qualitatively indicate the dilution factor for that day by the shading of the plot; low dilution factors have a lighter shading, while high dilution factors have a darker shading. Error bars are the SD of the beta distribution with Bayes' prior probability (see Methods).

87
88 Given that there was a single stable state at low dilution and a single stable state at high dilution,
89 we expected that alternating between the two dilution factors would also lead to a single stable
90 state (which could be survival of just one species or possibly stable coexistence of the two). To
91 our surprise, when we performed the coculture experiment alternating between the two dilution
92 factors, we instead observed the emergence of alternative stable states—only a single species
93 survived, but the surviving species depended on the species' initial fractions (Fig. 1C). We
94 therefore observe bistability in the fluctuating environment despite the fact that neither
95 environment alone showed bistability.

96 To explain a possible origin of this emergent bistability, we employed the Lotka-Volterra
97 (LV) competition model with added mortality, which previously provided powerful insight into
98 how microbial competitive outcomes shift with dilution rate²⁸. The two-species LV model with
99 added mortality is:

100
$$\frac{\dot{N}_i}{N_i} = r_i(1 - N_i - \alpha_{ij}N_j) - \delta, \quad (1)$$

101 where N_i is the abundance of species i normalized by its carrying capacity, r_i is the maximum
102 growth rate for species i , α_{ij} is the competition coefficient that determines how strongly species j
103 inhibits species i , and δ is the imposed mortality rate, which is experimentally controlled by the
104 dilution factor, which specifies the fraction of cells discarded per day. In the absence of added
105 mortality δ , the outcome is independent of growth rates r_i and solely determined by whether the
106 competition coefficients α_{ij} are greater or less than one: coexistence and bistability result when
107 both are less than or greater than one, respectively, and dominance results when only one
108 coefficient is greater than one (Fig. 2B). The presence of mortality makes the competition
109 coefficients, and thus the outcome, functions of growth and mortality, as can be seen in the
110 reparameterized model (Supplementary Note 1):

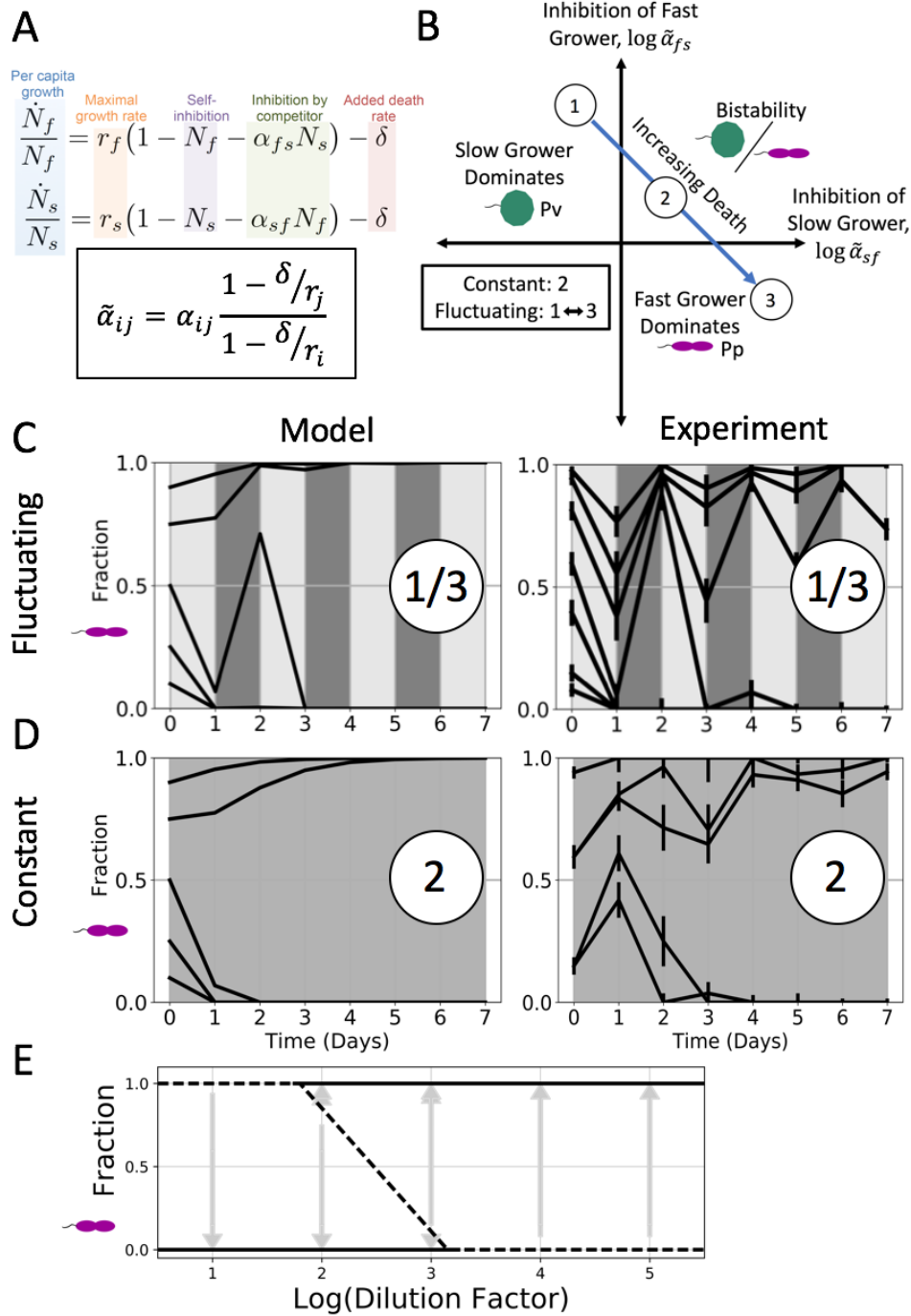
111
$$\frac{\dot{\tilde{N}}_i}{\tilde{N}_i} = \tilde{r}_i(1 - \tilde{N}_i - \tilde{\alpha}_{ij}\tilde{N}_j), \quad (2)$$

112 where

113
$$\tilde{\alpha}_{ij} = \alpha_{ij} \left(\frac{1 - \delta/r_j}{1 - \delta/r_i} \right). \quad (3)$$

114 If a slow grower dominates at low mortality/dilution, the model predicts that increasing dilution
115 will reverse the outcome and cause the fast grower to win, and at some range of intermediate
116 dilution the pair passes through either a region of bistability or coexistence (Fig. 2B). Moreover, a
117 fluctuating dilution rate will lead to the same outcome as the time-averaged rate in the absence of
118 fluctuations. This prediction arises because the per-capita growth rates, \tilde{N}_i/\tilde{N}_i , in the LV model
119 are linear and additive, and the steady state reached through a temporally fluctuating mortality δ is
120 the same state reached by its linear time-average $\langle \delta \rangle$ (Supplementary Note 2). The LV model thus
121 makes the simple prediction that an experiment alternating between dilution factors 10 and 10^5 , for
122 example, will end in the same state as one with a constant dilution factor of 10^3 . When averaging
123 dilution factors, we use the geometric mean of the dilution factors because of the logarithmic
124 relation between discrete dilution factor and equivalent continuous rate δ (Supplementary Note 2).

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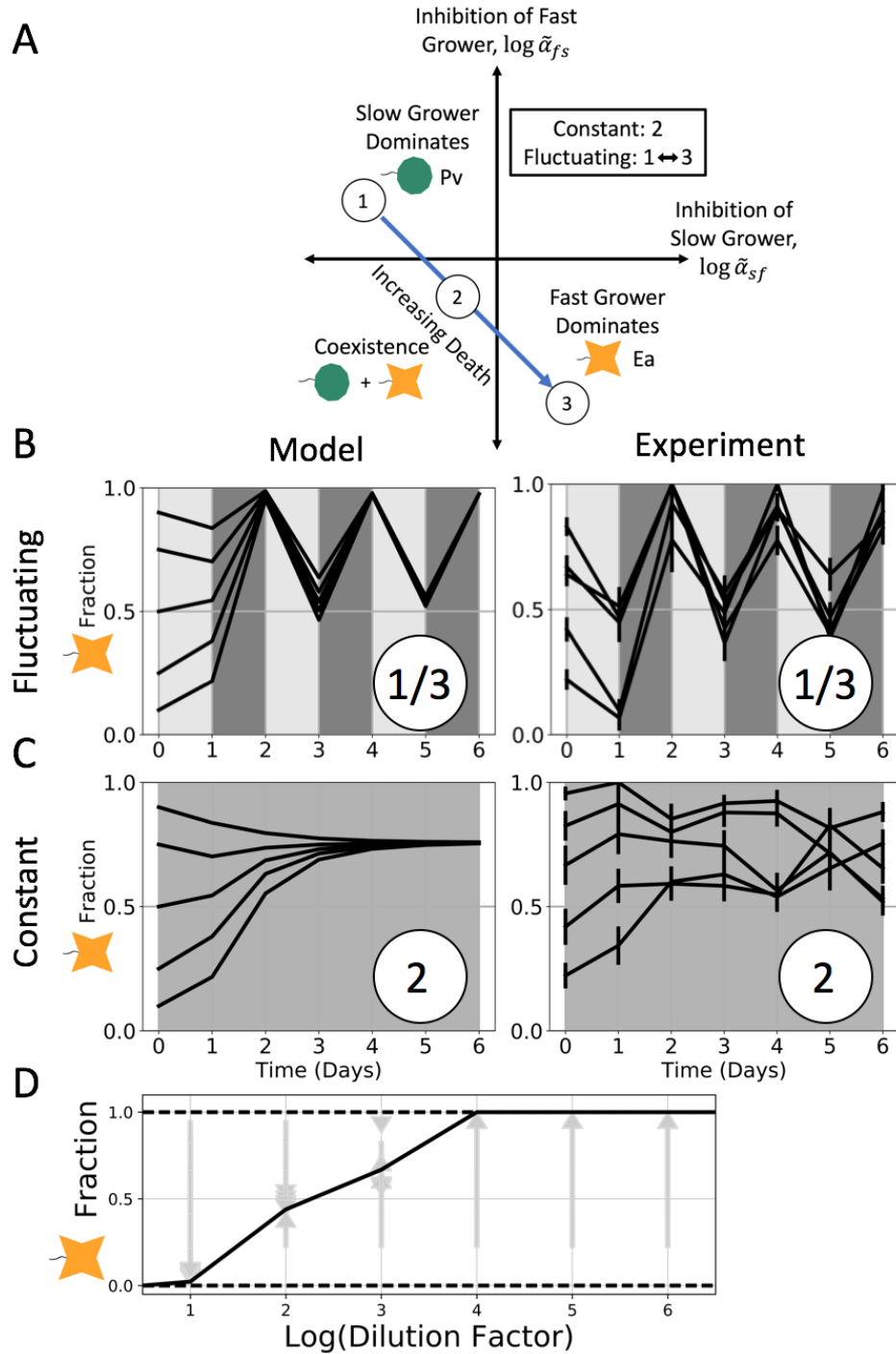
Figure 2: Bistability occurs in both fluctuating and average environments, confirming model prediction. To understand the results from Fig. 1, we employed a modified Lotka-Volterra model. **A:** We model daily dilutions by adding a community-wide death rate term δ to the two-species Lotka-Volterra (LV) competition model. The per-capita growth rate is a function of a species' maximum growth rate, self-inhibition, and competition with the other species. Because the per capita growth rate is linear and additive, the model predicts that the outcomes of a fluctuating environment should be the same as that of the time-averaged environment (Supplementary Note 1). **B:** The solutions to the LV model can be represented by a phase space of the re-parameterized competition coefficients α , which are functions of death δ and growth r . If a slow grower dominates at low or no added death, increasing mortality will favor the fast grower, causing the pair to pass through a region of bistability or coexistence on the way to fast grower dominance. Here we have illustrated the trajectory of a bistable pair. **C-D:** To test the prediction that the fluctuating and time-averaged environments are qualitatively equivalent, we cocultured *Pp* and *Pv* at a dilution factor equal to the time-average of the fluctuating dilution factors in Fig. 1, and we observed bistability, confirming this prediction (lower right). Additionally, we simulated daily dilutions of the model with both constant (upper left) and fluctuating (lower left) dilution factors and observed good agreement between the model and experimental results. **E:** A bifurcation diagram of the *Pp*-*Pv* outcomes at all constant dilution factors shows that the fast-growing *Pp* is favored as dilution increases. Arrows represent time trajectories from initial to final fractions; solid lines represent stable equilibria, and dotted lines represent unstable fractions. This diagram was used to estimate the competition coefficients used in simulations (see Methods and Supp. Fig. 1). Error bars are the SD of the beta distribution with Bayes' prior probability (see Methods).

127

128 Given our experimental observation of bistability in a fluctuating environment (Fig. 1), our next
129 step was to look for bistability in the constant average environment, as the model predicts. We
130 cocultured *Pp* and *Pv* at a range of constant and fluctuating daily dilution factors. The fluctuating
131 dilution factor experiments again led to bistability, replicating our previous results (Fig. 2C-D).
132 The constant dilution factor experiments revealed a range of fixed points varying with dilution
133 factor: in addition to domination of slow-growing *Pv* at low dilution and that of fast-growing *Pp* at
134 high dilution, we observed bistability at two intermediate dilution factors (Fig. 2E). The separatrix,
135 or starting fraction dividing the two stable states, shifts in favor of the fast grower at the higher of
136 these two dilution factors, consistent with the model. These experimental results confirm the
137 model's prediction that the dilution factor can be time-averaged to result in the same qualitative
138 outcome; namely, bistability (see Supp. Fig. 3).

139 As previously mentioned, the LV model also predicts that pairs of species can coexist at
140 intermediate mortality rates. Given this additional prediction, we sought to experimentally verify
141 that coexistence can also result from time-averaging the dilution factors. Based on results of
142 previous cocultured experiments in a similar growth medium²⁸, we chose another fast grower,
143 *Enterobacter aerogenes* (*Ea*), which is also excluded by the slow-growing *Pv* at low dilution
144 factor. At intermediate dilution factor *Ea* and *Pv* coexist, and *Ea* dominates at high dilution factor
145 (Fig. 3D). We then fluctuated between the dilution factors in which either species dominated, as in
146 our initial experiments. This time, we observed stable coexistence in the fluctuating environment
147 (Fig. 3). Furthermore, the stable fraction of *Ea* fluctuated in the neighborhood of the stable
148 fraction of *Ea* in the constant dilution experiment. In both the bistable pair (Fig. 2C-D) and the
149 coexisting pair (Fig. 3B-C), simulations match the experimental trajectories over time, in both

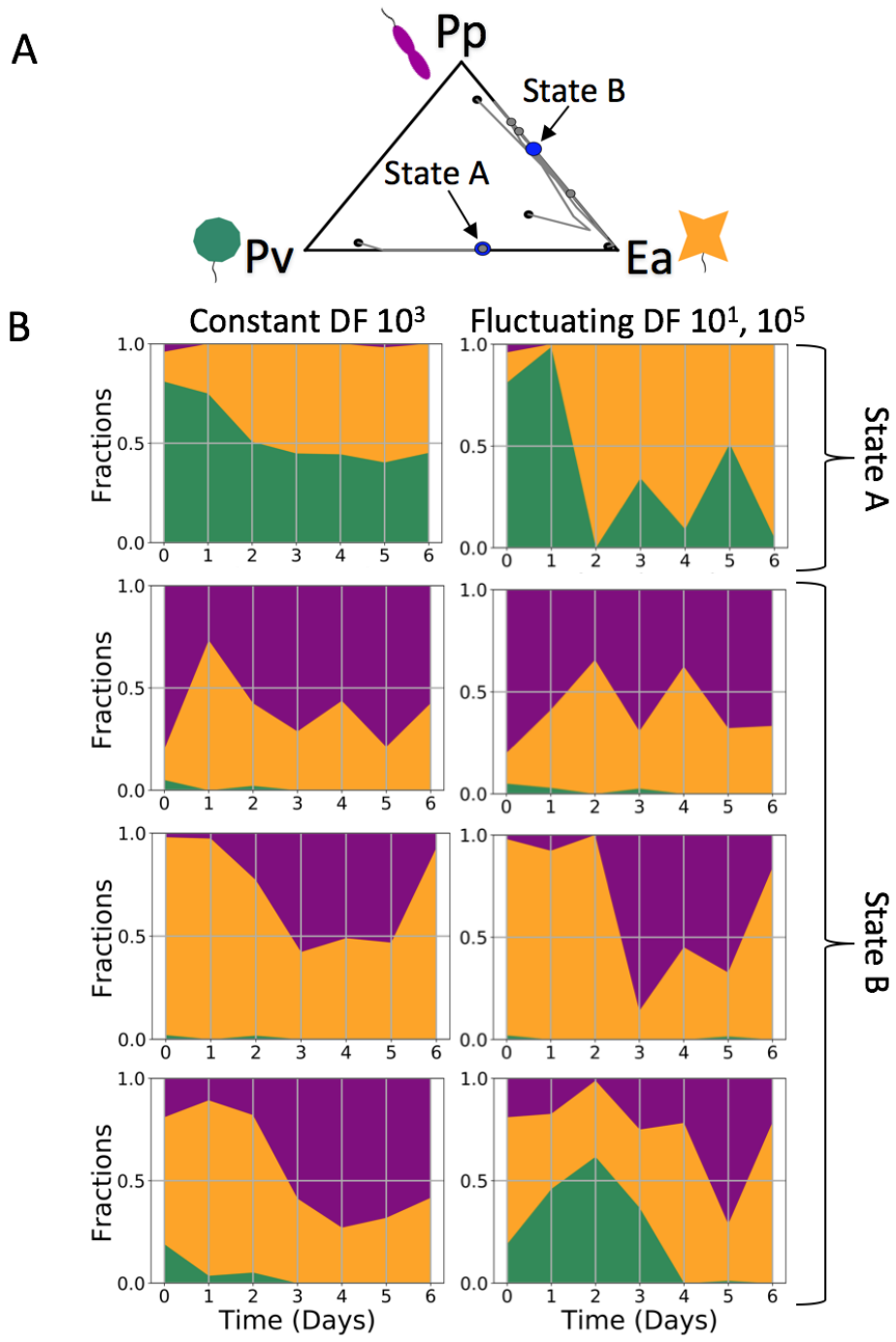
150 constant and fluctuating dilution factors. These examples of agreement between the model and
 151 experiments emphasize that in our simple bacterial communities, a fluctuating dilution factor may
 152 be time-averaged (see Supp. Fig. 4).



153

Figure 3: Coexistence occurs in both fluctuating and average environments, confirming model prediction. **A:** As in Fig. 2, the LV model phase space shows qualitative outcomes divided by the zero-points of the logarithm of the re-parameterized competition coefficients. Here we have illustrated the trajectory of a pair that passes through the coexistence region as the death rate increases. **B-C:** When we cocultured slow-growing *Pv* with another fast grower, *Ea*, we observed coexistence in an environment that fluctuated between dilution factors in which either species dominated (upper right). At a dilution factor equal to the mean of fluctuations, we also observed coexistence (lower right), confirming the model's prediction about a time-averaged environment for both types of trajectories across the phase space. Once again, simulations of daily dilutions showed good agreement between the model and experimental results (left). **D:** A diagram of outcomes at all constant dilution factors shows that fast-growing *Ea* is favored as dilution increases. Arrows represent time trajectories from initial to final fractions; solid lines represent stable equilibria, and dotted lines represent unstable fractions. We used this diagram to estimate the competition coefficients used in simulations (see Methods and Supp. Fig. 2). Error bars are the SD of the beta distribution with Bayes' prior probability (see Methods).

154
155 While pairwise interactions in a fluctuating environment appear to be well-described by the LV
156 model, the same might not be true of more complex communities. To address this question, we
157 cocultured all three of the previously mentioned species, *Ea*, *Pp*, and *Pv*. Across a range of
158 constant dilution factors, we saw changes in community outcome as a function of the dilution
159 factor (Supp. Fig. 6), shifting from a *Pv*-dominated final state at low dilution to coexistence of *Pp*
160 and *Ea* at high dilution. At an intermediate dilution factor, bistability occurs in the trio between a
161 *Pp-Ea* coexistence state and a *Pv-Ea* coexistence state (Fig. 4A). This bistability of coexisting
162 states can be predicted from the corresponding pairwise results, using our previously developed
163 community assembly rules²⁹. When we fluctuated the dilution factor between the low and high
164 values that average to this intermediate dilution factor, we again observed bistability, with each
165 initial condition ending in the same final state as it did in the constant environment (Fig. 4B).
166 These results show that even in a more complex community of three species, a fluctuating
167 mortality rate still leads to the same results as the constant average environment, which is in line
168 with the predictions of the modified LV model.



169

Figure 4: Fluctuating environment predictably leads to alternative stable states in a three-species community. To ensure that the time-averaging prediction is not only applicable to simple two-species communities, we tested our results in a three-species environment. **A:** A ternary plot shows the outcomes of a coculture with all three species, Pv, Pp, and Ea, in a constant environment with dilution factor (DF) 10^3 . The time trajectories, indicated by the grey lines, end at one of two alternative stable states (shown in blue; for state B, we plot the average of the three trajectories). In state A, Pv and Ea coexist, while Pp and Ea coexist in state B. **B:** Time series plots show the results of three-species coculture experiments in both constant (DF 10^3 ; left column) and fluctuating (between DF 10^3 and DF 10^5 ; right column) environments. As indicated in **A**, three initial fractions end in state B and one ends in state A. We find that the outcomes of a given initial fraction go to the same final state in both the constant and fluctuating environments. This suggests that the ability to time-average the outcomes extends to communities with more than two species (see Supp. Fig. 6).

170

171 Discussion

172 In criticizing the use of generic hypotheses, JW Fox posited in 2013, “The time is ripe for
173 ecologists to ... embark on a new research program testing the assumptions and predictions of
174 logically valid models of diversity and coexistence in fluctuating environments.”²¹ Our results
175 highlight a clear experimental demonstration of a simple theoretical prediction about the effects of
176 fluctuations on community structure. Using simple microbial communities with up to three
177 species, we have shown that an experiment with a dilution factor fluctuating between two
178 extremes, both of which typically lead to competitive exclusion, in fact leads to either coexistence
179 or bistability. The outcome of the fluctuating dilution factors is the same as that of the equivalent
180 time-averaged dilution factor, and therefore appears to be independent of fluctuations. Whether
181 the community coexists or forms alternative stable states in the fluctuating environment can be
182 predicted by the community state in a constant environment, providing evidence that a fluctuating
183 environment can be similar to the constant mean environment.

184 That the outcome of a fluctuating environment would be the same as that of the equivalent
185 constant environment is consistent with models that have linear and additive per-capita growth
186 rates^{21,30}. The Lotka-Volterra competition model, modified to include an added mortality rate, is
187 one such model, which we used for guidance. Other ecological competition models, such as those
188 that explicitly define resource consumption with the Monod equation, are not linear or additive,
189 and do not make this prediction³¹. Although our results rely on what some may call simplistic
190 predictions, our conclusions are strengthened by the simplicity of both the experimental system and
191 the model. More complex empirical studies that make no predictions about the effect of
192 fluctuations, or which seek to (dis)prove a generic prediction such as the intermediate disturbance
193 hypothesis (IDH)^{13,14,32–34}, lack clearly defined predictions and thus make less clear conclusions.
194 The IDH, which predicts a diversity peak at some undefined range of intermediate disturbance
195 frequencies, has left some unconvinced²¹, and a meta-study of the phenomenon found little
196 evidence of the trend³⁵. This paper provides a case study for how the effect of fluctuations can be

197 understood without adhering to any particular hypothesis of disturbance-diversity relationships,
198 with guidance from a simple phenomenological model.

199 The absence of resources from the LV model is not its only simplification—it also assumes
200 perfect logistic growth and interactions that are not density-dependent. While these assumptions
201 are clearly simplifications of the dynamics present within any actual community, the model
202 nonetheless worked well in predicting the outcome of fluctuations in the dilution rate in our
203 microbial community. We used regularly alternating and moderate disturbances, but as the
204 duration and intensity of disturbances increases one expects that eventually the model’s predictions
205 will fail due to stochastic extinction caused by finite population sizes. Still, its success here
206 highlights the relevance of simple, phenomenological models to biological systems.

207 Environmental disturbances have long been thought to weaken competition, thus leading to
208 increased biodiversity³⁶. This logic is oversimplified²², however, and has inhibited exploration of
209 other potential outcomes of fluctuations, such as alternative stable states. In our experiments, we
210 interpreted alternative stable states to be the result of simple time-averaging of dilution factors,
211 meaning that the outcome, whether bistability or coexistence, did not depend on fluctuations per
212 se. In the LV model, dilution δ can be time-averaged because it does not covary with other
213 quantities. Not all types of fluctuations would meet this criteria, however; for example, a
214 disturbance affecting the LV competition coefficients α_{ij} would lead to a covariance between the
215 coefficients and species’ abundances. As noted previously, temperature fluctuations led to stable
216 coexistence of two species of microalgae¹⁹, and in that case the outcome differed from the outcome
217 at the mean temperature. Indeed, a version of the LV model that assumes temperature acts only
218 upon growth rates r_i does not allow for linear time-averaging (Lax et. al., pre-print). A question
219 for future investigation is whether there are other simple examples of fluctuating parameters that
220 can be time-averaged.

221 The effect of environmental disturbances on community structure has become more urgent
222 as many habitats face the effects of climate change. The question of which types of disturbances
223 can be time-averaged is one that may provide insight into the effects of environmental fluctuations
224 on natural communities. Our system was composed of three species of soil bacteria, raising the
225 question of whether communities with more than three species would be similarly affected. A
226 linear time-dependence of interactions and growth rates on added mortality may be more dominant
227 in simple communities, while higher-order effects may become more important in complex
228 communities. Additionally, our simple community results do not mean that there were no
229 nonlinearities or covariances in our system, but only that they were not sufficiently large so as to
230 alter the experimental outcome. Many studies of natural systems have found evidence of
231 nonlinearities and covariances, such as the storage effect^{37–39}. In such systems, it may be difficult
232 to disentangle which effects are independent of fluctuations, but our results argue that in some
233 cases, simple models retain their predictive power even in fluctuating environments.

234

235 **Methods**

236 **Species and media**

237 The soil bacterial species used in this study were *Enterobacter aerogenes* (Ea, ATCC#13048),
238 *Pseudomonas putida* (Pp, ATCC#12633) and *Pseudomonas veronii* (Pv, ATCC#700474). All
239 species were obtained from ATCC. All coculture experiments were done in S medium,
240 supplemented with glucose and ammonium chloride. It contains 100 mM sodium chloride, 5.7
241 mM dipotassium phosphate, 44.1 mM monopotassium phosphate, 5 mg/L cholesterol, 10 mM
242 potassium citrate pH 6 (1 mM citric acid monohydrate, 10 mM tri-potassium citrate monohydrate),
243 3 mM calcium chloride, 3 mM magnesium sulfate, and trace metals solution (0.05 mM disodium
244 EDTA, 0.02 mM iron sulfate heptahydrate, 0.01 mM manganese chloride tetrahydrate, 0.01 mM
245 zinc sulfate heptahydrate, 0.01 mM copper sulfate pentahydrate), 0.93 mM ammonium chloride, 1
246 mM glucose. 1X LB broth was used for initial inoculation of colonies. Plating was done on
247 rectangular Petri dishes containing 45 ml of nutrient agar (nutrient broth (0.3% yeast extract, 0.5%
248 peptone) with 1.5% agar added), onto which diluted 96-well plates were pipetted at 10 ul per well.

249 **Growth rate measurements**

250 Growth curves were captured by measuring the optical density of monocultures (OD 600 nm) in
251 15-minute intervals over a period of ~40 hours (Fig. S3). Before these measurements, species were
252 grown in 1X LB broth overnight, and then transferred to the experimental medium for 24 hours.
253 The OD of all species was then equalized. The resulting cultures were diluted into fresh medium at
254 factors of 10^{-8} to 10^{-3} of the equalized OD. Growth rates were measured by assuming exponential
255 growth to a threshold of OD 0.1, and averaging across many starting densities and replicates (n =
256 16 for all species).

257 **Coculture experiments**

258 Frozen stocks of individual species were streaked out on nutrient agar Petri dishes, grown at room
259 temperature for 48 h and then stored at 4 °C for up to two weeks. Before competition experiments,
260 single colonies were picked and each species was grown separately in 50 ml Falcon tubes, first in 5
261 ml LB broth for 24 h and next in 5 ml of the experimental media for 24 h. During the competition
262 experiments, cultures were grown in 500 µl 96-well plates (BD Biosciences), with each well
263 containing a 200-µl culture. Plates were incubated at 25°C and shaken at 400 rpm, and were
264 covered with an AeraSeal film (Sigma-Aldrich). For each growth–dilution cycle, the cultures were
265 incubated for 24 h and then serially diluted into fresh growth media. Initial cultures were prepared
266 by equalizing OD to the lowest density measured among competing species, mixing by volume to
267 the desired species composition, and then diluting mixtures by the factor to which they would be
268 diluted daily (except for dilution factor 10^{-6} , which began at 10^{-5} on Day 0, to avoid causing
269 stochastic extinction of any species). Relative abundances were measured by plating on nutrient
270 agar plates. Each culture was diluted in phosphate-buffered saline prior to plating. Multiple

271 replicates were used to ensure that enough colonies could be counted. Colonies were counted after
272 48 h incubation at room temperature. The mean number of colonies counted, per plating, per
273 experimental condition, was 49. During competition experiments, we also plated monocultures to
274 determine whether each species could survive each dilution factor in the absences of other species.
275 *Pv* went extinct in the highest two dilution factors (10^{-5} and 10^{-6}); other species survived all
276 dilution factors.

277 **Estimating competition coefficients for simulations**

278 In order to simulate the effect of a fluctuating environment on the pairs (Fig. 2C-D, Fig. 3B-C), we
279 measured growth rates and carrying capacities (Supp. Fig. 5) and estimated the competition
280 coefficients α_{ij} . We used the diagrams of pairwise coculture outcomes at all constant dilution
281 factors (Fig. 2E, Fig. 3D) to estimate the competition coefficients as follows. The different
282 outcomes (dominance/exclusion, coexistence, and bistability) are divided by the zero points of
283 $\log(\tilde{\alpha}_{ij})$ and $\log(\tilde{\alpha}_{ji})$, meaning that the qualitative pairwise outcome changes at a dilution factor
284 where one of the reparamaterized coefficients $\tilde{\alpha}_{ij}$ is equal to one. We used Eqn. 3 of the main text
285 to solve for the competition coefficient at the boundary dilution factor where unstable (dotted) and
286 stable (solid) lines intersect on the diagrams (Fig. 2E, Fig. 3D).

287 **Statistical analysis**

288 The p-values given in Supplementary Figures 5 and 6 were obtained using two-tailed t-tests. The
289 error bars shown in the time-series plots in Fig. 1, Fig. 2, and Fig. 3 are the SD of the beta
290 distribution with Bayes' prior probability:

$$291 \quad \sigma = \sqrt{\frac{(\alpha + 1)(\beta + 1)}{(\alpha + \beta + 2)^2(\alpha + \beta + 3)}}$$

292 Here, α and β are the number of colonies of two different species.

293 **Code availability**

294 Code for data analysis is available upon request.

295 **Data availability**

296 The source data underlying all figures are provided as a Source Data File. Access to the data is
297 also publicly available at TBD. A reporting summary for this Article is available as a
298 Supplementary Information file.

299

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301 **References**

- 302 1. Olf, H., Pegtel, D. M., Van Groenendael, J. M. & Bakker, J. P. Germination Strategies During
303 Grassland Succession. *J. Ecol.* **82**, 69–77 (1994).
- 304 2. Rösel, S., Allgaier, M. & Grossart, H.-P. Long-Term Characterization of Free-Living and
305 Particle-Associated Bacterial Communities in Lake Tiefwaren Reveals Distinct Seasonal
306 Patterns. *Microb. Ecol.* **64**, 571–583 (2012).
- 307 3. Gilbert, J. A. *et al.* Defining seasonal marine microbial community dynamics. *ISME J.* **6**, 298–
308 308 (2012).
- 309 4. Lee, X., Wu, H.-J., Sigler, J., Oishi, C. & Siccama, T. Rapid and transient response of soil
310 respiration to rain. *Glob. Change Biol.* **10**, 1017–1026 (2004).
- 311 5. Blazewicz, S. J., Schwartz, E. & Firestone, M. K. Growth and death of bacteria and fungi
312 underlie rainfall-induced carbon dioxide pulses from seasonally dried soil. *Ecology* **95**, 1162–
313 1172 (2014).
- 314 6. Scheffer, M., Carpenter, S., Foley, J. A., Folke, C. & Walker, B. Catastrophic shifts in
315 ecosystems. *Nature* **413**, 591 (2001).
- 316 7. Wernberg, T. *et al.* Climate-driven regime shift of a temperate marine ecosystem. *Science* **353**,
317 169–172 (2016).
- 318 8. Levine, J. M. & Rees, M. Effects of Temporal Variability on Rare Plant Persistence in Annual
319 Systems. *Am. Nat.* **164**, 350–363 (2004).
- 320 9. Miller, A. D., Roxburgh, S. H. & Shea, K. Timing of disturbance alters competitive outcomes
321 and mechanisms of coexistence in an annual plant model. *Theor. Ecol.* **5**, 419–432 (2012).
- 322 10. Barabás, G., Meszéna, G. & Ostling, A. Community robustness and limiting similarity in
323 periodic environments. *Theor. Ecol.* **5**, 265–282 (2012).

- 324 11. Behar, H., Brenner, N., Ariel, G. & Louzoun, Y. Fluctuations-induced coexistence in public
325 goods dynamics. *Phys. Biol.* **13**, 056006 (2016).
- 326 12. Miller, E. T. & Klausmeier, C. A. Evolutionary stability of coexistence due to the storage
327 effect in a two-season model. *Theor. Ecol.* **10**, 91–103 (2017).
- 328 13. Sousa, W. P. Disturbance in Marine Intertidal Boulder Fields: The Nonequilibrium
329 Maintenance of Species Diversity. *Ecology* **60**, 1225–1239 (1979).
- 330 14. Flöder, S. & Sommer, U. Diversity in planktonic communities: An experimental test of the
331 intermediate disturbance hypothesis. *Limnol. Oceanogr.* **44**, 1114–1119 (1999).
- 332 15. D’Odorico Paolo & Bhattachan Abinash. Hydrologic variability in dryland regions: impacts
333 on ecosystem dynamics and food security. *Philos. Trans. R. Soc. B Biol. Sci.* **367**, 3145–3157
334 (2012).
- 335 16. Shimadzu, H., Dornelas, M., Henderson, P. A. & Magurran, A. E. Diversity is maintained
336 by seasonal variation in species abundance. *BMC Biol.* **11**, 98 (2013).
- 337 17. Catorci, A., Piermarteri, K., Penksza, K., Házi, J. & Tardella, F. M. Filtering effect of
338 temporal niche fluctuation and amplitude of environmental variations on the trait-related
339 flowering patterns: lesson from sub-Mediterranean grasslands. *Sci. Rep.* **7**, 12034 (2017).
- 340 18. Litchman, E. Competition and coexistence of phytoplankton under fluctuating light:
341 experiments with two cyanobacteria. *Aquat. Microb. Ecol.* **31**, 241–248 (2003).
- 342 19. Descamps-Julien, B. & Gonzalez, A. Stable Coexistence in a Fluctuating Environment: An
343 Experimental Demonstration. *Ecology* **86**, 2815–2824 (2005).
- 344 20. Chesson, P. L. & Warner, R. R. Environmental Variability Promotes Coexistence in Lottery
345 Competitive Systems. *Am. Nat.* **117**, 923–943 (1981).
- 346 21. Fox, J. W. The intermediate disturbance hypothesis should be abandoned. *Trends Ecol.*
347 *Evol.* **28**, 86–92 (2013).

- 348 22. Barabás, G., D’Andrea, R. & Stump, S. M. Chesson’s coexistence theory. *Ecol. Monogr.*
349 **88**, 277–303 (2018).
- 350 23. Tropini, C. *et al.* Transient Osmotic Perturbation Causes Long-Term Alteration to the Gut
351 Microbiota. *Cell* **173**, 1742-1754.e17 (2018).
- 352 24. Bello, M. D., Rindi, L. & Benedetti-Cecchi, L. Temporal clustering of extreme climate
353 events drives a regime shift in rocky intertidal biofilms. *Ecology* **100**, e02578 (2019).
- 354 25. Eberhardt, R. W. & Latham, R. E. Relationships among Vegetation, Surficial Geology and
355 Soil Water Content at the Pocono Mesic Till Barrens. *J. Torrey Bot. Soc.* **127**, 115–124 (2000).
- 356 26. Lefever, R. & Horsthemke, W. Bistability in fluctuating environments. Implications in
357 tumor immunology. *Bull. Math. Biol.* **41**, 469–490 (1979).
- 358 27. Holmgren, M. & Scheffer, M. El Niño as a Window of Opportunity for the Restoration of
359 Degraded Arid Ecosystems. *Ecosystems* **4**, 151–159 (2001).
- 360 28. Abreu, C. I., Friedman, J., Woltz, V. L. A. & Gore, J. Mortality causes universal changes in
361 microbial community composition. *Nat. Commun.* **10**, 2120 (2019).
- 362 29. Friedman, J., Higgins, L. M. & Gore, J. Community structure follows simple assembly
363 rules in microbial microcosms. *Nat. Ecol. Evol.* **1**, 0109 (2017).
- 364 30. Chesson, P. & Huntly, N. The Roles of Harsh and Fluctuating Conditions in the Dynamics
365 of Ecological Communities. *Am. Nat.* **150**, 519–553 (1997).
- 366 31. Stewart, F. M. & Levin, B. R. Partitioning of Resources and the Outcome of Interspecific
367 Competition: A Model and Some General Considerations. *Am. Nat.* **107**, 171–198 (1973).
- 368 32. Violle, C., Pu, Z. & Jiang, L. Experimental demonstration of the importance of competition
369 under disturbance. *Proc. Natl. Acad. Sci.* **107**, 12925–12929 (2010).
- 370 33. Gibbons, S. M. *et al.* Disturbance Regimes Predictably Alter Diversity in an Ecologically
371 Complex Bacterial System. *mBio* **7**, e01372-16 (2016).

- 372 34. Rodríguez-Verdugo, A., Vulin, C. & Ackermann, M. The rate of environmental
373 fluctuations shapes ecological dynamics in a two-species microbial system. *Ecol. Lett.* **0**,
- 374 35. Mackey, R. L. & Currie, D. J. The Diversity–Disturbance Relationship: Is It Generally
375 Strong and Peaked? *Ecology* **82**, 3479–3492 (2001).
- 376 36. Huston, M. A General Hypothesis of Species Diversity. *Am. Nat.* **113**, 81–101 (1979).
- 377 37. Sears, A. L. W. & Chesson, P. New Methods for Quantifying the Spatial Storage Effect: An
378 Illustration with Desert Annuals. *Ecology* **88**, 2240–2247 (2007).
- 379 38. Angert, A. L., Huxman, T. E., Chesson, P. & Venable, D. L. Functional tradeoffs determine
380 species coexistence via the storage effect. *Proc. Natl. Acad. Sci.* **106**, 11641–11645 (2009).
- 381 39. Usinowicz, J. *et al.* Temporal coexistence mechanisms contribute to the latitudinal gradient
382 in forest diversity. *Nature* **550**, 105–108 (2017).
- 383