

# 1 Modeling microbial metabolic trade-offs in a chemostat

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12 **ABSTRACT**

13 Microbes face intense competition in the natural world, and so need to wisely allocate their  
14 resources to multiple functions, in particular to metabolism. Understanding competition among  
15 metabolic strategies that are subject to trade-offs is therefore crucial for deeper insight into the  
16 competition, cooperation, and community assembly of microorganisms. In this work, we  
17 evaluate competing metabolic strategies within an ecological context by considering not only  
18 how the environment influences cell growth, but also how microbes shape their chemical  
19 environment. Utilizing chemostat-based resource-competition models, we exhibit a set of  
20 intuitive and general procedures for assessing metabolic strategies. Using this framework, we are  
21 able to relate and unify multiple metabolic models, and to demonstrate how the fitness landscape  
22 of strategies becomes intrinsically dynamic due to species-environment feedback. Such dynamic  
23 fitness landscapes produce rich behaviors, and prove to be crucial for ecological and  
24 evolutionary stable coexistence in all the models we examined.

25

26

## 27 INTRODUCTION

28 The way microbes respond to and shape their local environment influences their community  
29 structure (Callahan, Fukami, & Fisher, 2014). Such microbe-environment interactions depend on  
30 the allocation strategies of cells, i.e., how a cell allocates its internal resources into various  
31 cellular functions, such as transport, assimilation, reproduction, motility, maintenance, etc.  
32 (Bachmann, Bruggeman, Molenaar, dos Santos, & Teusink, 2016). Within a microbial cell,  
33 energy and biomass are limited, and trade-offs always exist in allocating these valuable internal  
34 resources into the various functions required for cell growth. Therefore, the growth rate of cells  
35 cannot increase without bound. Rather, evolution acts on cells' internal resource allocation to  
36 optimize growth and survival (S. Goyal, Yuan, Chen, Rabinowitz, & Wingreen, 2010;  
37 Liebermeister et al., 2014). To this end, in response to environmental changes, microbes rapidly  
38 adjust their metabolic strategies. For example, the yeast *Saccharomyces cerevisiae* switches from  
39 fermentation to respiration upon glucose depletion (Zaman, Lippman, Zhao, & Broach, 2008),  
40 and *Escherichia coli* exhibits drastic differences in ribosome content between different nutrient  
41 conditions (Li et al., 2018; Scott, Gunderson, Mateescu, Zhang, & Hwa, 2010). Moreover, in  
42 laboratory long-term evolution studies of microbes, adaptive mutations consistently emerge that  
43 reshape metabolism (Bachmann, Molenaar, dos Santos, & Teusink, 2017; Bajic & Sanchez,  
44 2019; Blount, Barrick, Davidson, & Lenski, 2012; Long & Antoniewicz, 2018). Such short-term  
45 and long-term adjustments of metabolic strategies presumably confer fitness benefits, and it is  
46 important to map metabolic strategies onto these benefits to better understand the regulation and  
47 evolution of microbial metabolism.

48  
49 The convergence towards steady state makes the chemostat an ideal experimental system to  
50 culture microorganisms and investigate their metabolic status (Wides & Milo, 2018; Ziv, Brandt,  
51 & Gresham, 2013). In a chemostat, fresh nutrients are supplied at a constant rate, while medium  
52 with cells is removed at the same rate to maintain constant volume. The metabolite  
53 concentrations in the chemostat constitute the chemical environment directly perceived by cells,  
54 and determine their growth rates. Importantly, cells also shape this environment through their  
55 consumption and secretion of metabolites. One advantage of a chemostat is the automatic  
56 convergence of cellular growth rates towards the controlled dilution rate. This convergence  
57 occurs through negative feedback between microbes and their environment: the higher the  
58 population, the worse the chemical environment, and the slower the growth rate. As a result  
59 (provided the nutrient supply allows for faster-than-dilution growth to prevent "washout"), the  
60 cells in the chemostat will reach the steady-state population that sustains growth at the dilution  
61 rate (De Leenheer, Levin, Sontag, & Klausmeier, 2006; Smith & Waltman, 1995). This  
62 stabilization of the cellular growth rate at the controlled dilution rate facilitates precise  
63 characterization of cellular physiology in a constant environment. However, it also imposes  
64 challenges in quantitatively understanding the advantages and disadvantages of various  
65 metabolic strategies: if all metabolic strategies lead to identical growth rates in a chemostat, how  
66 should we evaluate whether one strategy is "better" or "worse" than another? If strategies can be  
67 compared, are there "best" strategies, and how do these optima shift as the experimental  
68 conditions such as nutrient-supply concentrations and dilution rates change?

69  
70 If we evaluate metabolic strategies by the outcome of competition between species, many  
71 insights can be gained from theoretical ecology. For example, resource-competition models have

72 provided a simple context to explore competition dynamics in chemostat-like ecosystems such as  
73 lakes and rivers (Smith & Waltman, 1995). In such models, species interact only indirectly via  
74 consumption (and sometimes production) of a common pool of nutrients. A steady state can be  
75 reached if the species present can shape the nutrient concentration to support a growth rate equal  
76 to their dilution or death rate (Tilman, 1982). Resource-competition models underpin many  
77 ecosystem theories including contemporary niche theory as pioneered by MacArthur  
78 (MacArthur, 1970), popularized by Tilman (Tilman, 1980, 1982), and extended by Chase and  
79 Leibold (Chase & Leibold, 2003). A central component of contemporary niche theory is a  
80 graphical approach, generally consisting of three components: zero net growth isoclines (ZNGIs)  
81 in chemical space, an impact vector representing a species' nutrient consumption, and a supply  
82 point to describe the external resource supply (Koffel, Daufresne, Massol, & Klausmeier, 2016).  
83 This graphical approach is a powerful and intuitive way of assessing the outcome of competition,  
84 yet it is not yet commonly utilized in understanding microbial metabolic strategies with trade-  
85 offs.

86 Resource-competition models focusing on various aspects of cellular metabolism vary in their  
87 assumptions regarding species-environment interactions, and can lead to diverse results for  
88 community structure and population dynamics. In a model where species compete for essential  
89 resources, different nutrient requirements can produce intrinsically oscillatory or even chaotic  
90 dynamics (Huisman & Weissing, 1999, 2001). Alternatively, cross-feeding (Goldford et al.,  
91 2018; Pfeiffer & Bonhoeffer, 2004) can promote stable coexistence, while preferential nutrient  
92 utilization (A. Goyal, Dubinkina, & Maslov, 2018) can lead to multistability. With metabolic  
93 trade-offs, a model in which growth rate is additive in imported nutrients self-organizes to a state  
94 of unlimited stable coexistence (Posfai, Taillefumier, & Wingreen, 2017), while another model  
95 with convertible essential nutrients also allows evolutionarily stable coexistence but with a  
96 limited number of species (Taillefumier, Posfai, Meir, & Wingreen, 2017). This large variety of  
97 models and the richness of possible behaviors raises the question of unification: is there a simple  
98 framework that consolidates this diverse group of models into one easily understandable picture?

100 Continuity of the strategy space adds another layer of complexity in characterizing the “best”  
101 metabolic strategy or strategies. With infinite possibilities for allocating cellular resources, how  
102 should one pinpoint the optimal ones? Adaptive dynamics in ecological theory, also known as  
103 evolutionary invasion analysis, provides valuable guidance (Metz, 2012). This mathematical  
104 framework addresses the long-term evolution of traits in asexually reproducing populations by  
105 quantifying the fitness of each “trait” as a function of population composition. In this framework,  
106 “invasion fitness” is defined as the net-growth rate of a new variant when it is introduced into the  
107 resident population in an infinitesimally small amount, and a population allowing only non-  
108 positive invasion fitness for any new variant is considered to be “evolutionarily stable” (Doebeli,  
109 2002; Ispolatov, Madhok, & Doebeli, 2016; Rueffler, Van Dooren, & Metz, 2004). Such an  
110 evolutionarily stable point is valuable for defining optimal strategies, as a community adopting  
111 the most suitable metabolic strategies should not be invasible by any other strategies. As  
112 microbes in nature frequently experience environmental fluctuations, it is important to  
113 understand whether and how such “optimal metabolic strategies” change with external  
114 conditions. Nevertheless, in the standard modeling framework of adaptive dynamics, the  
115 environment is implicit, and species directly act on each other without the realistic constraints  
116 imposed by competition for resources. Combining the concept of invasion fitness with explicit

118 competition for resources, under the assumption of metabolic trade-offs, could therefore bring  
119 new insights into microbial metabolic strategies and community assemblies.

120  
121 In this work, we present a mathematical framework for analyzing competition for resources  
122 among various metabolic strategies in a chemostat setting. We combine and extend the graphical  
123 tools from resource-competition theory and the invasion-fitness approach from adaptive  
124 dynamics to relate and unify multiple models for microbial metabolic trade-offs. This  
125 combination provides an intuitive scheme to evaluate strategies under various external  
126 conditions. The center of the framework is the role of species in creating their own environment.  
127 Firstly, the chemical environment shaped by an endogenous species through growth and  
128 consumption can be inviting or prohibiting to an invader species, depending on the geometric  
129 relationship between the “zero net growth surface” of the invader and the environment created by  
130 the endogenous species. This geometry-dependent fitness leads to a general “rule of invasion” to  
131 compare pairs of strategies. In evaluating a continuum of strategies, the ensemble of their  
132 invasion fitnesses form a landscape, whose shape depends on the chemical environment. This  
133 intrinsically dynamic fitness landscape allows for the intransitivity of fitness (Soliveres et al.,  
134 2015). We demonstrate how such intransitivity can lead to rich ecosystem dynamics, including  
135 mutual invasion, multistability, and oscillations, and how all of these behaviors can be simply  
136 related via a graphical representation and the dynamic fitness landscape. Moreover, from the  
137 environment-dependent fitness landscape, we can define non-invasive/optimal metabolic  
138 strategies – namely, one or more strategies that construct a fitness landscape that places  
139 themselves on the top. The mathematical framework we present confers several advantages.  
140 Firstly, it separates the effect of changing dilution rate and of varying supply concentrations,  
141 facilitating quantitative interpretation as well as predictions for chemostat experiments.  
142 Secondly, it establishes an intuitive mapping from various metabolic models to population  
143 dynamics. Additionally, it reveals long-term implications, particularly in clarifying the general  
144 conditions for coexistence on both ecological and evolutionary time scales.  
145

146 **Results**

147 ***Metabolic trade-offs and metabolic strategies***

148 As discussed above, microorganisms need to allocate their limited internal resources into  
149 different cellular functions. In our models, we use  $\alpha_j$  to denote the fraction of internal resources  
150 allocated to the  $j$ -th metabolic function, with  $\vec{\alpha} = (\alpha_1, \alpha_2 \dots)$  representing a metabolic strategy.  
151 An exact metabolic trade-off is assumed, such that  $\sum_j \alpha_j = 1$ . All possible values of  $\vec{\alpha}$  define a  
152 continuous spectrum of metabolic strategies, which we name the strategy space. One major goal  
153 of our work is to construct a general and intuitive framework for evaluating strategies for a broad  
154 range of different metabolic models and experimental conditions.

155

156 ***Graphical representation of species creating their own chemical environment***

157 One way to evaluate metabolic strategies is by comparing the competitiveness of “species” with  
158 fixed strategies in chemostat-type resource-competition models. In an idealized model of a  
159 chemostat (Fig 1A),  $p$  types of nutrients are supplied at rate  $d$  and concentrations  $\vec{c}_{\text{supply}} =$   
160  $(c_{1, \text{supply}}, c_{2, \text{supply}}, \dots c_{p, \text{supply}})$ , meanwhile cells and medium are diluted at the same rate  $d$ . The  
161 chosen values of  $d$  and  $\vec{c}_{\text{supply}}$  constitute the “external condition” for a chemostat. Nevertheless,  
162 the chemical environment that directly impacts cells is the metabolite concentration inside the  
163 chemostat,  $\vec{c} = (c_1, c_2, \dots c_p)$ , which influences the intracellular metabolite concentrations  $\vec{q}_\sigma$  and  
164 the growth rate  $g_\sigma$  of the species  $\sigma$ . Accordingly, the biomass density  $m_\sigma$  of species  $\sigma$  adopting  
165 strategy  $\vec{\alpha}_\sigma$  in the chemostat obeys:

$$\frac{dm_\sigma}{dt} = m_\sigma \cdot (g(\vec{c}, \vec{q}_\sigma, \vec{\alpha}_\sigma) - d). \quad (1)$$

166 The concentration  $c_i$  of the  $i$ -th nutrient is a variable, influenced by its rate of consumption  $I_i$  per  
167 cell volume. In a chemostat occupied by a single species  $\sigma$ , the changing rate of  $c_i$  satisfies

$$\frac{dc_i}{dt} = d \cdot (c_{i, \text{supply}} - c_i) - m_\sigma / r \cdot I_i(\vec{c}, \vec{q}_\sigma, \vec{\alpha}_\sigma), \quad (2)$$

168 where  $r$  is a constant representing the biomass per cell volume. (If the volume of the chemostat is  
169  $V_{\text{chemostat}}$  and the total volume of cells is  $V_{\text{cells}}$ , the import flux of the  $i$ -th nutrient  $V_{\text{cells}} \cdot I_i$   
170 implies a rate of change of concentration inside cells of  $I_i$  and a corresponding rate of change of  
171 the concentration in the chemostat of  $V_{\text{cells}} / V_{\text{chemostat}} \cdot I_i = (m \cdot V_{\text{chemostat}} / r) / V_{\text{chemostat}} \cdot$   
172  $I_i = m / r \cdot I_i$ .) A negative value of  $I_i$  corresponds to secretion of the metabolite from cells into  
173 the environment.

174

175 In this manuscript, we define  $\vec{c}$  as the “chemical environment”, and all possible values of  $\vec{c}$   
176 constitute the “chemical space”. Within a cell, the concentration of metabolites is influenced by  
177 uptake/secretion rates, and influences the growth rate. Different metabolic models assume  
178 different forms for such influences, and we use  $\vec{f}(\vec{I}(\vec{c}, \vec{q}), \vec{q})$  to represent the rate of change in  $\vec{q}$ :

$$\frac{d\vec{q}_\sigma}{dt} = \vec{f}(\vec{I}(\vec{c}, \vec{q}_\sigma, \vec{\alpha}_\sigma), \vec{q}_\sigma). \quad (3)$$

179 Eqs. (1)-(3) represent a general chemostat model with a single species. The simplicity of the  
180 chemostat has inspired many theoretical studies of resource competition. Different model  
181 assumptions about how species grow and consume nutrients have produced a variety of  
182 intriguing behaviors and conclusions. However, the origins of these differences are not always  
183 simple to discern. To facilitate the evaluation of metabolic strategies, we next present a graphical

184 representation that allows ready visualization of the feedback between species and the  
185 environment in a chemostat.  
186  
187 The steady-state environment created by species  $\sigma$  can be obtained by setting Eqs. (1)-(3) to  
188 zero. First, when Eq. (3) is equal to zero,  $\vec{q}_\sigma$  can be solved as a function of  $\vec{c}$ , reducing  
189  $g(\vec{c}, \vec{q}_\sigma, \vec{\alpha}_\sigma)$  and  $I_i(\vec{c}, \vec{q}_\sigma, \vec{\alpha}_\sigma)$  to functions fully dependent on the variable  $\vec{c}$ , namely  $g(\vec{c}, \vec{\alpha}_\sigma)$  and  
190  $I_i(\vec{c}, \vec{\alpha}_\sigma)$ . Graphically, the steady state created by a single species can be visualized by the  
191 intersection of two nullclines, derived from Eq. (1) and Eq. (2), respectively (details in Methods).  
192  
193 Next, setting Eq. (1) to zero leads to a  $p$ -dimensional version of the ZNGI, which we name the  
194 “*growth contour*”. For a given metabolic strategy  $\vec{\alpha}_\sigma$ , the growth-rate function  $g(\vec{c}, \vec{\alpha}_\sigma)$  maps  
195 different points in the chemical space onto varying growth rates (background color in Fig 1B). At  
196 steady state, the relation  $dm_\sigma/dt = 0$  (Eq. (1)) requires the growth rate to be exactly equal to  
197 the dilution rate (assuming nonzero cell density). Therefore, the contour in chemical space  
198 satisfying  $g(\vec{c}, \vec{\alpha}_\sigma) = d$  indicates all possible environments that could support a steady state of  
199 the strategy  $\vec{\alpha}_\sigma$  (red curve in Fig 1B). This contour reflects how the chemical environment  
200 determines cell growth.  
201  
202 Thirdly, the nullcline derived from Eq. (2) reflects the impact of cellular metabolism on the  
203 chemical environment. At steady state, the nutrient influx should be equal to the summation of  
204 dilution and cellular consumption. When Eq. (2) is set to zero, varying values of cell density  $m$   
205 lead to different  $\vec{c}$  (Eq. (S5)), constituting a one-dimensional “*flux-balance curve*” in chemical  
206 space (purple, cyan, and blue curves in Fig 1B).  
207  
208 Finally, the steady-state chemical environment  $\vec{c}_{\sigma,ss}$  created by the species  $\sigma$  is the intersection of  
209 the growth contour and the flux-balance curve (Fig 1B, red dot). Changes in the chosen  
210 conditions  $d$  and  $\vec{c}_{\text{supply}}$  influence the shapes of the growth contour and the flux-balance curve  
211 separately, enabling clear interpretation of chemostat experiments under varied conditions  
212 (details in Methods). Sometimes for a given steady-state environment  $\vec{c}_{\sigma,ss}$ , one would like to  
213 derive the supply concentrations that ultimately lead to this environment. Setting Eq. (2) to zero  
214 and fixing  $\vec{c}_{\sigma,ss}$ , varying values of cell density  $m$  lead to a straight line in the space of supply  
215 concentrations, which we call the “*supply line*” (see Methods for details). Despite the fact that  
216 the supply space and the chemical space are distinct, they share the same units of concentration  
217 in each dimension. Therefore, for ease of visualization we typically show supply lines along with  
218 other features in the same nutrient space (Eq. (S6), black dashed line in Fig S1).  
219  
220 **Rule of invasion, and the environment-dependent fitness landscape**  
221 We use the outcome of competition between species to evaluate metabolic strategies, assuming  
222 each species  $\sigma$  adopts a fixed strategy  $\vec{\alpha}_\sigma$ . We first focus on the outcome of invasion, which  
223 corresponds to the “*invasion fitness*” in adaptive dynamics: here, “*invasion*” is defined as the  
224 introduction of a very small number of an “*invader*” species to a steady-state chemostat already  
225 occupied by a set of “*indigenous*” species, and the “*invasion growth rate*” is quantified by the  
226 growth rate of the invader species at the moment of introduction. Unlike adaptive dynamics, the  
227 invasion growth rate is evaluated with respect to the chemical environment created by the  
228 indigenous species, rather than with respect to the population composition of the indigenous  
229 species.

230  
231 In the chemical space, the outcome of an invasion can be summed up by a simple geometric rule,  
232 as demonstrated in Fig 1C and D. The growth contour of the invader (species *Red*) separates the  
233 chemical space into two regions: an “invasion zone” where the invader grows faster than dilution  
234 (green-colored region in Fig 1C and D), and “no-invasion zone” where the invader has a growth  
235 rate lower than dilution. If the steady-state environment constructed by the indigenous species  
236 (species *Blue*) is located within the invasion zone of the invader, the invader will initially grow  
237 faster than dilution. Therefore, the invader will expand its population and the invasion will be  
238 successful (Fig 1C). By contrast, if the steady-state chemical environment created by the  
239 indigenous species lies outside of the invasion zone, the invasion will be unsuccessful (Fig 1D,  
240 same species as in Fig 1C but with a different supply condition, and therefore a different steady  
241 state). (See Methods for details.)  
242  
243 In the steady-state environment created by the indigenous species, each strategy  $\vec{\alpha}$  has an  
244 invasion growth rate. We define an environment-dependent “fitness landscape” as the relation  
245 between the invasion growth rate and the metabolic strategy of invaders (Eq. (S8)-(S9), see  
246 Methods for details). Different indigenous species can create different chemical environments,  
247 and thus give rise to different shapes of the fitness landscapes.  
248  
249 ***Mutual invasion, a flat fitness landscape, and unlimited coexistence***  
250 The rule of invasion allows for easy assessment of competition dynamics. The emergence of  
251 complex dynamics generally requires that competitiveness be non-transitive. For example, if  
252 species *Red* can invade species *Blue*, that does not mean *Blue* cannot invade *Red*. Such mutual  
253 invasibility can be observed in substitutable-resource metabolic models, with a simple version  
254 illustrated in Fig 2A: two substitutable nutrients *a* and *b*, such as glucose and galactose,  
255 contribute linearly to biomass increase. Since a substantial investment of protein and energy is  
256 required for nutrient uptake, the model assumes a trade-off between the allocation of internal  
257 resources to import either nutrient. Specifically, a fraction of resources  $\alpha_a$  is allocated to  
258 import *a* and a fraction  $(1 - \alpha_a)$  to import *b*. As shown in Fig 2B, while the steady-state  
259 environment created by *Blue* is located within the invasion zone of *Red*, the steady-state  
260 environment created by *Red* is also located within the invasion zone of *Blue*. According to the  
261 rule of invasion, each species can therefore invade the steady-state environment created by the  
262 other. In the face of such successful invasions, the only possible stable chemical environment for  
263 this system is at the intersection of two growth contours, where the two species can coexist.  
264  
265 The environment-dependent fitness landscape readily explains this coexistence: In the steady-  
266 state environment created by *Red* ( $\alpha_a = 0.6$ ), strategies with smaller  $\alpha_a$  have higher fitness (Fig  
267 2C, upper panel). In the steady-state environment created by *Blue* ( $\alpha_a = 0.2$ ), the fitness  
268 landscape changes in shape, and strategies with larger  $\alpha_a$  have higher fitness (Fig 2C, middle  
269 panel). Therefore, each species creates an environment that is more suitable for its competitor,  
270 which leads to coexistence.  
271  
272 Typically in resource-competition models, the number of coexisting species cannot exceed the  
273 number of resources (Armstrong & McGehee, 1980; Hardin, 1960; Levin, 1970; McGehee &  
274 Armstrong, 1977). This conclusion can be understood intuitively from a graphical approach: The  
275 steady-state growth of a species can only be achieved on the growth contour of this species. The

276 stable-coexistence of  $N$  species can thus only occur at the intersection of their  $N$  corresponding  
277 growth contours. Generally, an  $N$ -dimensional chemical space allows a unique intersection of no  
278 more than  $N$  surfaces, and the diversity of species is therefore bounded by the number of  
279 metabolites in the environment. This theoretical restriction on biodiversity, made formal as the  
280 “competitive exclusion principle”, contradicts the tremendous biodiversity manifested in the real  
281 world (Friedman, Higgins, & Gore, 2017; Goldford et al., 2018; Maharjan, Seeto, Notley-  
282 McRobb, & Ferenci, 2006). There have been a multitude of theoretical efforts to reconcile this  
283 contradiction (Huisman & Weissing, 1999, 2001)(Goldford et al., 2018; Pfeiffer & Bonhoeffer,  
284 2004)(Beardmore, Gudelj, Lipson, & Hurst, 2011; Taillefumier et al., 2017).

285  
286 The mutual invasibility in linear-summation metabolic models with trade-offs presents one  
287 possibility resolution of the competitive-exclusion paradox. For the environment co-created by  
288 *Blue* and *Red* (Fig 2B, purple dot), the fitness landscape becomes flat (Fig 2C, bottom panel):  
289 species with any metabolic strategy will grow at the same rate as dilution in this environment.  
290 Therefore, in this system, once a pair of species with a mutual-invasion relationship construct the  
291 chemical environment together, all species become effectively neutral and can coexist  
292 indefinitely (see Methods for details). This graphical approach to mutual invasion and the flat  
293 fitness landscape provide an intuitive representation of species self-organizing to a state of  
294 unlimited coexistence beyond competitive exclusion.

295  
296 ***Rock-paper-scissor invasion loop and oscillation***  
297 Resource-competition models focusing on various aspects of cellular metabolism vary in their  
298 assumptions regarding  $g(\vec{c}, \vec{x}, \vec{\alpha})$ ,  $I(\vec{c}, \vec{x}, \vec{\alpha})$ , and  $f(\vec{c}, \vec{x}, \vec{\alpha})$ , and can lead to diverse results for  
299 community structure and coexistence. However, the above general “rule of invasion” allows us  
300 to treat these divergent resource-competition models in a unified framework. In the following  
301 example, we utilized a metabolic model slightly different from that of Fig 2, to show that a  
302 dynamic fitness landscape is indispensable for coexistence. In the metabolic model shown in Fig  
303 3A, three substitutable nutrients,  $a$ ,  $b$ , and  $c$ , contribute additively to cell growth. In this three-  
304 dimensional chemical space, the growth contour for each species is a two-dimensional surface  
305 (Fig 3B). In addition to requiring enzymes to import the raw forms of these nutrients as in the  
306 model of Fig 2A, enzymes are also required to convert the imported raw materials into biomass.  
307 In this model, a six-element  $\vec{\alpha}$  is required to describe the metabolic strategy, and there is the  
308 possibility of “mismatches” in the fraction of internal resources allocated to import and to  
309 convert the same nutrient. Such mismatches can produce a “rock-paper-scissor” invasion loop  
310 (Fig S3A): In the environment created by species 1, species 2 has a higher fitness but not species  
311 3; therefore species 2 can invade species 1 and establish its own environment; however, this  
312 environment lies within the invasion zone of species 3 (Fig 3B) but not of species 1, therefore  
313 species 3 subsequently invades; then in turn, species 3 create an environment where species 1 has  
314 the highest fitness. Such a loop of invasions leads to oscillatory population dynamics (Fig 3C,  
315 upper panel), with an ever-changing fitness landscape (Fig 3C, lower panel).

316  
317 Oscillation and even chaos in resource-competition model have been demonstrated by Huisman  
318 et al. (Huisman, van Oostveen, & Weissing, 1999), and shown to allow dynamical coexistence  
319 beyond competitive exclusion. The simple model presented here illustrates how oscillation can  
320 be understood as a loop of invasion creating an ever-changing fitness landscape.

321

322 **Multistability and chain of invasion**

323 When species create environments that are more favorable for their competitors, mutual-invasion  
324 and oscillations can occur. Can species create environments that are hostile to their competitors,  
325 and if so what will be the consequences?

326  
327 Figure 4A shows a simple metabolic model with two essential nutrients  $a$  and  $b$ , such as nitrogen  
328 and phosphorus (see Methods for details). Similar to the model in Fig 2A, the model assumes a  
329 trade-off between the allocation of internal resources to import nutrients, so that a resource  
330 allocation strategy is fully characterized by the fraction of resources  $\alpha_a$  allocated to import  
331 nutrient  $a$ . The growth rate is taken to be the minimum of the two input rates (Odum & Barrett,  
332 1971). As shown in Fig 4B, two species, *Red* and *Blue*, each creates a chemical environment  
333 outside of the invasion zone of each other. According to the rule of invasion, neither can be  
334 invaded by the other. Therefore, the steady state of the community depends on initial conditions  
335 – whichever species occupies the chemostat first will dominate indefinitely. It is worth noting  
336 that despite the fact that coexistence is excluded in a single chemostat under this metabolic  
337 model, the ability of species to create a self-favoring environment allows the spontaneous  
338 emergence of spatial heterogeneity and coexistence in an extended system with multiple linked  
339 chemostats (Fig S6). In ecology, the spontaneously emergence of spatial heterogeneity has been  
340 shown for species with the capacity to construct their own niches (Levin, 1970; Schertzer,  
341 Staver, & Levin, 2015), and the chain of chemostats provides a simple model for such spatial  
342 coexistence.

343  
344 From the perspective of the strategy-growth relationship (Fig 4B, inset), species *Red* ( $\alpha_a = 0.65$ )  
345 creates a fitness landscape where small  $\alpha_a$  is disfavored. Symmetrically, species *Blue* ( $\alpha_a =$   
346  $0.35$ ) creates a fitness landscape where large  $\alpha_a$  is disfavored. However, neither *Red* nor *Blue*  
347 sits on the top of the fitness landscape each one creates (Fig 4C). In the fitness landscape created  
348 by *Blue*, a slightly larger  $\alpha_a$  (green diamond in Fig 4C) has the highest growth rate.  
349 Consequently, species adopting the *Green* strategy can invade *Blue*. Nevertheless, species *Green*  
350 is not on the top of its own fitness landscape as an even larger  $\alpha_a$  (yellow diamond in Fig 4C)  
351 maximizes the growth rate in the environment created by *Green*. A series of replacements by the  
352 fastest-growing species in the environment created by the former species creates a chain of  
353 invasion.

354  
355 In this particular model after four steps of replacement, bistability appears. The species with  $\alpha_a$   
356 marked by *Deep Purple*, which is reached by the chain of invasion going from *Blue*, to *Green*, to  
357 *Yellow*, to *Deep Green*, cannot invade the original species *Blue*. A similar relationship holds  
358 between *Cyan* and *Red*. This phenomenon highlights the difference between ecological stability  
359 and evolutionary stability: Ecologically, as both *Blue* and *Deep Purple* create a fitness landscape  
360 where the other species grows slower than dilution, they constitute a bistable system. However,  
361 evolutionarily, “mutants” with slightly larger  $\alpha_a$  can invade *Blue*, bringing the system towards  
362 *Deep Purple* until bistability collapses.

363  
364 **Non-invasive strategies**

365 In this model, with symmetric parameters, the only evolutionarily stable strategy is  $\alpha_a = 0.5$   
366 (black diamond in Fig 4C). This is the only strategy that locates itself on the top of the fitness  
367 landscape it creates, and therefore cannot be invaded by any other species. This simple model

368 demonstrates a general definition of optimal (aka evolutionarily stable or non-invasive)  
369 strategies: those strategies that create a fitness landscape which places themselves on the top (Eq.  
370 (S10)).

371  
372 A chemical environment defines a fitness landscape, and the steady-state chemical environment  
373 created by species is influenced by supply condition, dilution rate, and the details of cell  
374 metabolism. Therefore, different chemostat parameters and different metabolic models lead to  
375 different optimal strategies. In the following, we described a generally applicable protocol for  
376 obtaining the non-invasive strategies, using the metabolic model in Fig 4A as the example (Fig  
377 4D, details in Methods): First, under a chemical environment  $\vec{c}$ , the maximal growth rate  
378  $g_{\max}(\vec{c})$  (background color in Fig 4D) and the corresponding resource allocation strategy  
379  $\vec{\alpha}_{\max}(\vec{c})$  can be obtained analytically or via numerical search through the strategy space (Eq.  
380 (S11)).  $g_{\max}(\vec{c})$  and  $\vec{\alpha}_{\max}(\vec{c})$  are independent of the chemostat parameters  $\vec{c}_{\text{supply}}$  and  $d$ .  
381 Second, the maximal growth contour for dilution rate  $d$  is defined as all chemical environments  $\vec{c}$   
382 that support a maximal growth rate of  $d$  (Eq. (S12)). Different maximizing strategies  $\vec{\alpha}_{\max}(\vec{c})$   
383 exist at different points of the maximal growth contour, as shown by the colors of the curve in  
384 Fig 4D. By definition, the maximal growth contour envelops the growth contour of any single  
385 strategy, and chemical environments on the maximal growth contour are outside of the invasion  
386 zone of any strategy. Therefore, if a species is able to create a steady-state environment on the  
387 maximal growth contour, it cannot be invaded. Finally, different  $\vec{c}_{\text{supply}}$  form different maximal  
388 flux-balance curves (Eq. (S14)), which intersect with the maximal growth contour at one point  
389  $\vec{c}_{\text{opt}}$ . Species  $\vec{\alpha}_{\max}(\vec{c}_{\text{opt}})$  that adopt the maximizing strategy at  $\vec{c}_{\text{opt}}$  create the environment  $\vec{c}_{\text{opt}}$ ,  
390 and are therefore immune to invasion. Under different  $\vec{c}_{\text{supply}}$ , different species become non-  
391 invasible (orange, green, and blue growth contours in Fig 4D), and the supply lines emanating  
392 from different points on the maximal growth contour indicate the supply conditions for which the  
393 corresponding strategies are optimal.

394  
395 ***Evolutionarily stable coexistence***  
396 Given  $d$  and  $\vec{c}_{\text{supply}}$ , the maximal growth contour and the maximal flux-balance curve are  
397 unique, therefore there is only one  $\vec{c}_{\text{opt}}$ . Does the uniqueness of  $\vec{c}_{\text{opt}}$  imply a single  
398 evolutionarily stable species? Or is coexistence still possible even in the face of evolution? In a  
399 recent work (Taillefumier et al., 2017), this question was addressed by modeling a population of  
400 microbes competing for steadily supplied resources. Through *in-silico* evolution and network  
401 analysis, the authors found that multiple species with distinct metabolic strategies can coexist as  
402 evolutionarily-stable co-optimal consortia, which no other species can invade.

403  
404 Using a simplified version of Taillefumier et al.'s model (Fig 5A), we employ the graphical  
405 approach to help identify the requirements for such evolutionarily-stable coexistence and the role  
406 of each species in supporting the consortium. In this model, at the cost of producing the  
407 necessary enzymes, cells are not only able to import external nutrients, but can also convert any  
408 one of the internal nutrients into any other. Meanwhile, nutrients passively diffuse in and out of  
409 the cell. The internal concentrations of nutrient  $a$  and nutrient  $b$  are both essential for cell growth  
410 (see Methods for detail). Therefore, metabolic trade-offs in this system have four elements: the  
411 fraction of internal resources allocated to import nutrient  $a$  ( $\alpha_a$ ) or nutrient  $b$  ( $\alpha_b$ ) and/or convert

412 one nutrient into another ( $\alpha_{ab}$  converts internal  $b$  into  $a$ , and  $\alpha_{ba}$  converts internal  $a$  into  $b$ ).  
413 Each species is defined by its internal resource allocation strategy  $\vec{\alpha} = (\alpha_a, \alpha_b, \alpha_{ab}, \alpha_{ba})$ .  
414  
415 Following the general protocol described in the previous section, we first identified the maximal  
416 growth rates  $g_{\max}(\vec{c})$  and the corresponding strategy or strategies  $\vec{\alpha}_{\max}(\vec{c})$  at each point  $\vec{c}$  in the  
417 chemical space, and generated maximal growth contours for different dilution rates (Fig 5B).  
418 The maximal growth contours are not smoothly continuous, nor are the corresponding strategies.  
419 In chemical space, three distinct sectors of maximizing strategies appear (Fig 5B, Fig S6A):  
420 When nutrient  $a$  is very low compared to  $b$ , the maximizing strategy is a “ $b$ - $a$  converter” which  
421 imports  $b$  and converts it into  $a$  (blue sector, only  $\alpha_b$  and  $\alpha_{ab}$  are non-zero). Symmetrically,  
422 when  $a$  is comparatively high, the optimal strategy is a “ $a$ - $b$  converter” (green sector, only  $\alpha_a$   
423 and  $\alpha_{ba}$  are non-zero). Otherwise, the maximizing strategy is an “importer” which imports both  
424 nutrients without conversion (red sector, only  $\alpha_a$  and  $\alpha_b$  are non-zero). On the border between  
425 sectors, the maximal growth contour has a discontinuous slope.  
426  
427 Optimal coexistence occurs at these discontinuous points. If an environment point  $\vec{c}_0$  is located  
428 in a continuous region of the maximal growth contour, only one maximizing strategy  $\vec{\alpha}_{\max}(\vec{c}_0)$   
429 exists for that environment (maximizing strategies along the maximal growth contour are  
430 indicated by colored squares in Fig 5C). Supply conditions that make  $\vec{\alpha}_{\max}(\vec{c}_0)$  the optimal  
431 strategy (i.e. allow  $\vec{\alpha}_{\max}(\vec{c}_0)$  to create the steady-state environment  $\vec{c}_0$ ) constitute the supply line  
432 for  $\vec{c}_0$  and  $\vec{\alpha}_{\max}(\vec{c}_0)$ . However, at the discontinuous points of the maximal growth contour,  
433 where two classes of strategies meet, two different strategies are both maximizing. For example,  
434 at the purple dot in Fig 5C a strategy belonging to the “ $b$ - $a$  converter” class (species *Blue*) and  
435 one belonging to the “importer” class (species *Red*) are both maximizing strategies. Each  
436 strategy derives a supply line from the purple dot (black dashed line, Fig 5C). The two supply  
437 lines span a gray region where no supply line from any single strategy enters. Correspondingly,  
438 for any supply conditions inside the gray region, no single species can alone create an  
439 environment on the maximal growth contour. For example, under the supply condition shown by  
440 the black open circle in the gray region, species *Blue* and species *Red* both create chemical  
441 environments that lie within the maximal growth contour (blue and red dots, Fig 5C), and are  
442 thus subject to invasion by other species. Nevertheless, the species-specific growth contours of  
443 *Blue* and of *Red* intersect at the purple point on the maximal growth contour. Therefore, only  
444 when *Blue* and *Red* coexist can they co-create an environment on the maximal growth contour,  
445 and thus be resistant to invasion from any other species. Indeed, when we simulate multiple  
446 species with different maximizing strategies under the supply condition indicated by the open  
447 black circle, species *Blue* and species *Red* are the only two that survive (Fig 5C, inset).  
448  
449 The optimal coexistence of species *Blue* and species *Red* can be understood intuitively from the  
450 dynamic fitness landscape. Given a chemical environment, the relation between  $\alpha_a$  and growth  
451 rate of importer (red curve) or  $a$ - $b$  converter (green curve), and that between  $\alpha_b$  and growth rate  
452 of  $b$ - $a$  converter (green curve) constitute the fitness landscape of species adopting different  
453 possible maximizing strategies (Fig 5D). In the environment created by species *Blue* (blue dot in  
454 Fig 5C), not only will some importers grow faster than *Blue*, species *Blue* (strategy marked by  
455 blue diamond) is not even on the fitness peak of its own class (Fig 5D, upper box). Similarly, in  
456 the environment created by species *Red*, the strategy of *Red* is not at the top of the fitness  
457 landscape (Fig 5D, middle box). By contrast, in the environment co-created by species *Blue* and

458 *Red* (purple dot in Fig 5C), their strategies are at the top of the fitness landscapes of their own  
459 classes and at equal height. For all supply conditions in the gray region, species *Blue* and species  
460 *Red* jointly drive the nutrient concentrations to the discontinuous point of the optimal growth  
461 contour, and thereby achieve evolutionarily stable coexistence.  
462

#### 463 ***Species creating a new nutrient dimension***

464 One possible solution to the competitive-exclusion paradox is the creation of new nutrient  
465 “dimensions” by species secreting metabolites that can be utilized by other species. For example,  
466 *E. coli* secretes acetate as a by-product of glucose metabolism. Accumulation of acetate impedes  
467 the growth of *E. coli* on glucose (Luli & Strohl, 1990), but the acetate can be utilized as a carbon  
468 source, e.g. by mutant strains that emerge in long-term evolution experiments (D'Souza et al.,  
469 2018; Rosenzweig, Sharp, Treves, & Adams, 1994).  
470

471 To explore the possibilities of evolutionarily stable coexistence when species create new  
472 nutrients, we used a simplified model to represent multi-step energy generation with a dual-role  
473 intermediate metabolite (Fig 6A). A single chemical energy source S is supplied into the  
474 chemostat. The pathway for processing S consists of four relevant reactions driven by designated  
475 enzymes: External S can be imported and converted into intermediate  $I_{int}$  to generate ATP (with a  
476 corresponding fraction of the enzyme budget  $\alpha_{ATP1}$ ). The intermediate has a dual role in energy  
477 production: on the one hand, it positively contributes to ATP production via a downstream  
478 reaction (with a fraction of the enzyme budget  $\alpha_{ATP2}$ ); on the other hand, it negatively  
479 contributes to ATP production through product inhibition of the first energy-producing reaction.  
480 To deal with this negative effect of internal intermediate, cells may synthesize transporters (with  
481 a fraction of the enzyme budget  $\alpha_{exp}$ ) to export intermediate out into environment, where it  
482 becomes external intermediate  $I_{ext}$ . By this reaction, cells can increase the dimension of chemical  
483 space from one (S) into two (S and  $I_{ext}$ ). Cells can also import  $I_{ext}$  into  $I_{int}$  (fraction of enzyme  
484 budget  $\alpha_{imp}$ ), then use  $I_{int}$  as an energy source via the second reaction. (See Methods for details.)  
485

486 The metabolic strategy  $\vec{\alpha}$  in this model has four components:  $\vec{\alpha} = (\alpha_{ATP1}, \alpha_{ATP2}, \alpha_{exp}, \alpha_{imp})$ .  
487 When we examine the maximizing strategies and maximal growth rates in the chemical space,  
488 three distinct classes of strategy emerge (Fig 6B). When S is abundant and  $I_{ext}$  is low, the  
489 maximizing strategies have only two non-zero components,  $\alpha_{ATP1}$  and  $\alpha_{exp}$  (Fig S6B), meaning  
490 this class of species only imports S, for the first energy-generating reaction, then exports  
491 intermediate as waste. Therefore, we call strategies in this class “polluters” (blue section in Fig  
492 6B, Fig S3C). When  $I_{ext}$  is high while S is low, the maximizing strategies have two different non-  
493 zero components,  $\alpha_{ATP2}$  and  $\alpha_{imp}$  (Fig S6B), meaning this class of species relies solely on  $I_{ext}$  as  
494 its energy source. We call these strategies “cleaners” as they clean up  $I_{ext}$  from the environment,  
495 (green section in Fig 6B, Fig S6C). When there are comparable amounts of S and  $I_{ext}$  present, a  
496 third class of maximizing strategies appears: these cells neither export nor import intermediates,  
497 but rather allocate all their enzyme budget to  $\alpha_{ATP1}$  and  $\alpha_{ATP2}$  to carry out both energy-  
498 producing reactions. We call species in this class “generalists” (red section in Fig 6B, Fig S6C).  
499

500 As shown in Fig 6B, on the borders between classes of strategies in chemical space, the maximal  
501 growth contours turn discontinuously. These points of discontinuity, as in the example in the  
502 previous section, are chemical environments corresponding to evolutionarily stable coexistence  
503 of species from distinct metabolic classes. The classes of optimally coexisting species change

504 with dilution rate. When the dilution rate is low ( $d = 0.4$ , Fig 6C), at the discontinuous point of  
505 the maximal growth contour, the corresponding two maximizing strategies are one polluter  
506 (species *Blue*) and one cleaner (species *Green*). Their supply lines span a gray region where both  
507 species *Blue* and species *Green* are required to create a steady-state environment on the maximal  
508 growth contour. As by assumption we are only supplying the system with S, the supply condition  
509 always lies on the x-axis of concentration space. For the supply condition shown by the black  
510 open circle in Fig 6C, polluter *Blue* creates a chemical environment (blue dot) far from the  
511 maximal growth contour. When the cleaner *Green* is added to the system, not only does the  
512 biomass of *Blue* increase (inset), but also the steady-state chemical environment moves to the  
513 discontinuous point of the maximal growth contour (cyan dot), where both *Blue* and *Green*  
514 occupy the peaks of their fitness landscapes (Fig 6D). This result is consistent with the long-term  
515 evolution experiment of *E. coli* and also intuitive: polluter *Blue* and cleaner *Green* form a  
516 mutually beneficial relationship by, respectively, providing nutrients and cleaning up waste for  
517 each other, thereby reaching an optimal cooperative coexistence.  
518

519 A quite different coexistence occurs at higher dilution rate ( $d = 0.6$ , Fig 6E). Growth contours at  
520 this dilution rate show two turning points, but neither are between the polluter and the cleaner  
521 class. One discontinuous point is between the cleaner class (green squares) and the generalist  
522 class (red squares), but the gray region spanned by the corresponding supply lines does not cover  
523 the x-axis and so does not represent an attainable coexistence when only S is supplied. The other  
524 discontinuous point is between the generalist class and the polluter class (blue squares). The gray  
525 region spanned by the supply lines of the corresponding two maximizing strategies of generalist  
526 class (species *Red*) and polluter class (species *Blue*) does cover the x-axis. Therefore, a supply  
527 condition with only S within the gray region (e.g., the black open circle) leads to the optimal  
528 coexistence of generalist *Red* and polluter *Blue* on the discontinuous point (purple dot), despite  
529 the fact that they do not directly benefit each other. Indeed, when the generalist *Red* is added to a  
530 system with polluter *Blue* and a cleaner *Green*, the cleaner *Green* goes extinct and the biomass of  
531 the polluter *Blue* decreases (inset). Nevertheless, the steady-state chemical environment is moved  
532 from a cyan dot lying inside the maximal growth contour to the purple dot lying on the maximal  
533 growth contour. In the environment of the cyan dot created by cleaner *Green* and polluter *Blue*,  
534 *Blue* is not on the top of the fitness landscape of the polluter class (Fig 6F, upper box). By  
535 contrast, for the fitness landscape created by polluter *Blue* and generalist *Red* (Fig 6F, lower  
536 box), despite being lower in biomass, *Blue* occupies the top of the landscape. Therefore, the  
537 optimal coexistence of this polluter and this generalist does not arise from direct cooperation, but  
538 rather from collaborating to defeat other competitors.

539 **DISCUSSION**

540 Evaluating microbial metabolic strategies within an ecological context is the major focus of this  
541 work. Due to the intensity of competition in the microbial world, it is accepted that natural  
542 selection has extensively shaped microorganisms' internal resource allocation strategies and the  
543 regulatory mechanisms controlling these strategies (S. Goyal et al., 2010; Liebermeister et al.,  
544 2014). Therefore, a quantitative mapping from metabolic strategies to fitness consequences can  
545 further our understanding of both regulation and evolution (Bajic & Sanchez, 2019). Many  
546 previous studies of metabolic strategies directly assumed the optimization goal of microbial  
547 metabolism to be biomass gain, with the chemical environment acting as a fixed input ({Scott,  
548 2014 #86}{Wang & Tang, 2017}) {Schuetz, 2012 #84} (Roller, Stoddard, & Schmidt,  
549 2016){Brophy, 2014 #85}, which simplifies the problem into a search for a maximum on a static  
550 "fitness landscape". However, in the natural world where metabolic strategies compete and  
551 evolve, the feedback between species and their environment produces an intrinsically dynamic  
552 fitness landscape in which the actions of one species can influence the fitness of all species  
553 (Bajic, Vila, Blount, & Sanchez, 2018). One profound example is the Great Oxygenation Event,  
554 when cyanobacteria created an oxygen-rich atmosphere (Kasting & Siefert, 2002), causing a  
555 massive extinction of anaerobic bacteria but also stimulating an explosion of biodiversity  
556 (Schirrmeyer, de Vos, Antonelli, & Bagheri, 2013). Therefore, metabolic strategies need to be  
557 assessed within an ecological context, taking into consideration not only how species respond to  
558 the environment, but also how species construct their own environment.  
559

560 For the past thirty years, researchers have been utilized various mathematical tools to study  
561 flexible fitness landscapes that change with space, time, and population composition. The  
562 prerequisite for a intrinsically dynamical fitness landscape – that the species composition  
563 influences the fitness of all species in the system – takes a particularly simple form in chemostat-  
564 type resource-competition models: Microbes shape their local environment by exchanging  
565 metabolites within a shared chemical environment, which determines the growth rate of all cells.  
566 Focusing on the chemical environment created by microbial metabolism, we exhibited a set of  
567 intuitive and general procedures for analyzing strategies within various metabolic models.  
568 Namely, we showed that to compare a set of fixed strategies, the geometric relationships between  
569 their growth contours and their steady-state chemical environments yield an immediate  
570 prediction for the outcome of competitions. In searching for optimality over the continuous  
571 family of strategies, the "maximal growth contour" envelope of all growth contours provides  
572 candidates, and the supply condition selects the non-invasive strategy or strategies from among  
573 these candidates via the flux-balance curve. Such selection of optimal strategies also supports the  
574 conclusion that having the fastest growth rate in an environment does not necessarily imply  
575 being the most competitive strategy, as this strategy may shift the environment in an unfavorable  
576 direction. To be non-invasive, strategies also need to be able to construct the environment for  
577 which they are best suited.  
578

579 From the perspective of chemostat-related experiment, this work demonstrates the subtleties of  
580 controlling nutrient limitation in chemostats. The capacity of species to shape their own  
581 environment, even in a system as simple as a chemostat, presents challenges to controlling which  
582 nutrient or nutrients are limiting. By traditional definition, if increasing a certain nutrient leads to  
583 an increase of a cell's growth rate, that nutrient is considered "limiting". However, growth rate is

584 invariant in a chemostat, being set experimentally by the dilution rate, so inferring nutrient  
585 limitation requires special attention. For example, if one sees the same cellular responses under  
586 different nutrient supplies, what can one conclude? Cells may be creating the same chemical  
587 environment out of different supply conditions (*cf.* Fig S1A), or alternatively cells may be  
588 transducing different chemical environments into the same physiological response through  
589 mechanisms such as “ratio sensing” (Escalante-Chong et al., 2015). Our graphical approach  
590 combined with direct measurements of steady-state nutrient concentrations in the chemostat can  
591 precisely define and help guide the control of nutrient limitation (Boer et al., 2010). As described  
592 above, changes in supply concentrations shift the flux-balance curve, but do not change the shape  
593 of the growth contour. Therefore, by experimentally varying the supply conditions and  
594 measuring the chemical environment created by cells, the shape of the growth contour can be  
595 obtained. The resulting slope of the growth contour provides information on nutrient limitation  
596 even in the absence of detailed knowledge about a cell’s metabolism. For example, in the  
597 nutrient *a* - nutrient *b* plane, a near-horizontal growth contour indicates *b*-limited growth while a  
598 near-vertical growth contour means *a*-limited growth, and an intermediate slope implies that the  
599 two nutrients are co-limiting.  
600

601 Our work also has implications for microbial community assembly. The competitive exclusion  
602 principle poses a long-term puzzle in ecology: since species are in constant competition in the  
603 natural world, why doesn’t the fittest species outcompete the others and become the sole  
604 survivor? Actually, on the basis of simple resource-competition models, it has been argued that  
605 the number of stably coexisting species cannot exceed the number of resources, leading to the so-  
606 called competitive exclusion principle (Armstrong & McGehee, 1980; Hardin, 1960; Levin,  
607 1970; McGehee & Armstrong, 1977). Nevertheless, tremendous biodiversity manifests in the  
608 real world, from environmental surveys to controlled lab experiments (Friedman et al., 2017;  
609 Goldford et al., 2018; Maharjan et al., 2006). Our work is not aimed at adding another solution to  
610 the paradox of the plankton; rather, our framework helps in summarizing the criteria for  
611 coexistence on both ecological and evolutionary timescales. On the ecological scale where a  
612 limited number of species with different strategies compete for resources, the intransitivity of  
613 competitiveness as a result of fitness landscape deformability is the key to coexistence. Given  
614 resource allocation trade-offs, the growth contours of any pair of strategies must intersect, clearly  
615 demonstrating why trade-offs prevent a single species from unconditional dominance, allowing  
616 various forms of intransitivity under different metabolic models. In the case of substitutable  
617 nutrients, the fitness landscape can even be made flat – allowing unlimited coexistence. When  
618 the rule of invasion allows non-transitive loops, oscillations and chaos can occur, which have  
619 been shown to allow coexistence beyond competitive exclusion (Huisman & Weissing, 1999,  
620 2001). In addition, when multiple nutrients are all essential, the ability of each species to create  
621 an environment that favors itself allows for the spontaneous emergence of spatial heterogeneity  
622 in an extended system (Fig S5). Meanwhile, on the evolutionary time scale where  
623 mutation/adaptation allows searches for the “most suitable” strategies among infinite  
624 possibilities, an ongoing threat to diversity is that selection may produce a supreme winner that  
625 takes over the habitat. With the dynamic fitness landscape and the maximal growth contour  
626 approach, we showed that the condition for evolutionarily stable coexistence is indeed more  
627 restricted, occurring only at the discontinuous points of the maximal growth contour.  
628 Nevertheless, via the species-environment feedback, a large number of supply conditions can

629 self-organize to the discontinuous point, where multiple species co-create a non-invasive  
630 environment where they jointly locate on the peak of the fitness landscape.

631  
632 Many future directions can follow this work. From the perspective of experiment, our framework  
633 can assist in analyzing and interpreting results of microbial evolution in the lab (Van den Bergh,  
634 Swings, Fauvart, & Michiels, 2018), where the continual emergence of new mutants under  
635 defined experimental conditions suggests an intrinsically dynamic fitness landscape. From the  
636 perspective of theory, we do not yet have a rigorous mathematical theorem concerning the  
637 conditions for discontinuity of the maximal growth contour, nor proof that discontinuity  
638 necessarily leads to evolutionarily stable coexistence. Theoretical developments paralleling those  
639 on the general existence of ecologically stable states (De Leenheer et al., 2006; Marsland III,  
640 Cui, & Mehta, 2019) would bring a more comprehensive understanding of evolutionarily optimal  
641 states in metabolic models. Besides, the metabolic models considered in this work are highly  
642 simplified. Going forward, more detailed and experimentally-based models can be examined  
643 using the same graphical and dynamical fitness landscape framework.

644

645

646 **METHODS**

647 Programs for this work are coded in MATLAB R2018a. A repository of all tools used to  
 648 generate the results can be found at: <https://github.com/zhiyuanli1987/Qbiotoolbox.git>

649  
 650 **Total RNA and total protein measurements**

651 The methods for total RNA and protein measurements shown in Figure S2 are described in Li et  
 652 al., 2018.

653

654 **Supplemental Table 1: Symbols**

Chemostat parameters	
$\vec{c}_{\text{supply}} = (c_{1,\text{supply}}, c_{2,\text{supply}}, \dots c_{p,\text{supply}})$	Nutrient supply. $c_{i,\text{supply}}$ is the concentration of the $i$ -th nutrient in the supply.
$d$	Dilution rate (same as supply influx rate to keep volume fixed).
Chemostat variables	
$\vec{c}_k = (c_{1,k}, c_{2,k}, \dots c_{p,k})$	Chemical environment inside the $k$ -th chemostat. $c_{i,k}$ is the concentration of the $i$ -th nutrient within the medium of the $k$ -th chemostat. All possible $\vec{c}$ constitute the “chemical space”.
$m_{\sigma,k}$	Biomass density of species $\sigma$ in the $k$ -th chemostat.
Species-specific quantities	
$\vec{\alpha}_\sigma = (\alpha_{1,\sigma}, \alpha_{2,\sigma} \dots)$	Resource allocation strategy of species $\sigma$ . $\alpha_{j,\sigma}$ is the fraction of internal resources allocated to the $j$ -th cellular function by species $\sigma$ .
$\vec{q}_\sigma$	Intracellular concentrations of growth-related metabolites for species $\sigma$ .
$g(\vec{c}, \vec{q}, \vec{\alpha})$	Growth rate as a function of $\vec{c}$ , $\vec{q}$ , and $\vec{\alpha}$ .
$I_i(\vec{c}, \vec{q}, \vec{\alpha})$	Intake rate per biomass of the $i$ -th nutrient as a function of $\vec{c}$ , $\vec{q}$ , and $\vec{\alpha}$ . $I_i(\vec{c}, \vec{q}, \vec{\alpha})$ can be negative to describe cells exporting secondary metabolites.
$r$	Biomass concentration within an average cell, taken to be a constant that is always equal to 100.
$\vec{f}(\vec{I}(\vec{c}, \vec{q}, \vec{\alpha}), \vec{q}, \vec{\alpha})$	Functions defining the changing rate of intracellular metabolite concentrations $\vec{q}$ , as a function of $\vec{c}$ , $\vec{q}$ , and $\vec{\alpha}$ .
$GC_\sigma$	Growth-rate contour of species $\sigma$ .
$FB_\sigma$	Flux-balance curve of species $\sigma$ .
$\vec{c}_{\sigma, \text{ss}}$	The steady-state environment created by one species $\sigma$ .
$SL_\sigma(\vec{c})$	The supply line for species $\sigma$ in environment $\vec{c}$ .

$\{\sigma^*\}$	A set of species stably surviving in a chemostat. The set can contain one or more species.
$\vec{c}_{\{\sigma^*\},ss}$	The steady-state environment created by a set of species $\{\sigma^*\}$ .

655

656 **Metabolic model and resource allocation strategy**

657 In modeling population dynamics in a chemostat, multiple assumptions need to be made  
 658 concerning how cells sense the environment, import nutrients, export metabolites, utilize  
 659 resources, and grow in biomass. Different assumptions result in different metabolic models.  
 660 Some metabolic models focus on trade-offs in resource allocation, as the amount of internal  
 661 resources “owned” by a cell, including proteins and energy, is limited. Cells need to allocate  
 662 these limited resources into different cellular functions, such as metabolism, gene expression,  
 663 reproduction, motility, maintenance, etc. We use  $\alpha_{j,\sigma}$  to represent the fraction of internal  
 664 resources allocated to the  $j$ -th cellular function of species  $\sigma$ , with  $\vec{\alpha}_\sigma = (\alpha_{1,\sigma}, \alpha_{2,\sigma} \dots)$   
 665 representing the resource allocation strategy of species  $\sigma$ . For simplicity, we assume each species  
 666 has a fixed resource allocation strategy.

667

668 **Dynamic equations for a single species in a chemostat**

669 In a chemostat with nutrient supply  $\vec{c}_{\text{supply}}$ , dilution rate  $d$  and a single species  $\sigma$  with fixed  
 670 strategy  $\vec{\alpha}_\sigma$  and intracellular metabolite concentration  $\vec{q}_\sigma$ , the cell biomass density  $m_\sigma$  and the  
 671 chemostat nutrient concentrations  $\vec{c}$  are generally described by the following equations:

$$\frac{dm_\sigma}{dt} = m_\sigma \cdot (g(\vec{c}, \vec{q}_\sigma, \vec{\alpha}_\sigma) - d), \quad (\text{S1})$$

$$\frac{d\vec{c}}{dt} = d \cdot (\vec{c}_{\text{supply}} - \vec{c}) - m_\sigma / r \cdot \vec{I}(\vec{c}, \vec{q}_\sigma, \vec{\alpha}_\sigma). \quad (\text{S2})$$

672 In considering the details of cellular metabolism, one may choose to incorporate the dynamics of  
 673 intracellular metabolites that originate from nutrient import and influence cell growth. We make  
 674 the assumption that the biomass concentration  $r$ , e.g. protein concentration, is constant for cells  
 675 under all growth conditions. Thus, an increase of total cell mass induces a corresponding  
 676 increase of total cell volume.  $m_\sigma$  is the cell mass per element of volume in the chemostat, and  $r$   
 677 is the cell mass per element of volume within a cell. For a chemostat-to-cell flux of mass  $J$ , the  
 678 concentration of the metabolite in the chemostat decrease by  $J/V_{\text{chemostat}}$  while the  
 679 concentration in cell increase by  $J/V_{\text{cell}}$ . As a result, the metabolites imported into cells are  
 680 enriched by a factor of  $r$ , and metabolites secreted by cells are diluted by  $1/r$ . Also, all  
 681 intracellular metabolites are diluted by cellular growth, which is generally a slow process  
 682 compared to metabolic reactions and can be ignored in most cases. We use a function  
 683  $\vec{f}(\vec{I}(\vec{c}, \vec{q}, \vec{\alpha}), \vec{q}, \vec{\alpha})$  to represent the rate of change of intracellular metabolite concentration  $\vec{q}_\sigma$ :

$$\frac{d\vec{q}_\sigma}{dt} = \vec{f}(\vec{I}(\vec{c}, \vec{q}_\sigma, \vec{\alpha}_\sigma), \vec{q}_\sigma, \vec{\alpha}_\sigma). \quad (\text{S3})$$

684 Eq. (S2) represents  $p$  equations for  $p$  types of nutrients, and Eq. (S3) represents  $h$  equations for  
 685  $h$  growth-related intracellular metabolites.

686

687 **Species-specific steady state**

688 In the steady state of a chemostat, Eqs. (S1)- (S3) will all be equal to zero.

689 For intracellular metabolites, as  $\vec{f}(\vec{I}(\vec{c}, \vec{q}_\sigma, \vec{\alpha}_\sigma), \vec{q}_\sigma, \vec{\alpha}_\sigma) = 0$  as a result of Eq. (S3) being equal to  
 690 zero, given an environment  $\vec{c}$ , the steady state of  $\vec{q}_\sigma$  can be expressed as a function of  $\vec{c}$ :  $\vec{q}_\sigma^* =$   
 691  $\vec{f}^{-1}(\vec{c}, \vec{\alpha}_\sigma)$ .

692 Growth contour (GC): From the perspective of the environment influencing species, at each  
 693 constant environment, the steady-state growth rate is fully determined by  $\vec{c}$ :  $g^*(\vec{c}, \vec{q}_\sigma) =$   
 694  $g(\vec{c}, \vec{f}^{-1}(\vec{c}, \vec{\alpha}_\sigma))$ . If the biomass of a species is non-zero ( $m \neq 0$ ), Eq. (S1) requires  $g^* = d$ . In  
 695 the  $p$ -dimensional chemical space, this requirement defines a  $(p - 1)$ -dimensional surface,  
 696 constituted by all environments  $\vec{c}$  that support an equal-to-dilution growth rate. This surface  
 697 reduces to the zero-growth isoclines in contemporary niche theory when the chemical space is  
 698 two-dimensional and the growth rate  $g$  solely relies on  $\vec{c}$  monotonically, but is not necessarily  
 699 limited by the nutrient dimension or the form of the growth function. For convenience, we name  
 700 this surface the “growth contour” (GC) for species  $\sigma$ :

$$GC_\sigma := \{\vec{c} \mid g(\vec{c}, \vec{f}^{-1}(\vec{c}, \vec{\alpha}_\sigma), \vec{\alpha}_\sigma) = d\}. \quad (S4)$$

701 An example of growth contours is shown in Fig 1D.

702  
 703 Flux-balance curve (FB): Eq. (S2) describes how species act on the environment. In steady state,  
 704 the influx, out-flux, and consumption by species should be balanced for each nutrient, which  
 705 enables calculation of the biomass density-to-dilution ratio for every  $i$ :  $\frac{m_\sigma}{d \cdot r} = \frac{c_{i, \text{supply}} - c_i}{I_i(\vec{c}, \vec{f}^{-1}(\vec{c}, \vec{\alpha}_\sigma), \vec{\alpha}_\sigma)}$ .

706 For a  $p$ -dimensional chemical space, there are  $p$  equations for the same value of  $\frac{m}{d \cdot r}$ . This leads to  
 707 a one-dimensional curve in the chemical space, which we name the “flux-balance curve” (FB),  
 708 defined as:

$$FB := \{\vec{c} \mid \frac{c_{i, \text{supply}} - c_i}{I_i(\vec{c}, \vec{f}^{-1}(\vec{c}, \vec{\alpha}_\sigma), \vec{\alpha}_\sigma)} = \frac{m_\sigma}{d \cdot r} \text{ AND } c_i < c_{i, \text{supply}}\}. \quad (S5)$$

709 For example, for two nutrients  $a$  and  $b$ , the flux balance curve is:  $\frac{c_{a, \text{supply}} - c_a}{I_a(\vec{c}, \vec{f}^{-1}(\vec{c}, \vec{\alpha}_\sigma), \vec{\alpha}_\sigma)} -$   
 710  $\frac{c_{a, \text{supply}} - c_a}{I_a(\vec{c}, \vec{f}^{-1}(\vec{c}, \vec{\alpha}_\sigma), \vec{\alpha}_\sigma)} = 0$ , as demonstrated in Fig 1B and Figure S1.

711 In chemical space, the steady-state environment  $(\vec{c}_{\sigma, \text{ss}})$  with non-zero biomass of species  $\sigma$  will  
 712 be located at the intersection of the growth contour and the flux-balance curve. This environment  
 713 is constructed by the species  $\sigma$  via its consumption of nutrients. If  $\vec{c}_{\sigma, \text{ss}}$  exists, this species can  
 714 survive in the chemostat. Otherwise, this species will be washed out by dilution even without  
 715 competition from other species. For the following discussion, we only consider species that can  
 716 survive when alone in a chemostat.

717  
 718 Supply line (SL): The flux-balance curve is determined by the supply condition  $\vec{c}_{\text{supply}}$ . In many  
 719 cases, it is helpful to derive the supply conditions that enable a species  $\sigma$  to construct a steady-  
 720 state environment  $\vec{c}_{\sigma, \text{ss}}$ . All possible values of  $\vec{c}_{\text{supply}}$  that can produce a given  $\vec{c}_{\sigma, \text{ss}}$ , form a  
 721 straight line in the space of supply concentrations, described by:

$$SL := \{\vec{c}_{\text{supply}} \mid \vec{c}_{\text{supply}} = \frac{m_\sigma}{d \cdot r} \cdot \vec{I}(\vec{c}_{\sigma, \text{ss}}, \vec{f}^{-1}(\vec{c}_{\sigma, \text{ss}}, \vec{\alpha}_\sigma), \vec{\alpha}_\sigma) + \vec{c}_{\sigma, \text{ss}}\}, \quad (S6)$$

722 with varying non-negative values of  $m_\sigma/d$ . An example of a supply line is shown in  
 723 Fig S1A.

724

725 **Dynamic equations for multiple species in a chemostat**

726 In nutrient competition models, multiple species ( $\sigma = 1 \dots n$ ) each with biomass density  $m_\sigma$   
 727 compete for resources. They have species-specific growth rates  $g(\vec{c}, \vec{q}_\sigma, \vec{\alpha}_\sigma)$  and import rates  
 728  $\vec{I}(\vec{c}, \vec{q}_\sigma, \vec{\alpha}_\sigma)$ , yet all experience the same chemical environment  $\vec{c}$ . Therefore, Eq. (S1) and Eq.  
 729 (S3) remain the same for each species, while the rate of change of chemostat nutrient  
 730 concentrations is influenced by the summed action of all species:

$$\frac{d\vec{c}}{dt} = d \cdot (\vec{c}_{\text{supply}} - \vec{c}) - \sum_{\sigma=1}^n m_\sigma / r \cdot \vec{I}(\vec{c}, \vec{q}_\sigma, \vec{\alpha}_\sigma). \quad (\text{S7})$$

731  
 732 **Multiple species steady state**  
 733 Multiple species, even if each alone can survive in chemostat, do not generally coexist when  
 734 competing together. For a system starting with  $n$  different species, the stable steady state  
 735 contains  $n^*$  ( $1 \leq n^* \leq n$ ) species with non-zero biomass. We define these  $n^*$  surviving species  
 736 as a stable species set  $\{\sigma^*\}$ , and mark the steady-state environment created by this set as  $\vec{c}_{\{\sigma^*\},\text{ss}}$ .  
 737 If  $n > 1$ , according to Eq. (S1),  $\vec{c}_{\{\sigma^*\},\text{ss}}$  must be located at the common intersection of growth  
 738 contours formed by every species in  $\{\sigma^*\}$ .  
 739

740 **Invasion**  
 741 Invasion is defined as introducing a small number of invaders (with biomass density  $m_{\text{inv}}$ ) to a  
 742 steady-state chemostat occupied by a set of local species. At the time of introduction, if the  
 743 invader can increase in biomass ( $\frac{dm_{\text{inv}}}{dt} > 0$ ), the invasion is successful; otherwise if the invader  
 744 decreases in biomass ( $\frac{dm_{\text{inv}}}{dt} < 0$ ), the invasion is unsuccessful. If the biomass stays constant  
 745 ( $\frac{dm_{\text{inv}}}{dt} = 0$ ), the species is neutral with respect to the local species.

746 In evaluating invasion by a species  $\sigma$  with strategy  $\vec{\alpha}_\sigma$  of any environment  $\vec{c}$ , we make two  
 747 assumptions:  
 748 1. The biomass of the invader is so small that it does not disturb the environment at the time of  
 749 introduction.  
 750 2. There is a separation of timescales such that the concentrations of intracellular metabolites  
 751 reach equilibrium instantaneously at the time of introduction of the invader, therefore Eq. (S3) is  
 752 always equal to zero and  $\vec{q}_\sigma = \vec{f}^{-1}(\vec{c}, \vec{\alpha}_\sigma)$  holds.  
 753 Therefore, we define the “invasion growth rate” of a species  $\sigma$  with strategy  $\vec{\alpha}_\sigma$  introduced into  
 754 environment  $\vec{c}$  as:

$$g_{\text{inv}}(\vec{\alpha} | \vec{c}) = g(\vec{c}, \vec{f}^{-1}(\vec{c}, \vec{\alpha}), \vec{\alpha}). \quad (\text{S8})$$

755 **Invasion zone:** By definition, the growth contour of the invader  $GC_{\text{inv}}$  divides the chemical space  
 756 into two regions: an “invasion zone” that includes all environments where the invader has an  
 757 invasion growth rate higher than dilution, and “no-invasion zone” where the invader has an  
 758 invasion growth rate lower than dilution. If the steady-state environment constructed by local  
 759 species  $\vec{c}_{\{\text{local}\},\text{ss}}$  is located within the invasion zone of the invader,  $g_{\text{inv}}(\vec{\alpha}_{\text{inv}} | \vec{c}_{\{\text{local}\},\text{ss}}) > d$ ,  
 760 therefore  $\frac{dm_{\text{inv}}}{dt} > 0$  by Eq. (S1), and the invasion is successful; otherwise, if  $\vec{c}_{\{\text{local}\},\text{ss}}$  is located  
 761 outside of the invasion zone of the invader,  $g_{\text{inv}}(\vec{\alpha}_{\text{inv}} | \vec{c}_{\{\text{local}\},\text{ss}}) < d$ , and the invasion is  
 762 unsuccessful. If  $\vec{c}_{\{\text{local}\},\text{ss}}$  locate exactly on the growth contour, it is neutral.  
 763 Two examples of this rule of invasion are presented in Fig 1C and 1D.

764 If the growth rate monotonically increases with the concentration of each nutrient, it can be  
 765 proven that the invasion zone is always above the growth contour of the invader (an environment  
 766  $\vec{c}_+$  “above” the growth contour  $GC_{\text{inv}}$  is defined as  $\exists \vec{c}_0 \in GC_{\text{inv}}, \text{s.t. } c_{i,+} \geq c_{i,0} \forall i$ ). If the  
 767 growth rate is not monotonically increasing with nutrient concentrations, identifying the invasion  
 768 zone requires more model-specific analysis.  
 769

770 ***Fitness landscape***

771 We quantified the fitness landscapes in the chemostat via the relationship between metabolic  
 772 strategies  $\vec{\alpha}$  and the invasion growth rates of an invader adopting strategy  $\vec{\alpha}$  in a given chemical  
 773 environment  $\vec{c}$ . Specifically,

774

$$\text{Fitness landscape} := g_{\text{inv}}(\vec{\alpha}|\vec{c}). \quad (\text{S9})$$

775 Each environment  $\vec{c}$  defines a fitness landscape. A set of species  $\{\sigma^*\}$  constructs a steady-state  
 776 environment  $\vec{c}_{\{\sigma^*\},\text{ss}}$  and a corresponding fitness landscape  $g_{\text{inv}}(\vec{\alpha}|\vec{c}_{\{\sigma\},\text{ss}})$ . Some examples of  
 777 fitness landscapes are shown in Figs 2C, 3C, 4B-C, 5D and 6D-F.  
 778

779 ***Non-invasive /optimal/ evolutionarily stable strategies***

780 A set of species  $\{\sigma^*\}_{\text{opt}}$  is non-invasive, aka optimal or evolutionarily stable, if no other species  
 781 can invade the steady-state environment constructed by  $\{\sigma^*\}_{\text{opt}}$ :

$$g_{\text{inv}}\left(\vec{\alpha}_\sigma|\vec{c}_{\{\sigma^*\}_{\text{opt},\text{ss}}}\right) < d, \forall \sigma \notin \{\sigma^*\}_{\text{opt}}. \quad (\text{S10})$$

782 Equivalently, Eq. (S10) can be expressed as “a set of species  $\{\sigma^*\}_{\text{opt}}$  construct a fitness  
 783 landscape which places themselves on the top”, according to Eq. (S9).  
 784

785 The steady state constructed by  $\{\sigma^*\}$  is influenced by the supply  $\vec{c}_{\text{supply}}$  and the dilution rate  $d$ .  
 786 For different chemostat parameters, the non-invasive species set  $\{\sigma^*\}_{\text{opt}}$  may be different. In the  
 787 following steps, we described a generally applicable protocol for obtaining the non-invasive  
 788 strategies:  
 789

790 ***1. Maximal growth rates and maximizing strategies***

791 In a metabolic model with trade-offs in resource allocation, the maximizing resource allocation  
 792 strategy  $\vec{\alpha}_{\text{max}}$  under a given environment  $\vec{c}$  is defined as the strategy that maximizes invasion  
 793 growth rate:

$$\begin{aligned} g_{\text{max}}(\vec{c}) &:= \max_{\vec{\alpha}}(g_{\text{inv}}(\vec{\alpha}_\sigma|\vec{c})) \\ \vec{\alpha}_{\text{max}}(\vec{c}) &:= \arg \max_{\vec{\alpha}}(g_{\text{inv}}(\vec{\alpha}_\sigma|\vec{c})). \end{aligned} \quad (\text{S11})$$

794

795 ***2. Maximal growth contour***

796 For a given dilution rate  $d$ , all environments that support a maximal growth rate of  $d$  constitute  
 797 the “maximal growth contour”:

$$GC_{\text{max}} := \{\vec{c}_0 \mid g_{\text{max}}(\vec{c}_0) = d\}. \quad (\text{S12})$$

798  $GC_{\text{max}}$  is generally formed by many species, with each species adopting the maximizing  
 799 strategies  $\vec{\alpha}_{\text{max}}(\vec{c}_0)$  corresponding to one environment  $\vec{c}_0$  on the maximal growth contour.

800  $GC_{\max}$  is outside of the invasion zone for any species  $\sigma$ . (Otherwise, if a species  $\sigma$  could invade  
 801 an environment  $\vec{c}_0$  on  $GC_{\max}$ ,  $g_{\text{inv}}(\vec{\alpha}_\sigma | \vec{c}_0 \in GC_{\max}) > d$ , this would directly violate the  
 802 requirement by Eqs. (S11) and (S12) that  $\max_{\vec{\alpha}}(g_{\text{inv}}(\vec{\alpha}_\sigma | \vec{c}_0)) = d$ .) Therefore, the necessary and  
 803 sufficient condition for a set of species to be evolutionarily stable, is to construct a steady-state  
 804 environment on the maximal growth contour:

$$\vec{c}_{\{\sigma^*\}_{\text{opt,ss}}} \in GC_{\max}. \quad (\text{S13})$$

805 Therefore, a strategy belonging to the non-invasive set must be a maximizing strategy.  
 806 An example of maximal growth contour is shown in Figs 4D, 5B-C, and 6D

### 808 3. Non-invasive strategy

809 Nevertheless, adopting one of the maximizing strategies along the maximal growth contour does  
 810 not guarantee that a species will satisfy Eq. (S13) and become non-invasive, as a maximizing  
 811 strategy for environment  $\vec{c}_1$  may end up constructing a different environment  $\vec{c}_2$ . To identify a  
 812 non-invasive species for supply condition  $\vec{c}_{\text{supply}}$ , the flux-balance condition needs to be  
 813 considered, with the strategies maximized at each environment. This requirement forms a  
 814 “maximal flux-balance curve” in the chemical space:

$$FB_{\max} := \{\vec{c} \mid \frac{c_{i, \text{supply}} - c_i}{I_i(\vec{c}, \vec{f}^{-1}(\vec{c}, \vec{\alpha}_{\max}(\vec{c})), \vec{\alpha}_{\max}(\vec{c}))} = \frac{m}{d \cdot r} \text{ AND } c_i < c_{i, \text{supply}}\}. \quad (\text{S14})$$

815 If the intersection of the maximal growth contour and the maximal flux-balance curve exists, it is  
 816 the evolutionarily stable environment under dilution rate  $d$  and supply condition  $\vec{c}_{\text{supply}}$ ,  
 817  $\vec{c}_{\text{opt}}$ . The maximizing strategy for this environment,  $\vec{\alpha}_{\text{opt}} = \vec{\alpha}_{\max}(\vec{c}_{\text{opt}})$ , constructs the  
 818 environment  $\vec{c}_{\text{opt}}$ , and is evolutionarily stable.

### 819 4. Evolutionarily stable coexistence at the discontinuous points of the maximal growth contour

820 Inversely, for each environment  $\vec{c}_0$  on the maximal growth contour, all supply conditions that  
 821 enable the maximizing strategy of  $\vec{c}_0$  to become the non-invasive strategy can be calculated  
 822 from the supply line according to Eq. (S6):

$$SL(\vec{c}_0) := \{\vec{c}_{\text{supply}} \mid \vec{c}_{\text{supply}} = x \cdot \vec{I}(\vec{c}_0, \vec{f}^{-1}(\vec{c}_0, \vec{\alpha}_{\max}(\vec{c}_0)), \vec{\alpha}_{\max}(\vec{c}_0)) + \vec{c}_0\}, \quad (\text{S15})$$

824 for any non-zero value of  $x$ . Some examples are shown in Fig 4D.  
 825 When there are discontinuous points on the maximal growth contour, there can be “gaps” in the  
 826 nutrient supply space, where no single strategy on the maximal growth contour satisfies Eq.  
 827 (S14). Under this condition, more than one strategy is required to co-create an environment on a  
 828 discontinuous point of the maximal growth contour. Therefore, discontinuous points of the  
 829 maximal growth contour permit evolutionarily stable coexistence, where  $\{\sigma^*\}_{\text{opt}}$  contains more  
 830 than one species. Two examples of such discontinuities and coexistence are shown in Fig 5 and  
 831 Fig 6.

### 832 Metabolic models

833 Different assumptions can be made regarding the metabolic models  $\vec{f}(\vec{c}, \vec{x}, \vec{\alpha})$ ,  $g(\vec{c}, \vec{x}, \vec{\alpha})$ , and  
 834  $\vec{I}(\vec{c}, \vec{x}, \vec{\alpha})$ , focusing on various aspects of cellular growth. Different assumptions lead to distinct  
 835 classes of metabolic models with various results. Nevertheless, our analysis schemes, including  
 836 the invasion geometry, fitness landscape, and evolutionary stable strategies, are generally  
 837

838 applicable for various metabolic models. In this work, we used five metabolic models to  
839 illustrate multiple aspects of the species-environment feedback:  
840

841 *1. Metabolic model with two essential nutrients*

842 When two nutrients are both essential for growth, such as nitrogen and phosphorus, and both  
843 require a substantial allocation of resources for import, the system can be abstractly modeled as  
844 shown in Fig 4A. In this metabolic model, we assume an exact trade-off between the allocation  
845 of limited resources to import nutrient  $a$  or nutrient  $b$ . The fraction of resources allocated to  
846 import nutrient  $a$  is represented by  $\alpha_a$ , thus leaving a fraction  $\alpha_b = 1 - \alpha_a$  to import nutrient  $b$ .  
847 The import rate of nutrient  $i$  is assumed to follow the Monod equation as a function of nutrient  
848 concentration, and is proportional to  $\alpha_i$ :

$$I_i(\vec{c}) = \alpha_i \cdot \frac{c_i}{c_i + K_i} \text{ for } i = a, b. \quad (\text{S16})$$

849 Import of both nutrients is required for cell growth:

$$g(\vec{c}) = \gamma \cdot \min(I_a(\vec{c}), I_b(\vec{c})). \quad (\text{S17})$$

850 For this model, for simplicity we do not explicitly consider intracellular metabolites. Rather,  
851 import directly determines growth.

852 In this model, a “species” is defined by its value of  $\alpha_a$ .

853 Nutrient limitation can be clearly quantified in this system: if  $I_a(\vec{c}) > I_b(\vec{c})$ , the system is  
854 limited by nutrient  $b$ ; if  $I_a(\vec{c}) < I_b(\vec{c})$ , the system is limited by nutrient  $a$ .  
855

856 A species with the following parameters was used to generate Fig S1, focusing on how supply  
857 conditions and dilution rate influence nutrient limitation:

$K_a$	$K_b$	$\gamma$	$\alpha_a$
0.7	1.3	10	0.3

858 In Fig S1A, to demonstrate how species construct the same environment out of different supply  
859 conditions, the chemostat dilution rate was set to  $d = 1$ , and three supply conditions were used:  
860  $\vec{c}_{\text{supply}} = [0.6, 0.3546]$  (purple),  $\vec{c}_{\text{supply}} = [0.8, 0.5273]$  (cyan), and  $\vec{c}_{\text{supply}} = [1, 0.7]$  (blue).

861 In Fig S1B, to demonstrate how dilution rates may switch the limiting nutrient, we used the same  
862 supply condition as the blue condition in Fig 1D ( $\vec{c}_{\text{supply}} = [1, 0.7]$ ), and three dilution rates: 0.5  
863 (yellow), 1 (red), and 1.6 (deep red).

864

865 Species with following parameters were used to generate Fig 4B-D:

$K_a$	$K_b$	$\gamma$
0.5	0.5	10

866 The strategy  $\alpha_a$  varies for different species. In Fig 4B, Species *Blue* has  $\alpha_a = 0.35$ , species *Red*  
867 has  $\alpha_a = 0.65$ . In Fig 4C, we started with species *Blue* and species *Red*. We then generated the  
868 fitness landscape for each species at the steady-state environment it constructed, then chose the  
869 strategy that maximized invasion growth rate for this fitness landscape to generate a new species,  
870 and iterated this process five times. The species *Black* has  $\alpha_a = 0.5$ .

871 In generating Fig 4D, we followed the protocols described in section “Non-invasive /  
872 evolutionarily stable strategies”.

873

874 *2. Metabolic model with substitutable nutrients*

875 When two nutrients are mutually substitutable for growth, such as glucose and galactose, the  
 876 system can be described by metabolic model as shown in Fig 2A. The trade-off and import  
 877 functions are taken to be the same as in Model 1: *metabolic model with two essential nutrients*.  
 878 However, import of the two nutrients contributes additively toward growth rate:

$$g(\vec{c}) = \gamma \cdot (I_a(\vec{c}) + I_b(\vec{c})). \quad (\text{S18})$$

879 A species is defined by its value of  $\alpha_a$ .

880 For this model, all growth contours intersect at one point. The growth contour of species  $\sigma$   
 881 satisfies the equation:  $\alpha_a \cdot \frac{c_a}{c_a + K_a} + (1 - \alpha_a) \cdot \frac{c_b}{c_b + K_b} = d/\gamma$ . Regardless of the value of  $\alpha_a$ , the  
 882 environment  $[\frac{K_a}{\gamma-1}, \frac{K_b}{\gamma-1}]$  is always on the growth contour.

883 Species with the following parameters were used to generate Fig 1C-D and Figure 2.:

$K_a$	$K_b$	$\gamma$
1.2	0.8	3

884 The strategy  $\alpha_a$  varies for different species. In Fig 1C-D and Fig 2B-C, Species *Blue* has  $\alpha_a =$   
 885 0.2, species *Red* has  $\alpha_a = 0.6$ . Supply conditions are different among the three figures: in Fig  
 886 1C,  $\vec{c}_{\text{supply}} = [0.5, 1]$ ; in Fig 1D,  $\vec{c}_{\text{supply}} = [1, 0.5]$ ; in Fig 2B,  $\vec{c}_{\text{supply}} = [1, 1]$ .

887 All conditions in Fig 2D are the same as in Fig 2B other than that five additional species are  
 888 added to the system. Their strategies are indicated by the legend at the right.

### 3. Metabolic model with substitutable nutrients that require assimilation

889 In cells, the assimilation of imported raw material, such sugars, into biomass such as proteins,  
 890 takes multiple steps and enzymes and consumes a considerable amount of energy. When the  
 891 resources allocated to nutrient assimilation are considered, a cell's strategy becomes more  
 892 complex. A mathematical model involving three substitutable nutrients  $a, b, c$  that need  
 893 assimilation is shown in Fig 3A, with  $\alpha_{i1}$  represents the fraction of resources allocated to  
 894 importing nutrient  $i$  into internal metabolite, and  $\alpha_{i2}$  represents the fraction of resources  
 895 allocated to assimilate the internal  $i$  into biomass. In this model, the import rate has a similar  
 896 form to the previous two models,

$$I_i(c_i) = V \cdot \alpha_{i1} \cdot \frac{c_i}{c_i + K_i}. \quad (\text{S19})$$

897 The internal metabolite concentration  $c_{i,\text{internal}}$  has an influx of  $r \cdot I_i(c_i)$ , meanwhile, it is diluted  
 898 by cell growth at the rate  $g$ . We assume all nutrients are substitutable therefore the internal pools  
 899 contribute via summation to growth, and are converted into biomass at a rate  $k \cdot \alpha_{i2} \cdot c_{i,\text{internal}}$ :

$$\frac{dc_{i,\text{internal}}}{dt} = I_i(c_i) - g(\vec{c}_{\text{internal}}) \cdot c_{i,\text{internal}} - k \cdot \alpha_{i2} \cdot c_{i,\text{internal}}. \quad (\text{S20})$$

900 Therefore, the mass converted into biomass per unit time per unit volume is:  $\sum_i (k \cdot \alpha_{i2} \cdot$   
 901  $c_{i,\text{internal}})$ , and the growth rate defined as the relative gain of total biomass  $M$  is:

$$g(\vec{c}_{\text{internal}}) = \frac{\frac{dM}{dt}}{M} = \frac{k}{r} \cdot \sum_i (\alpha_{i2} \cdot c_{i,\text{internal}}). \quad (\text{S21})$$

902 In generating Fig 3B-C, the chemostat parameters were:  $\vec{c}_{\text{supply}} = [1, 1, 1]$ , and  $d = 1$ , and the  
 903 species parameters were:

$V$	$K_i$ ( $i = a, b, c$ )	$k$
1000	0.5	11

904 The three species allocate their resources differently:

Strategies	$\alpha_{a1}$	$\alpha_{a2}$	$\alpha_{b1}$	$\alpha_{b2}$	$\alpha_{c1}$	$\alpha_{c2}$
------------	---------------	---------------	---------------	---------------	---------------	---------------

<i>Red</i>	0.15	0.2	0.1	0.25	0.26	0.04
<i>Green</i>	0.26	0.04	0.15	0.2	0.1	0.25
<i>Blue</i>	0.1	0.25	0.26	0.04	0.15	0.2

908

909 To generate the Fig S3, all other parameters are the same, other than  $k = 10$ .

910

911

#### 912 4. Metabolic model with essential nutrients that can be interconverted

913 If two nutrients are both essential for growth, and a cell is able to convert one nutrient into  
 914 another albeit at a certain cost, as shown in Fig 5A, metabolic trade-offs involve the following  
 915 four elements of the allocation strategy  $\vec{\alpha}$  :

916  $\alpha_a$ : Fraction of resources allocated to import nutrient  $a$ .

917  $\alpha_b$ : Fraction of resources allocated to import nutrient  $b$ .

918  $\alpha_{ab}$ : Fraction of resources allocated to convert internal  $b$  into  $a$ .

919  $\alpha_{ba}$ : Fraction of resources allocated to convert internal  $a$  into  $b$ .

920 To implement trade-offs, the sum of elements of  $\vec{\alpha} = (\alpha_a, \alpha_b, \alpha_{ab}, \alpha_{ba})$  is taken to be equal to 1.  
 921 In this metabolic model, cells internalize nutrient  $a$  and nutrient  $b$  from the chemostat to supply  
 922 internal concentration of nutrients,  $c_{a,\text{internal}}$  and  $c_{b,\text{internal}}$ . Meanwhile, the internal nutrients  
 923 can be converted into each other. Nutrients also diffuse in and out of the cell passively with rate  
 924  $\beta$ . Cell growth requires both internal nutrients, and depletes them in a fixed proportion.

925 In this model, the growth rate of a cell is taken to be:

$$g(\vec{c}_{\text{internal}}) = \frac{\gamma}{\frac{K_a}{c_{a,\text{internal}}} + \frac{K_b}{c_{b,\text{internal}}}} \quad (\text{S22})$$

926 The net import rate, including passive diffusion, is:

$$I_i = (\alpha_i + \beta) \cdot c_i - \beta \cdot c_{i,\text{internal}}, i = a, b. \quad (\text{S23})$$

927 Therefore, the dynamical equations for the internal nutrients are:

$$\frac{dc_{a,\text{internal}}}{dt} = I_a + \alpha_{ab} \cdot c_{b,\text{internal}} - \alpha_{ba} \cdot c_{a,\text{internal}} - g/K_a, \quad (\text{S24})$$

$$\frac{dc_{b,\text{internal}}}{dt} = I_b + \alpha_{ba} \cdot c_{a,\text{internal}} - \alpha_{ab} \cdot c_{b,\text{internal}} - g/K_b. \quad (\text{S25})$$

928

929 A species is defined by its value of  $\vec{\alpha}$ .

930

931 This metabolic model was used to demonstrate how to obtain locally optimal strategies and  
 932 cartels, as shown in Fig 5. The parameter values used to generate the plots in Fig 5B-D were:

$\gamma$	$K_i (i = a, b)$	$\beta$
1	1	0.2

933 In generating Fig 5B, we searched for the maximizing strategies in the chemical space, and  
 934 classified them by their non-zero values. Maximal growth contours for four dilution rates: 0.1,  
 935 0.2, 0.3, 0.4 are shown from black to gray and white colors.

936 In generating Fig 5C, the chemostat parameters were set to  $\vec{c}_{\text{supply}} = [0.5, 1]$ , and  $d = 0.2$ . The  
 937 maximal growth contours for  $d = 0.2$  were drawn, along with maximizing strategies along the  
 938 contour shown as squares with colors corresponding to their sub-classes. At the discontinuous  
 939 point of the maximal growth contour where the “converter” and the “importer” converge, the

940 distinct two maximizing strategies are denoted species *Red* and species *Blue*. In generating the  
941 competition dynamics in inset, additional to the species *Red* and species *Blue*, ten other  
942 maximizing strategies along the maximal growth contours were chosen.  
943

944 5. Metabolic model with multiple energy generating steps

945 Cell growth is also tightly coupled with energy production. For example, with a single carbon  
946 supply as the energy source, cells employ multi-step reactions to generate multiple ATP  
947 molecules. Each step requires dedicated enzymes. The reaction intermediates, such as acetate,  
948 usually have dual roles: on the one hand, they positively contribute to ATP production via  
949 downstream reactions; on the other hand, they negatively contribute to ATP production by  
950 hampering upstream reactions. To deal with the negative effects of intermediates, cells may  
951 transport them out into the environment, generally with some metabolic cost for transporters. On  
952 the other hand, cells can also uptake such intermediates and use them as an energy source.  
953 We abstract such a process by the model shown in Fig 6A. A single chemical energy source S is  
954 supplied into the chemostat, which can be converted into intermediate I by cells. Four reactions  
955 are possible in this model, each mediated by a specific enzyme:

956 1. Import the resource S into the cell and convert it into internal intermediate  $I_{int}$  to extract  
957 energy (e.g. ATP). The fraction of the model enzyme budget allocated to this reaction is  $\alpha_{ATP1}$ .  
958 We assume the reaction is reversible, with the concentration of S contributing positively to the  
959 reaction rate while the concentration of  $I_{int}$  contributes negatively:

$$J_1 = \alpha_{ATP1} \cdot V_1 \cdot \frac{[S] - \frac{[I_{int}]}{K_3}}{K_1 + [S] + \frac{[I_{int}]}{K_5}} \quad (S26)$$

960 2. Process  $I_{int}$  via a downstream reaction to obtain more energy. The fraction of enzymes being  
961 allocated to this reaction is  $\alpha_{ATP2}$ . For this model system, it does not qualitatively influence the  
962 final results whether this reaction is product inhibited, so we neglect product inhibition. For  
963 simplicity, we assume this reaction has Michaelis–Menten form:

$$J_2 = \alpha_{ATP2} \cdot V_2 \cdot \frac{[I_{int}]}{K_2 + [I_{int}]} \quad (S27)$$

964 3. Export the internal intermediate out into the environment by diffusion, with a fraction of  
965 proteins  $\alpha_{exp}$  allocated to channels that allow the excretion of the intermediate into the  
966 environment to become external intermediate  $I_{ext}$ .

$$J_3 = \alpha_{exp} \cdot k \cdot ([I_{int}] - [I_{ext}]) \quad (S28)$$

967 4. Import the external intermediate into cells, with a fraction of proteins  $\alpha_{imp}$  allocated to the  
968 import process. To reflect the property of the internal intermediate in inhibiting this transport  
969 reaction, the rate for this process is also product-inhibited:

$$J_4 = \alpha_{imp} \cdot V_4 \cdot \frac{[I_{ext}] - \frac{[I_{int}]}{K_6}}{K_4 + [I_{ext}] + \frac{[I_{int}]}{K_7}} \quad (S29)$$

970 Under this model, the rate of change of the concentration of the energy source S in the chemostat  
971 is:

$$\frac{d[S]}{dt} = d \cdot ([S_{\text{supply}}] - [S]) - \frac{m}{r} \cdot J_1. \quad (\text{S30})$$

972 The rate of change of the external intermediate concentration in the chemostat is:

$$\frac{d[I_{\text{ext}}]}{dt} = d \cdot (-[I_{\text{ext}}]) - m/r \cdot (J_4 - J_3). \quad (\text{S31})$$

973

974 The concentration of the intracellular metabolite  $I_{\text{int}}$  follows the equation:

$$\frac{d[I_{\text{int}}]}{dt} = J_1 - J_2 - J_3 + J_4. \quad (\text{S32})$$

975

976 The growth rate is a weighted sum of the ATP produced by  $J_1$  and  $J_2$ :

$$g = n_{\text{ATP1}} \cdot J_1 + n_{\text{ATP2}} \cdot J_2. \quad (\text{S33})$$

977

978 In generating plots in Fig 6B-F, the species parameters were:

$V_1$	$V_2$	$k$	$V_4$	$K_1$	$K_2$	$K_3$	$K_4$	$K_5$	$K_6$	$K_7$	$n_{\text{ATP1}}$	$n_{\text{ATP2}}$
5	1	8	10	0.5	0.5	0.5	0.1	0.5	15	10	1	1

979

980 Maximal growth contours for dilution rates 0.2, 0.4, and 0.6 are shown in Fig 6B.

981 For Fig 6C-D, the chemostat parameters are:  $S_{\text{supply}} = 1, d = 0.4$ .

982 For Fig 6E-F, the chemostat parameters are:  $S_{\text{supply}} = 1.8, d = 0.6$ .

983 A summary of maximizing strategies in chemical space is shown in Fig S4.

984

### 985 ***Dynamic equations for multiple species in a chain of chemostats***

986 Real ecosystems seldom exist in isolation. We modeled interconnected ecosystems via a chain of  
 987 chemostats labeled  $k = 1$  to  $k_{\text{tot}}$  (Fig S5A). Each chemostat exchanges medium and cells at  
 988 leakage rate  $l$  with its two neighboring chemostats (if  $k = 1$  or  $k = k_{\text{tot}}$ , there is only one  
 989 neighbor). The chemostat parameters  $\vec{c}_{\text{supply}}$  and  $d$  are taken to be identical for all chemostats.

990

991 For the  $k$ -th chemostat, the dynamical equations for the biomass density of species  $\sigma$  and the  
 992 concentration of the  $i$ -th nutrient are:

$$\frac{dm_{\sigma,k}}{dt} = m_{\sigma,k} \cdot (g_{\sigma}(\vec{c}_k) - d) + l \cdot (m_{\sigma,k-1} + m_{\sigma,k+1} - 2 \cdot m_{\sigma,k}), \quad (\text{S34})$$

$$\frac{dc_{i,k}}{dt} = d \cdot (c_{i,\text{supply}} - c_{i,k}) - \sum_{\sigma=1}^n m_{\sigma,k} \cdot I_{i,\sigma}(\vec{c}_k) + l \cdot (c_{i,k+1} + c_{i,k-1} - 2 \cdot c_{i,k}). \quad (\text{S35})$$

993 A steady-state solution to these equations is shown in Fig S5, using the same growth and import  
 994 models and parameters as in Fig 4, with the leakage rate set to be  $l = 1$ .

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1000

1001 **COMPETING INTERESTS**

1002 The authors declare that they have no conflict of interest.

1003

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1006 **REFERENCES**

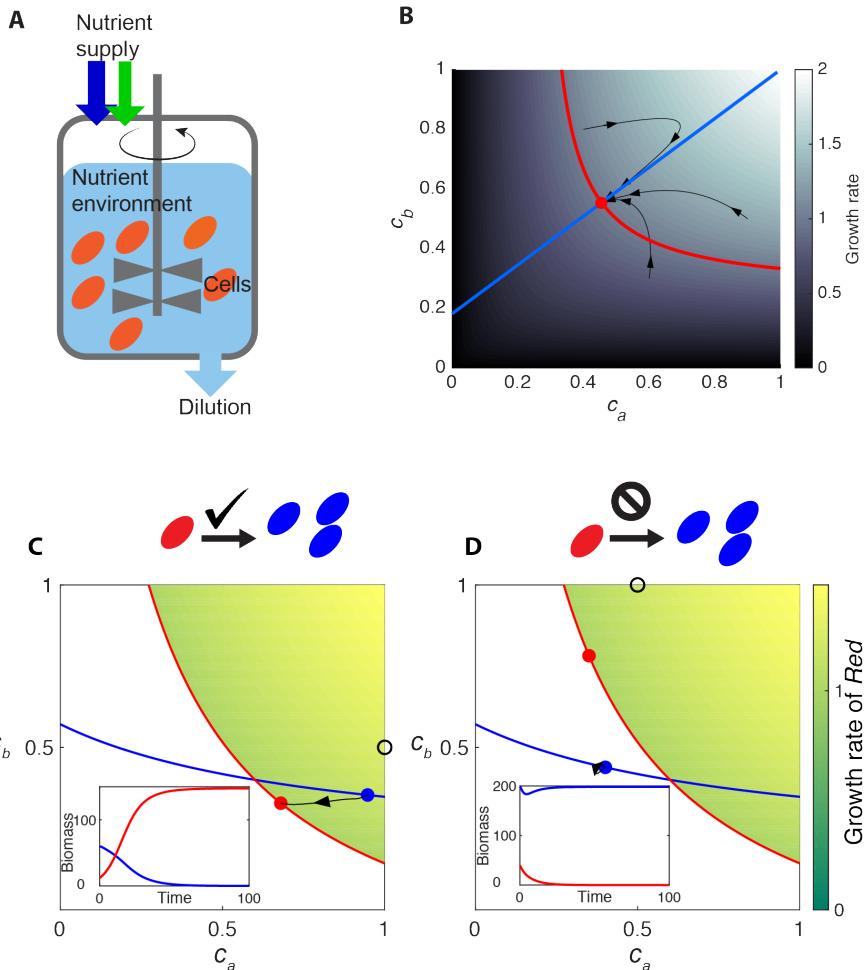
- 1007 Armstrong, R. A., & McGehee, R. (1980). Competitive exclusion. *The American Naturalist*,  
1008 115(2), 151-170.
- 1009 Bachmann, H., Bruggeman, F. J., Molenaar, D., dos Santos, F. B., & Teusink, B. (2016). Public  
1010 goods and metabolic strategies. *Current Opinion in Microbiology*, 31, 109-115.  
1011 doi:10.1016/j.mib.2016.03.007
- 1012 Bachmann, H., Molenaar, D., dos Santos, F. B., & Teusink, B. (2017). Experimental evolution  
1013 and the adjustment of metabolic strategies in lactic acid bacteria. *Fems Microbiology  
1014 Reviews*, 41, S201-S219. doi:10.1093/femsre/fux024
- 1015 Bajic, D., & Sanchez, A. (2019). The ecology and evolution of microbial metabolic strategies.  
1016 *Curr Opin Biotechnol*, 62, 123-128. doi:10.1016/j.copbio.2019.09.003
- 1017 Bajic, D., Vila, J. C. C., Blount, Z. D., & Sanchez, A. (2018). On the deformability of an  
1018 empirical fitness landscape by microbial evolution. *Proc Natl Acad Sci U S A*, 115(44),  
1019 11286-11291. doi:10.1073/pnas.1808485115
- 1020 Beardmore, R. E., Gudelj, I., Lipson, D. A., & Hurst, L. D. (2011). Metabolic trade-offs and the  
1021 maintenance of the fittest and the flattest. *Nature*, 472(7343), 342-346.  
1022 doi:10.1038/nature09905
- 1023 Blount, Z. D., Barrick, J. E., Davidson, C. J., & Lenski, R. E. (2012). Genomic analysis of a key  
1024 innovation in an experimental *Escherichia coli* population. *Nature*, 489(7417), 513-+.  
1025 doi:10.1038/nature11514
- 1026 Boer, V. M., Crutchfield, C. A., Bradley, P. H., Botstein, D., & Rabinowitz, J. D. (2010).  
1027 Growth-limiting intracellular metabolites in yeast growing under diverse nutrient  
1028 limitations. *Mol Biol Cell*, 21(1), 198-211. doi:10.1091/mbc.E09-07-0597
- 1029 Callahan, B. J., Fukami, T., & Fisher, D. S. (2014). Rapid evolution of adaptive niche  
1030 construction in experimental microbial populations. *Evolution*, 68(11), 3307-3316.  
1031 doi:10.1111/evol.12512
- 1032 Chase, J. M., & Leibold, M. A. (2003). *Ecological niches: linking classical and contemporary  
1033 approaches*: University of Chicago Press.
- 1034 D'Souza, G., Shitut, S., Preussger, D., Yousif, G., Waschina, S., & Kost, C. (2018). Ecology and  
1035 evolution of metabolic cross-feeding interactions in bacteria. *Nat Prod Rep*, 35(5), 455-  
1036 488. doi:10.1039/c8np00009c
- 1037 De Leenheer, P., Levin, S. A., Sontag, E. D., & Klausmeier, C. A. (2006). Global stability in a  
1038 chemostat with multiple nutrients. *Journal of Mathematical Biology*, 52(4), 419-438.  
1039 doi:10.1007/s00285-005-0344-4
- 1040 Doebeli, M. (2002). A model for the evolutionary dynamics of cross-feeding polymorphisms in  
1041 microorganisms. *Population Ecology*, 44(2), 59-70. doi:DOI 10.1007/s101440200008
- 1042 Escalante-Chong, R., Savir, Y., Carroll, S. M., Ingraham, J. B., Wang, J., Marx, C. J., &  
1043 Springer, M. (2015). Galactose metabolic genes in yeast respond to a ratio of galactose  
1044 and glucose. *Proc Natl Acad Sci U S A*, 112(5), 1636-1641.  
1045 doi:10.1073/pnas.1418058112
- 1046 Friedman, J., Higgins, L. M., & Gore, J. (2017). Community structure follows simple assembly  
1047 rules in microbial microcosms. *Nature ecology & evolution*, 1(5), 0109.
- 1048 Goldford, J. E., Lu, N., Bajić, D., Estrela, S., Tikhonov, M., Sanchez-Gorostiaga, A., . . .  
1049 Sanchez, A. (2018). Emergent simplicity in microbial community assembly. *Science*,  
1050 361(6401), 469-474.

- 1051 Goyal, A., Dubinkina, V., & Maslov, S. (2018). Multiple stable states in microbial communities  
1052 explained by the stable marriage problem. *The ISME Journal*. doi:10.1038/s41396-018-  
1053 0222-x
- 1054 Goyal, S., Yuan, J., Chen, T., Rabinowitz, J. D., & Wingreen, N. S. (2010). Achieving optimal  
1055 growth through product feedback inhibition in metabolism. *PLoS computational biology*,  
1056 6(6), e1000802.
- 1057 Hardin, G. (1960). The competitive exclusion principle. *Science*, 131(3409), 1292-1297.
- 1058 Huisman, J., van Oostveen, P., & Weissing, F. J. (1999). Species dynamics in phytoplankton  
1059 blooms: incomplete mixing and competition for light. *The American Naturalist*, 154(1),  
1060 46-68.
- 1061 Huisman, J., & Weissing, F. J. (1999). Biodiversity of plankton by species oscillations and  
1062 chaos. *Nature*, 402(6760), 407-410. doi:10.1038/46540
- 1063 Huisman, J., & Weissing, F. J. (2001). Biological conditions for oscillations and chaos generated  
1064 by multispecies competition. *Ecology*, 82(10), 2682-2695.
- 1065 Ispolatov, I., Madhok, V., & Doebeli, M. (2016). Individual-based models for adaptive  
1066 diversification in high-dimensional phenotype spaces. *Journal of theoretical biology*,  
1067 390, 97-105. doi:10.1016/j.jtbi.2015.10.009
- 1068 Kasting, J. F., & Siefert, J. L. (2002). Life and the evolution of Earth's atmosphere. *Science*,  
1069 296(5570), 1066-1068. doi:10.1126/science.1071184
- 1070 Koffel, T., Daufresne, T., Massol, F., & Klausmeier, C. A. (2016). Geometrical envelopes:  
1071 Extending graphical contemporary niche theory to communities and eco-evolutionary  
1072 dynamics. *Journal of theoretical biology*, 407, 271-289.
- 1073 Levin, S. A. (1970). Community equilibria and stability, and an extension of the competitive  
1074 exclusion principle. *The American Naturalist*, 104(939), 413-423.
- 1075 Li, S. H.-J., Li, Z., Park, J. O., King, C. G., Rabinowitz, J. D., Wingreen, N. S., & Gitai, Z.  
1076 (2018). Escherichia coli translation strategies differ across carbon, nitrogen and  
1077 phosphorus limitation conditions. *Nature microbiology*, 3(8), 939.
- 1078 Liebermeister, W., Noor, E., Flamholz, A., Davidi, D., Bernhardt, J., & Milo, R. (2014). Visual  
1079 account of protein investment in cellular functions. *Proceedings of the National Academy  
1080 of Sciences*, 111(23), 8488-8493.
- 1081 Long, C. P., & Antoniewicz, M. R. (2018). How adaptive evolution reshapes metabolism to  
1082 improve fitness: recent advances and future outlook. *Current Opinion in Chemical  
1083 Engineering*, 22, 209-215. doi:10.1016/j.coche.2018.11.001
- 1084 Luli, G. W., & Strohl, W. R. (1990). Comparison of growth, acetate production, and acetate  
1085 inhibition of Escherichia coli strains in batch and fed-batch fermentations. *Applied and  
1086 environmental microbiology*, 56(4), 1004-1011.
- 1087 MacArthur, R. (1970). Species packing and competitive equilibrium for many species.  
1088 *Theoretical population biology*, 1(1), 1-11.
- 1089 Maharjan, R., Seeto, S., Notley-McRobb, L., & Ferenci, T. (2006). Clonal adaptive radiation in a  
1090 constant environment. *Science*, 313(5786), 514-517. doi:10.1126/science.1129865
- 1091 Marsland III, R., Cui, W., & Mehta, P. (2019). The Minimum Environmental Perturbation  
1092 Principle: A New Perspective on Niche Theory. *arXiv preprint arXiv:1901.09673*.
- 1093 McGehee, R., & Armstrong, R. A. (1977). Some mathematical problems concerning the  
1094 ecological principle of competitive exclusion. *Journal of Differential Equations*, 23(1),  
1095 30-52.
- 1096 Metz, J. (2012). Adaptive dynamics.

- 1097 Odum, E. P., & Barrett, G. W. (1971). *Fundamentals of ecology* (Vol. 3): Saunders Philadelphia.  
1098 Pfeiffer, T., & Bonhoeffer, S. (2004). Evolution of cross-feeding in microbial populations. *The  
1099 American Naturalist*, 163(6), E126-E135.  
1100 Posfai, A., Taillefumier, T., & Wingreen, N. S. (2017). Metabolic Trade-Offs Promote Diversity  
1101 in a Model Ecosystem. *Physical Review Letters*, 118(2).  
1102 doi:10.1103/PhysRevLett.118.028103  
1103 Roller, B. R. K., Stoddard, S. F., & Schmidt, T. M. (2016). Exploiting rRNA operon copy  
1104 number to investigate bacterial reproductive strategies. *Nature Microbiology*, 1(11).  
1105 doi:Unsp 16160  
1106 10.1038/Nmicrobiol.2016.160  
1107 Rosenzweig, R. F., Sharp, R., Treves, D. S., & Adams, J. (1994). Microbial evolution in a simple  
1108 unstructured environment: genetic differentiation in *Escherichia coli*. *Genetics*, 137(4),  
1109 903-917.  
1110 Rueffler, C., Van Dooren, T. J. M., & Metz, J. A. J. (2004). Adaptive walks on changing  
1111 landscapes: Levins' approach extended. *Theoretical Population Biology*, 65(2), 165-178.  
1112 doi:10.1016/j.tpb.2003.10.001  
1113 Schertzer, E., Staver, A. C., & Levin, S. A. (2015). Implications of the spatial dynamics of fire  
1114 spread for the bistability of savanna and forest. *J Math Biol*, 70(1-2), 329-341.  
1115 doi:10.1007/s00285-014-0757-z  
1116 Schirrmeister, B. E., de Vos, J. M., Antonelli, A., & Bagheri, H. C. (2013). Evolution of  
1117 multicellularity coincided with increased diversification of cyanobacteria and the Great  
1118 Oxidation Event. *Proc Natl Acad Sci U S A*, 110(5), 1791-1796.  
1119 doi:10.1073/pnas.1209927110  
1120 Scott, M., Gunderson, C. W., Mateescu, E. M., Zhang, Z. G., & Hwa, T. (2010). Interdependence  
1121 of Cell Growth and Gene Expression: Origins and Consequences. *Science*, 330(6007),  
1122 1099-1102. doi:10.1126/science.1192588  
1123 Smith, H. L., & Waltman, P. (1995). *The theory of the chemostat: dynamics of microbial  
1124 competition* (Vol. 13): Cambridge university press.  
1125 Soliveres, S., Maestre, F. T., Ulrich, W., Manning, P., Boch, S., Bowker, M. A., . . . Allan, E.  
1126 (2015). Intransitive competition is widespread in plant communities and maintains their  
1127 species richness. *Ecology letters*, 18(8), 790-798. doi:10.1111/ele.12456  
1128 Taillefumier, T., Posfai, A., Meir, Y., & Wingreen, N. S. (2017). Microbial consortia at steady  
1129 supply. *eLife*, 6, e22644.  
1130 Tilman, D. (1980). Resources: a graphical-mechanistic approach to competition and predation.  
1131 *The American Naturalist*, 116(3), 362-393.  
1132 Tilman, D. (1982). *Resource competition and community structure*: Princeton university press.  
1133 Van den Bergh, B., Swings, T., Fauvert, M., & Michiels, J. (2018). Experimental design,  
1134 population dynamics, and diversity in microbial experimental evolution. *Microbiol. Mol.  
1135 Biol. Rev.*, 82(3), e00008-00018.  
1136 Wang, X., & Tang, C. (2017). Optimal growth of microbes on mixed carbon sources. *arXiv  
1137 preprint arXiv:1703.08791*.  
1138 Wides, A., & Milo, R. (2018). Understanding the dynamics and optimizing the performance of  
1139 chemostat selection experiments. *arXiv preprint arXiv:1806.00272*.  
1140 Zaman, S., Lippman, S. I., Zhao, X., & Broach, J. R. (2008). How *Saccharomyces* Responds to  
1141 Nutrients. *Annual Review of Genetics*, 42, 27-81.  
1142 doi:10.1146/annurev.genet.41.110306.130206

1143 Ziv, N., Brandt, N. J., & Gresham, D. (2013). The Use of Chemostats in Microbial Systems  
1144 Biology. *Jove-Journal of Visualized Experiments*(80). doi:UNSP e50168  
1145 10.3791/50168  
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1148 **FIGURES**  
Figure 1



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1150

1151 **Figure 1 - Chemostat behavior represented in chemical space.**

1152

1153 A. Schematic diagram of a chemostat occupied by a single microbial species. In the well-mixed  
1154 medium (pale blue) of a chemostat, cells (orange ellipses) consume nutrients and grow. An  
1155 influx of nutrients with fixed concentrations (blue and green arrows) is supplied at the same rate  
1156 as dilution, keeping the medium volume constant.

1157

1158 B. Visual representation of how a species creates its own chemostat environment. Background  
1159 color indicates the growth rate of cells as a function of metabolite concentrations  $c_a$  and  $c_b$ , with  
1160 the growth contour shown by the red curve. The flux-balance curve is shown in blue. Black  
1161 curves with arrows show the time trajectories of chemostat simulation.

1162

1163 C. Example of successful invasion of species *Blue* by species *Red*. A small amount of species  
1164 *Red* is introduced to a steady-state chemostat of species *Blue*. Growth contours and steady-state  
1165 environments of species *Blue* and species *Red* are shown as curves and dots in the corresponding  
1166 colors (colored background indicates the “invasion zone” of *Red*, and represents the growth rate

1167 of *Red* in this zone). The supply condition is marked by a black circle. Black curves with arrows  
1168 show the time trajectory of the invasion in chemical space. Inset: time course of species biomass  
1169 in the chemostat during the invasion.

1170

1171 D. Same as (C), except that because the supply condition (black circle) is different, the attempted  
1172 invasion by species *Red* is unsuccessful.

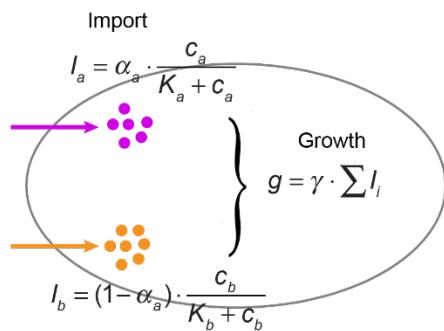
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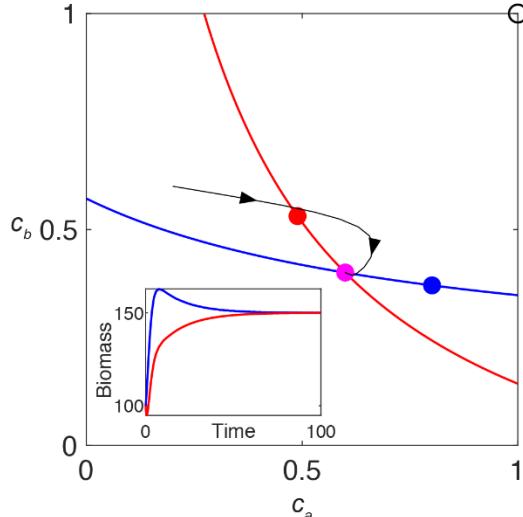
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Figure 2

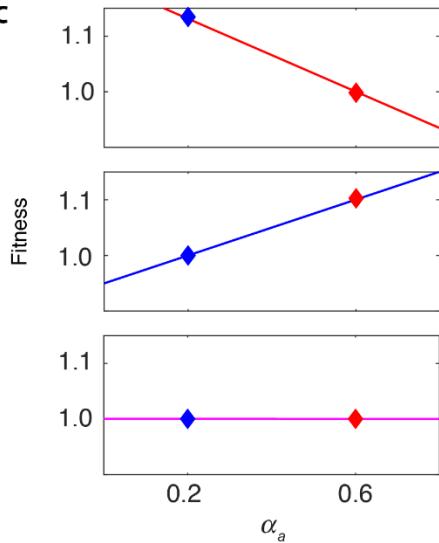
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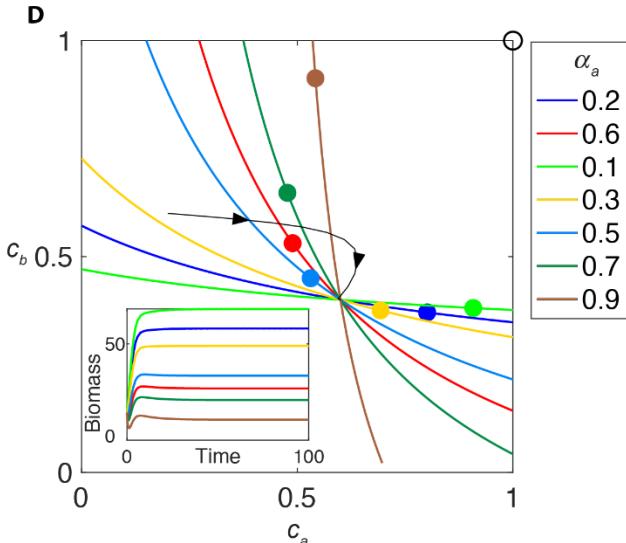
B



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D



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1178 **Figure 2 – Metabolic models with substitutable nutrients and coexistence in a chemostat.**

1179

1180 A. Example of a metabolic model with a trade-off in allocation of internal resources for import  
1181 of two substitutable nutrients, with both nutrients contributing additively to growth. Species *Red*  
1182 and species *Blue* allocate resources differently (indicated by parameter  $\alpha_a$ , see Methods).

1183

1184 B. Growth contours and the steady-state environments created by *Red* or *Blue* alone, under the  
1185 supply condition shown by the black circle. Black curve with arrows shows a trajectory in  
1186 chemical space. Purple dot indicates the steady-state environment created by *Red* and *Blue*  
1187 together. Lower inset: time course of species biomass.

1188

1189 C. From top panel to bottom panel: the fitness landscape created by *Red* alone (for the red dot in  
1190 (B)), created by *Blue* alone (for the blue dot in (B)), and created by both species (for the purple

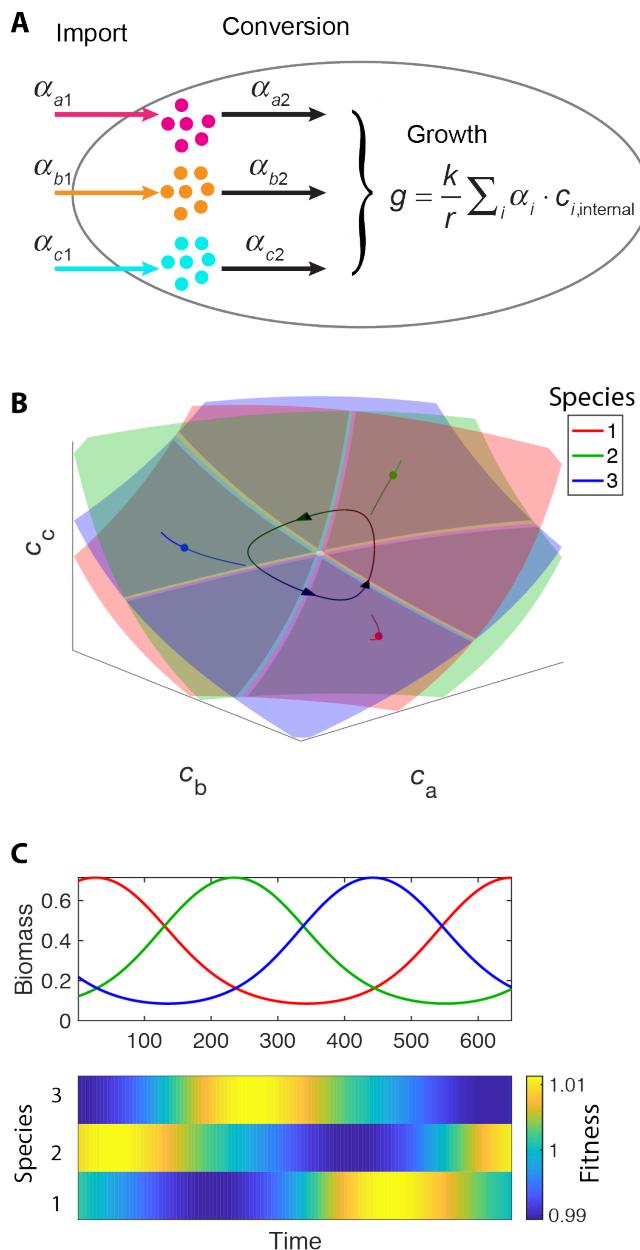
1191 dot in (B)). Diamonds mark the locations of *Red* and *Blue* strategies and their corresponding  
1192 fitness in each fitness landscape.

1193  
1194 D. Growth contours and the species-specific steady-state environments for seven different  
1195 species alone, under the supply condition shown by the black circle. Black curve with arrows  
1196 shows a trajectory in chemical space. Lower inset: time course of species biomass in the  
1197 chemostat.

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Figure 3



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Figure 3 - Rock-paper-scissors oscillations.

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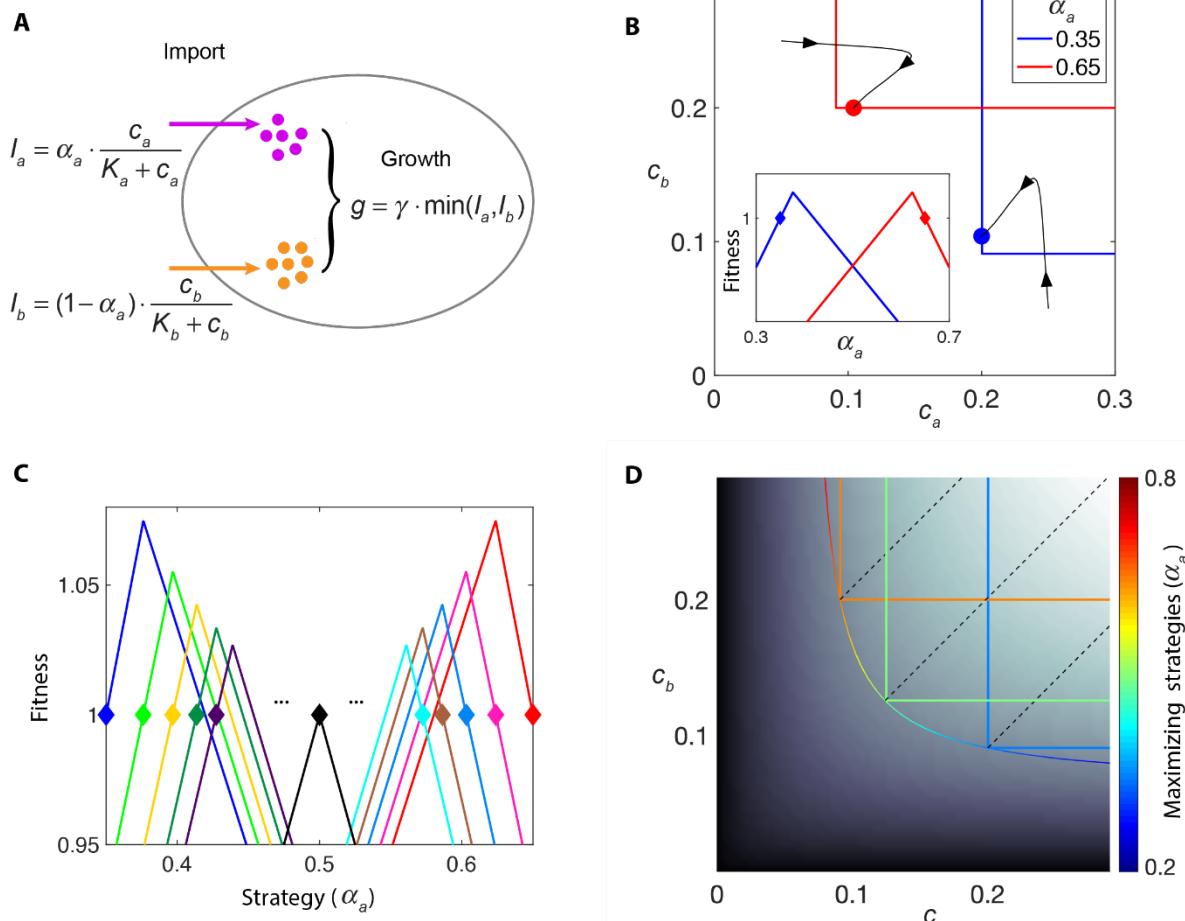
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A. Example of a metabolic model with a trade-off in allocation of internal resources for import and assimilation of three substitutable nutrients, with all three nutrients contributing additively to growth. Species *Red*, species *Blue*, and species *Green* allocate resources differently (see Methods).

- 1209 B. Growth contours (surfaces), flux-balance curves (lines), and steady-state nutrient  
1210 concentrations (dots) for the three species in a three-dimensional chemical space. Black curves  
1211 with arrows show the system's limit-cycle trajectory.  
1212  
1213 C. The top panel shows the time course of species biomass in the chemostat for the limit cycle in  
1214 (B). The bottom panel shows how the fitness landscape changes with time over one period of the  
1215 oscillation.  
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Figure 4



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**Figure 4 – Multistability, chain of invasion, and non-invasive strategy.**

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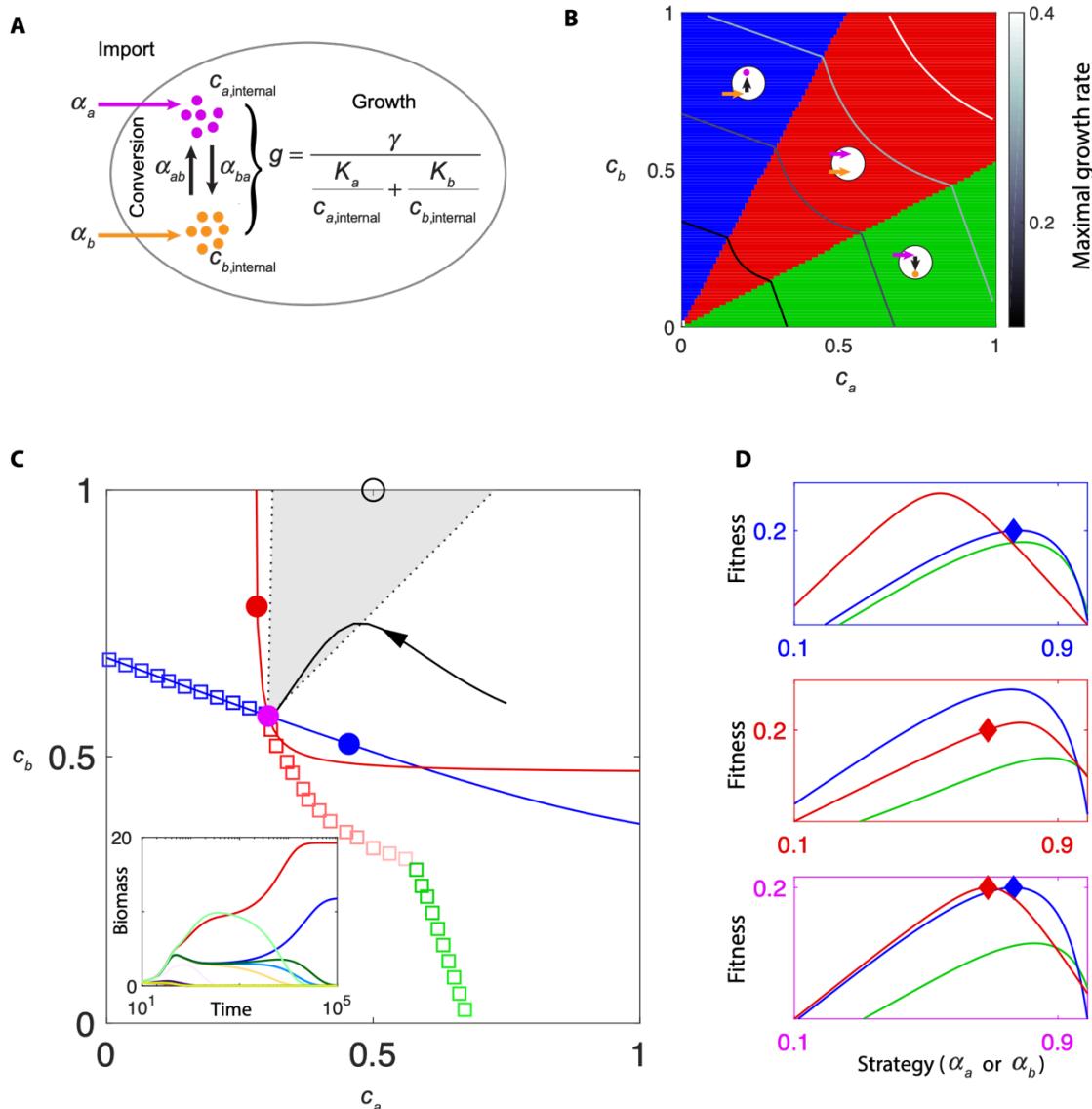
A. Example of bistability for a metabolic model with a trade-off in allocation of resources for import of two essential nutrients, with the lower of the two import rates determining growth rate. Species *Red* and species *Blue* allocate resources differently (indicated by parameter  $\alpha_a$ , see Methods).

B. Bistability of the system in (A) shown in chemical space. Black curves with arrows show the trajectories of simulations with different initial conditions. Inset: the fitness landscape created by species *Red* or *Blue* alone, with colors corresponding to the steady-state environments shown by colored dots in the main panel.

C. A chain of invasion. Fitness landscape created by species with different internal resource allocation strategies (marked by diamond shapes). Starting from species *Blue*, the species having the highest growth rate in the fitness landscape created by the “former” species is chosen. This creates a chain of invasion from *Blue* to *Light Green*, *Yellow*, *Deep Green*, *Deep Purple*, all the way (intermediate processes omitted) to the species *Black*, which places itself on the peak of its own fitness landscape. The same procedure is also performed starting with species *Red*.

1239 D. Depiction of non-invasive strategies under different supply conditions. Black-white  
1240 background indicates the maximal growth rate of model in (A) under each environment, and the  
1241 contour of maximal growth rates contains different strategies (represented by red-to-blue color).  
1242 Growth contours of three species adopting one of the “maximizing strategies” are colored by  
1243 their strategies. The supply conditions allowing these strategies to be “non-invasive” are marked  
1244 by dashed black lines.  
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Figure 5



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**Figure 5 - Non-invasive cartels.**

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A. Metabolic model with a trade-off in allocation of internal resources for import of two nutrients plus their interconversion, with both nutrients necessary for growth.

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B. Three subclasses of maximizing metabolic strategies in chemical space are indicated by background color, and circles with arrows illustrate the metabolic strategies of each subclass. The maximal growth contours for four growth rates (0.1, 0.2, 0.3, 0.4) are marked by gray colors.

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C. Two maximizing strategies co-creating a non-invasive steady state. At dilution rate 0.2, the maximal growth contour and the corresponding maximizing strategies are shown as colored squares. At a discontinuous point of the growth contour, the supply lines of two distinct

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metabolic strategies (Red and Blue) span a gray region, where any supply condition (e.g. black

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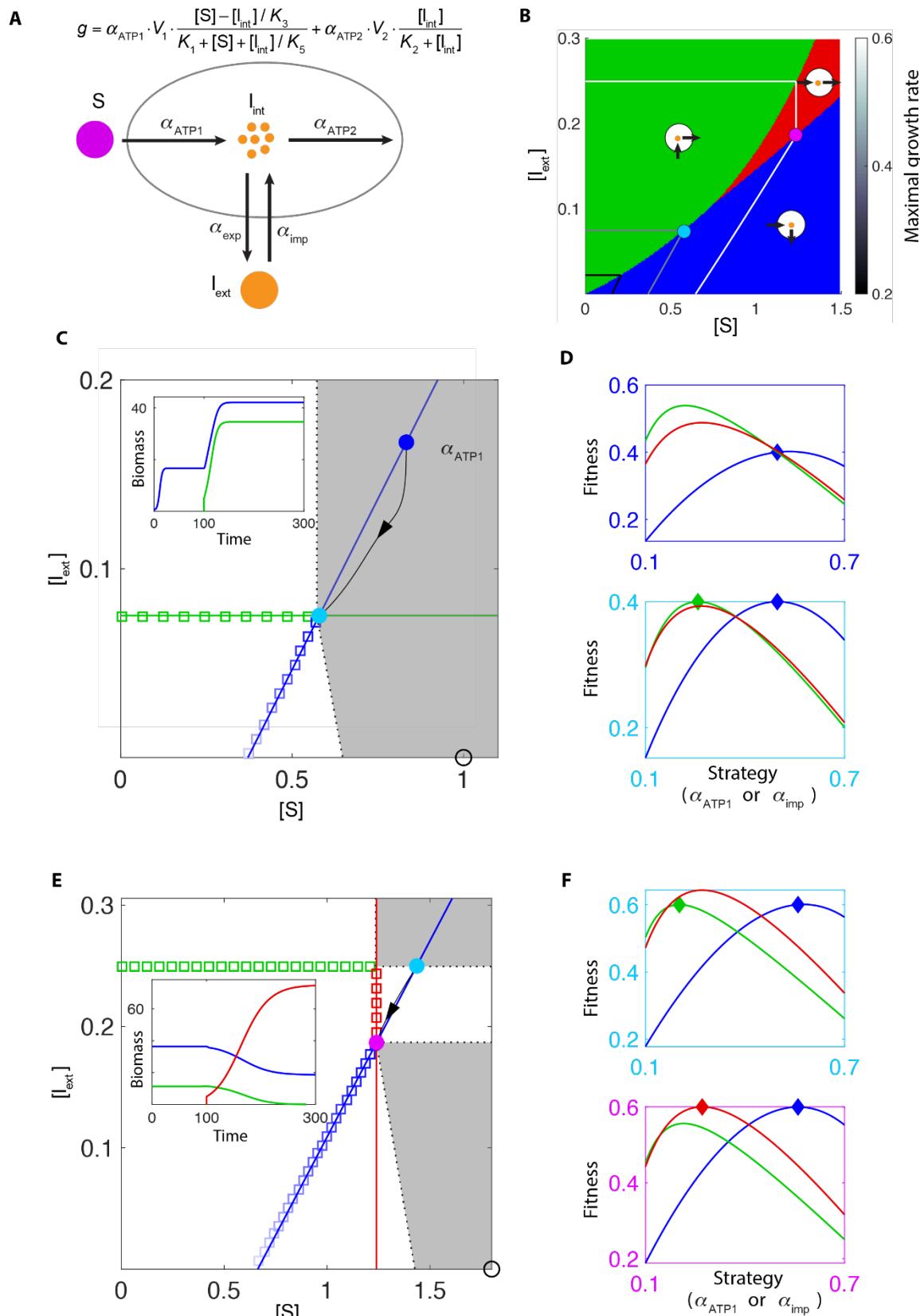
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1263 circle) requires the two maximizing strategies to co-create the environment on the discontinuous  
1264 point. Red and blue dots mark the environments created by species *Red* and species *Blue* alone,  
1265 and the purple dot marks the environment co-created by *Red* and *Blue*. Black curve with arrows  
1266 shows a trajectory in chemical space. Inset: competition dynamics of species *Red* and species  
1267 *Blue* together with 10 other maximizing species with different strategies.  
1268

1269 D. The fitness landscapes for the three environments in (C) indicated by corresponding box  
1270 colors. For class Green and Red, the strategy is represented by  $\alpha_a$ , for class Blue, the strategy is  
1271 represented by  $\alpha_b$ .  
1272

Figure 6



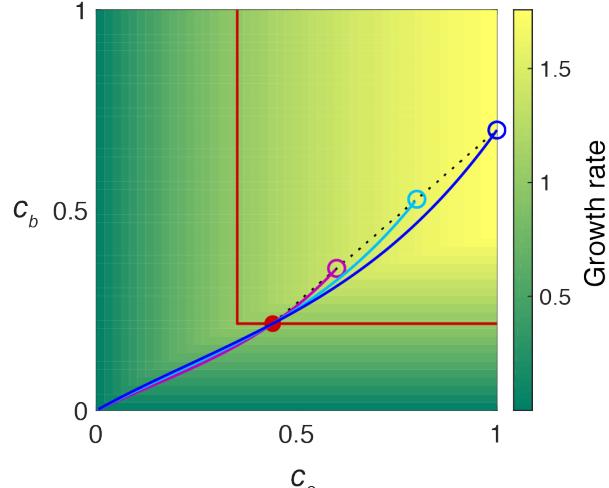
- 1274 **6 - Species creating new nutrient dimensions and achieving evolutionarily stable**  
1275 **coexistence.**  
1276  
1277 A. Metabolic model with a single supplied nutrient S. Cells allocate enzymes to convert S into  
1278 internal intermediate  $I_{int}$  and produce energy (denoted as “ATP”), export internal intermediate  
1279 into the chemostat to become  $I_{ext}$ , import external intermediate, or consume  $I_{int}$  to produce ATP.  
1280 The growth rate is the sum of ATP production (see Methods).  
1281  
1282 B. Three subclasses of maximizing metabolic strategies in chemical space are indicated by  
1283 background color, and circles with arrows illustrate the metabolic strategies of each subclass.  
1284 The maximal growth contours for three growth rates (0.2, 0.4, 0.6) are marked by black-to-white  
1285 colors.  
1286  
1287 C. At dilution rate 0.4, two maximizing strategies co-create a non-invasive environment. The  
1288 maximal growth contour and the corresponding maximizing strategies are shown as colored  
1289 squares. At a discontinuous point of the growth contour, the supply lines of two distinct  
1290 metabolic strategies (*Green* and *Blue*) span a gray region, where any supply condition (e.g. black  
1291 circle) requires two maximizing strategies to co-create the environment at the discontinuous  
1292 point. Blue dot marks the environment created by species *Blue* alone, and the cyan dot marks the  
1293 environment co-created by *Blue* and *Green*. Black curve with arrows shows a trajectory in  
1294 chemical space. Inset: time course of species biomass, with species *Green* added to the  
1295 chemostat at time 100.  
1296  
1297 D. The fitness landscapes for two environments in (C) indicated by corresponding colors of the  
1298 boxes, reflecting the relationship between instantaneous growth rate and resource allocation  
1299 strategy. For class *Blue* and *Red*, the strategy is represented by  $\alpha_{ATP1}$ ; for class *Green* the  
1300 strategy is represented by  $\alpha_{imp}$ .  
1301  
1302 E. Same as (C), except that the dilution rate is 0.6. Inset: time course of species biomass, starting  
1303 with *Blue* and *Green*, with species *Red* added to the chemostat at time 100.  
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1305 F. Same as (D), except corresponding to the two steady-state environments shown in (E).  
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1308 **SUPPLEMENTAL FIGURES**

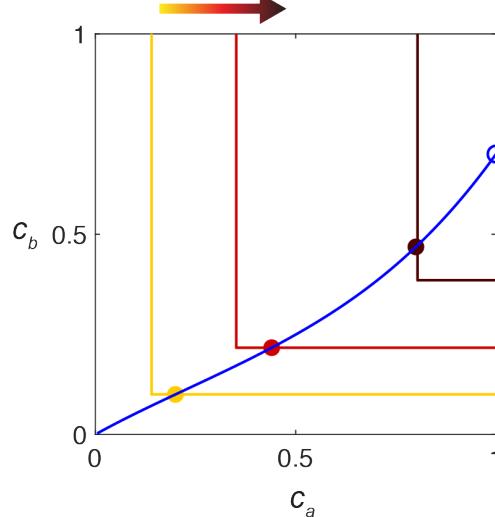
1309

Figure S1

A



B



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**Figure S1 - How supply concentrations and dilution rate separately influence the shapes of nullclines and the steady-state environment.**

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1313

A. Various supply concentrations can lead to the same steady-state chemical environment.

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Background color indicates the growth rate of cells as a function of nutrient concentrations  $c_a$  and  $c_b$ , with the growth contour shown by the red curve. The supply line for the steady-state environment (purple dot) is shown as a dotted black line. Different supply concentrations ( $c_{a,\text{supply}}$  and  $c_{b,\text{supply}}$ ) along the supply line are marked by purple, cyan, and blue circles, with the corresponding flux-balance curves shown in the same colors.

1315

B. Dilution rate can flip nutrient limitation. The external supply condition is marked by a blue circle, and the flux-balance curve for this supply is shown in the same color. Three growth contours with increasing dilution rates are shown from yellow to deep red, and the corresponding steady-state environments are shown in colored dots.

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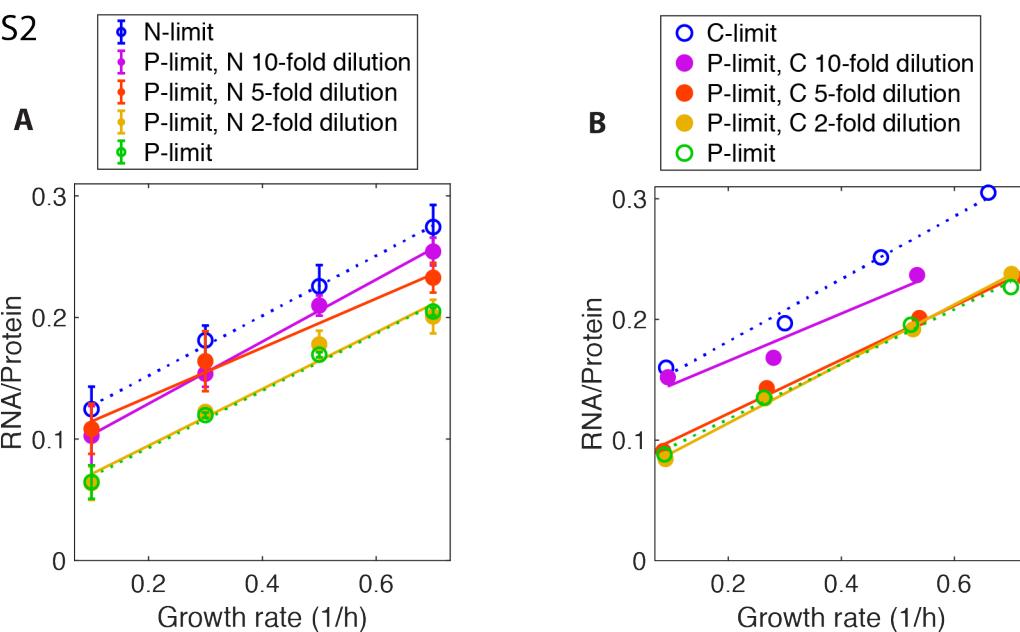
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Figure S2



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1332 **Figure S2 - Nutrient supply shifts the relationship between RNA/Protein ratio and growth**  
1333 **rate in chemostat.**

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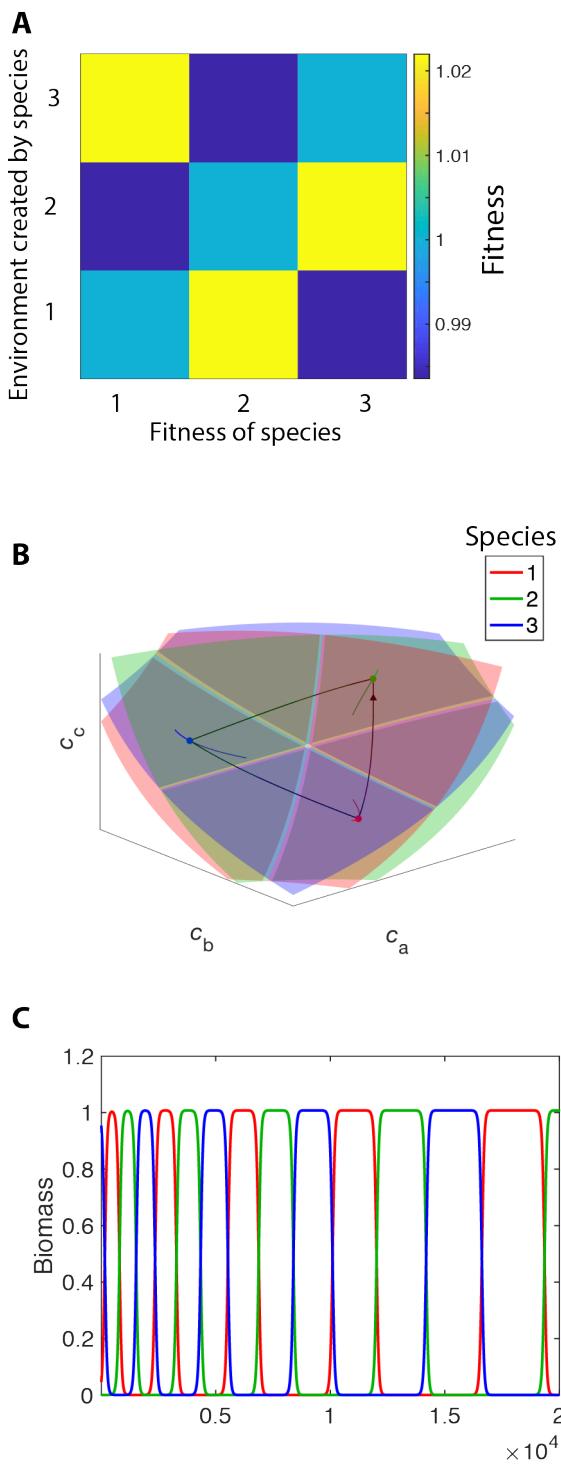
1335 A. The relationship between ribosome abundance represented by RNA/Protein ratio (y-axis) and  
1336 growth rate (x-axis) of *E. coli* cultured in chemostats from phosphorus limitation (P-limited,  
1337 green open circles and dotted line) to nitrogen limitation (N-limited, blue open circles and dotted  
1338 line). Starting from the P-limited condition, data for decreasing the supply concentration of  
1339 nitrogen by 2, 5, and 10-fold are shown as solid dots and corresponding best-fit lines. Each  
1340 measurement was repeated three times and standard errors are shown by bars.

1341

1342 C. Same as (B), but for phosphorus and carbon limitation instead of phosphorus and nitrogen  
1343 limitation. Starting from the P-limited condition, data for decreasing the supply concentration of  
1344 carbon by 2, 5, and 10-fold are shown as solid dots and corresponding best-fit lines.

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Figure S3



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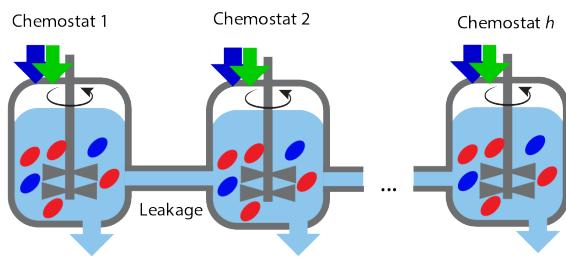
Figure S3 - Rock-paper-scissor fitness landscape and heteroclinic cycle

A. The fitness of Species 1, 2, and 3 in the steady-state environment constructed by species 1, 2, and 3 for the model in Fig 3.

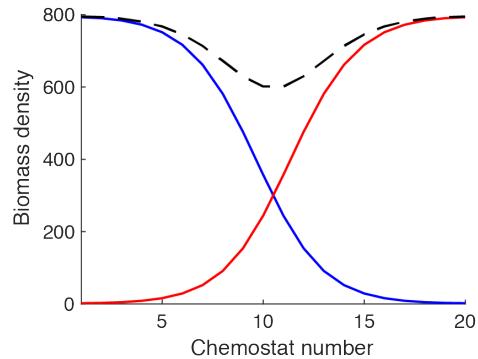
1352 B. Growth contours (surfaces), flux-balance curves (lines), and steady-state nutrient  
1353 concentrations (dots) for three species in a three-dimensional nutrient space, with a different  
1354 conversion speed ( $k = 10$ ) than in Fig. 3 ( $k = 1$ ) (see Methods). Black curves with arrows show  
1355 the system's oscillatory trajectory.  
1356  
1357 C. course of species biomass in the chemostat over a long duration for species in (B).  
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Figure S4

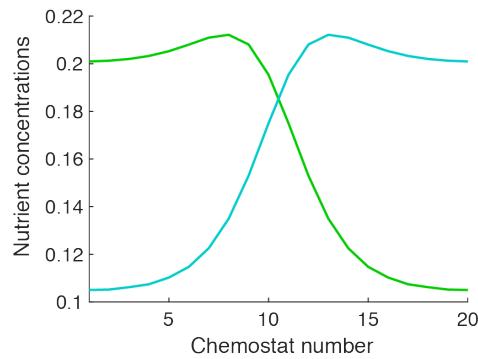
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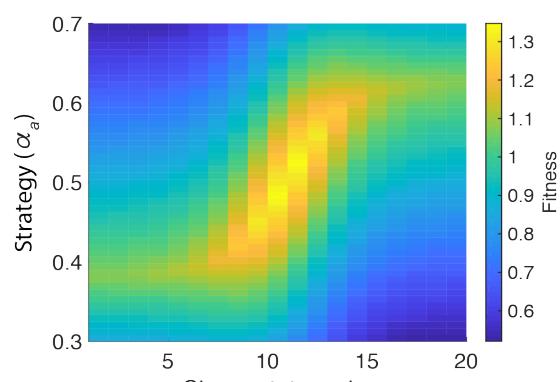
B



C



D



1362 **Figure S4 - Steady-state spatial heterogeneity for linked chemostats.**

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1364 With initial seeding of two species, one at each of the two ends of a chain of chemostats, a  
1365 steady-state gradient of species biomass density spontaneously emerges accompanied by a  
1366 gradient of nutrient concentrations, even though the supply conditions and dilution rates are  
1367 identical for all the chemostats.

1368

1369 A. Schematic of  $k_{\text{tot}}$  linked chemostats exchanging medium and cells via leakage, described by  
1370 Eqs. S34-S35. The two species in the chemostats (*Blue* and *Red*) are the same bistable pair as in  
1371 Fig 4B and the leakage rate is  $l = 1$ .

1372

1373 B. The species composition along 20 linked chemostats for the system in (A). Species colors  
1374 correspond to those in Fig 4B, with species *Blue* having  $\alpha_a = 0.35$  and species *Red* having  $\alpha_a =$   
1375 0.65. The dashed black curve shows the sum of the two biomass densities. The initial condition  
1376 was cell-free chemostats with a small amount of *Blue* added to Chemostat 1 and small amount of  
1377 *Red* added to Chemostat 20.

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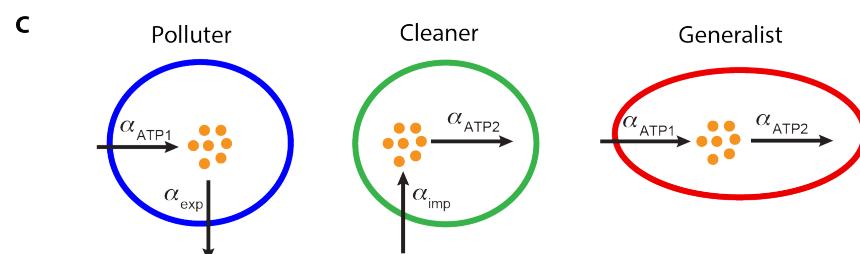
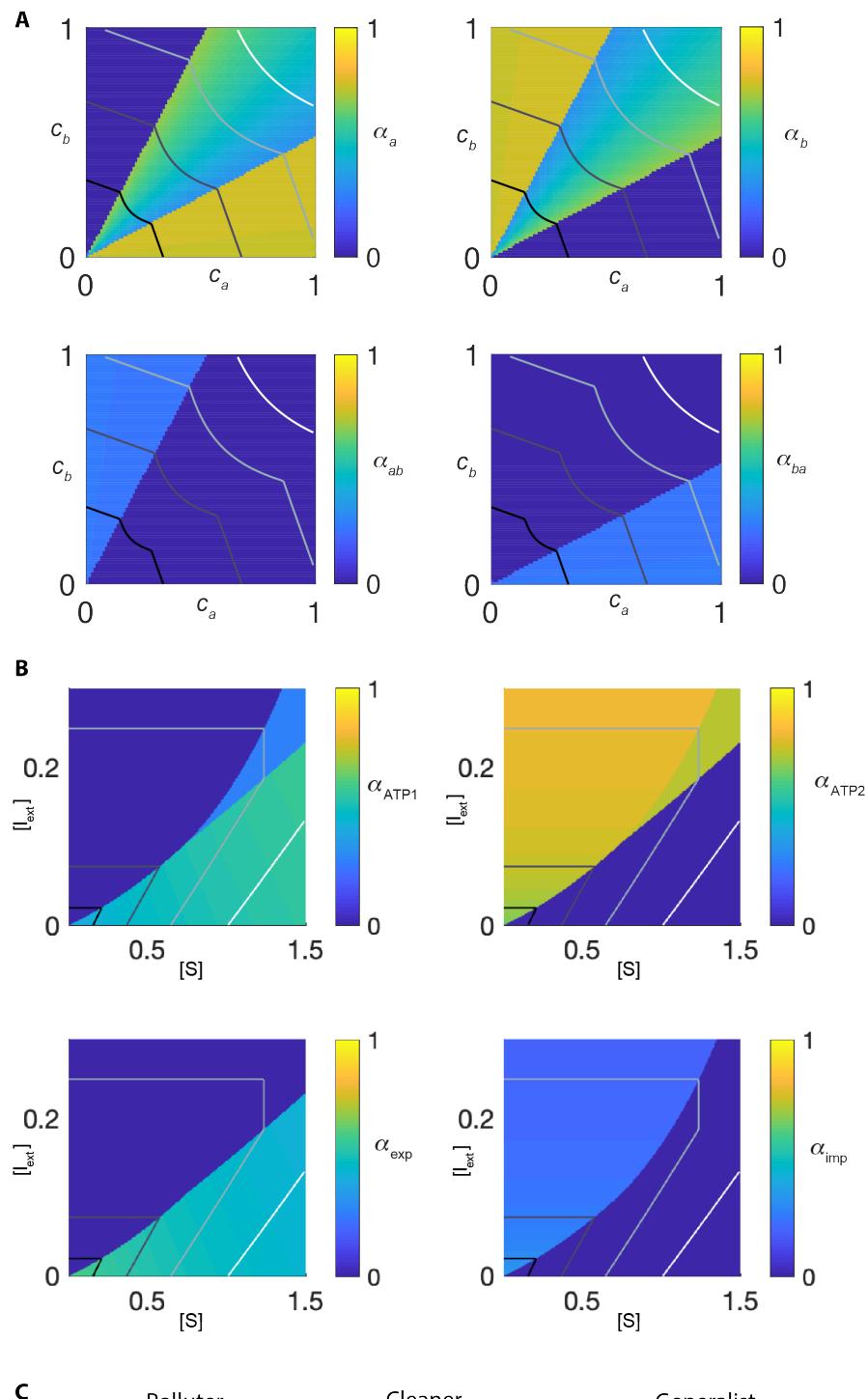
1379 C. Concentrations along the 20 chemostats for nutrient *a* (green) and nutrient *b* (cyan) for system  
1380 in (A).

1381

1382 D. The fitness landscape along the chain of chemostats. The *x*-axis is the 20 linked chemostats,  
1383 and the *y*-axis is the metabolic strategy represented by  $\alpha_a$ . Color indicates the growth rate of  
1384 species adopting the given strategy in the  $k$ -th chemostat.

1385

Figure S5



1387 **Figure S5 – Maximizing strategies in chemical space.**

1388

1389 A. For each environment in the chemical space, the maximizing resource allocation strategies  
1390 that maximize growth rates for the model in Fig 5A. Each strategy is represented by the four  
1391 elements  $[\alpha_a, \alpha_b, \alpha_{ab}, \alpha_{ba}]$ , and values for each element are shown by a heatmap. Black-to-  
1392 white curves are the maximal growth contours for  $d = 0.1, 0.2, 0.3, 0.4$ .

1393

1394 B. For each environment in the chemical space, the maximizing resource allocation strategies  
1395 that maximize growth rates for the model in Fig 6A. Each strategy is represented by the four  
1396 elements  $[\alpha_{ATP1}, \alpha_{ATP2}, \alpha_{exp}, \alpha_{imp}]$ , and values for each element are shown by a heatmap.  
1397 Black-to-white curves are the maximal growth contours for  $d = 0.2, 0.4, 0.6$ .

1398

1399 C. Schematic representations of the three classes of maximizing strategies appearing in (B).

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