

The Environmental Games

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Abstract

For over 4 billion years, the environment has guided evolutionary selection by presenting biological entities with opportunities and challenges and by mediating their interactions. In evolutionary game theory (EGT), the replicator equation defines the game according to direct interactions between biological entities. Eco-evolutionary models have described environmental feedback by modeling environmental states dynamically. Yet, these models channel environmental pressures through the direct interaction coefficients instead of creating a distinct game. Here, I invent Pure Environmental Games (PEGs) and formally introduce the environment as a strategy in EGT. The PEGs describe the neutral selection game and environmental games operating in parallel. Through the net specific accumulation rates of microbes parametrized by the environmental state, Environmental Biotechnology — an engineering discipline designing wastewater treatment plants worldwide — offers a collection of instances of PEGs. The environmental strategy resists invasion from biological strategies when it is a strict Nash equilibrium. The PEGs provide a template for identifying regimes of environmental selection within an environmental game (e.g., the r/K selection game). This mapping identifies persistence as a new dynamic regime under extinction. In a closed system, life persists by partnering with the environmental strategy consistent with Schrödinger's negative entropy.

Keywords: evolutionary game theory, environmental biotechnology, replicator dynamics, microbial ecology, thermodynamic boundaries

Introduction

The environment is the “invisible” hand directing evolution. As early lifeforms invaded uninhabited frontiers, the environment selected its new residents by presenting both opportunities and challenges. Yet, the environment has not been formally introduced as a strategy in evolutionary game theory (EGT).

The replicator equation (Figure 1a) and Lotka-Volterra model (LVM) (Figure 1b) are foundational equations in the EGT and population ecology, respectively. Hofbauer and Sigmund¹ proved the equivalence of the two models with n LVM species mapping onto $n + 1$ replicator strategies. This mapping separates the intrinsic growth rates under S_0 from the direct interaction coefficients in the payoff matrix (S_n for $n \geq 1$, Figure 1d). Traditionally, the direct interaction coefficients define the game². When the intrinsic growth rates are assumed to be identical across different strategies, traditional EGT classifications map to replicator dynamics (Supplementary Note S2;³).

Among EGT classifications, neutral selection corresponds to a game where deterministic replicator dynamics no longer control biological behavior (Figure 2b; Chapter 4 of Nowak³). However, when environmental conditions differentiate the intrinsic growth rates of biological entities^{2,4}, the intrinsic growth rates can drive selection even in the absence of replicator dynamics². A differential in intrinsic growth rates alters the selection baseline against which direct interspecies interactions operate; thereby, mapping between the game and EGT classifications is disrupted². Therefore, Tarnita and Traulsen² call for the field of EGT to embrace approaches that incorporate a more nuanced ecology, such as Lotka-Volterra or adaptive dynamics.

Past eco-evolutionary models have dynamically tracked absolute quantities (such as population density and environmental state) and modified direct interaction coefficients to describe the environmental feedback^{5–8}. While this approach has the potential to bring nuanced ecology, it

47 does not dissolve the difficulty of mapping the game to EGT classification when the intrinsic rate
48 differentials exist.

49 Here, in response, I invent a new evolutionary game beyond the direct interaction coefficients
50 in two steps. Step 1 derives an ideal LVM framework by setting the direct-interaction submatrix
51 to null. Step 2 sets the payoffs that biological strategies derive from the environmental strategy
52 to the net specific accumulation rates parameterized by the environmental state, yielding the Pure
53 Environmental Games (PEGs). Thereby, the PEGs will provide a more ecologically nuanced
54 interpretation of the neutral selection game by visiting the evolutionarily stable strategy (ESS)
55 classifications and the r/K selection game as an instance of PEGs. While the main text provides
56 high-level ideas, the Supplementary Note provides the following domain-specific concepts and
57 derivations for a broad readership:

- 58 • Note 1 provides a technical overview.
- 59 • Notes 2–3 establish EGT foundations and the replicator equation and Lotka-Volterra equiva-
60 lence.
- 61 • Note 4 derives the ideal LVM, which serves as a skeleton and a precursor to the PEGs.
- 62 • Note 5 defines the PEGs and identifies Environmental Biotechnology as a collection of
63 instances of PEGs to import environmental selection principles and conservation laws.
- 64 • Note 6 dynamically models environmental selection operating in parallel to the limiting case
65 of neutral selection using *Methanosarcina-Methanotherix* as a canonical example.

66 This work aims to serve as a foundation for cross-disciplinary conversations between EGT, En-
67 vironmental Biotechnology, and Microbial Ecology — and other curious minds — rather than to
68 claim novel predictions.

69 Pure Environmental Games

70 Imagine biological entities as gas molecules. At low temperatures or under high density, gas
71 molecules interact and behave non-ideally. At high temperatures or under low density, the inter-
72 actions become negligible, revealing the ideal gas law. In a similar spirit, removing interactions
73 between biological entities “purifies” environmental selection.

74 Begin by setting the game to the limiting case of neutral selection where $a = b = c = d = 0$
75 (Figure 2a). This produces the ideal LVM, where deterministic selection can continue via the
76 intrinsic growth rates (u and v in Figure 1d). Equating the intrinsic growth rates to the payoffs that
77 biological strategies receive from the environmental strategy, $\mu_{a,i}(\mathbf{E})$, produces the PEGs (Figures
78 1c and 2a).

79 The general payoff matrix shows the relationship between PEGs and traditional EGT (Figure
80 1d) in two parts: the direct interaction submatrix defines the game in traditional EGT; the intrinsic
81 growth submatrix defines the environmental game. By setting the game to null, the PEGs describe
82 the neutral selection game operating in parallel to the environmental game. The PEGs depart from
83 the traditional EGT by making S_0 the environmental strategy and x_0 the environmental frequency.
84 The asymmetry between one environmental strategy and n biological strategies indicates that all
85 biological strategies share the same local environment. Multiple environmental strategies may
86 exist in the presence of environmental variation, though spatial games are beyond the scope of this
87 work. As with the traditional EGT, the biological strategies still describe a fixed or “hardwired”
88 phenotype. The payoffs can change, but the underlying representation does not. Thereby, $\mu_{a,i}(\mathbf{E})$
89 provides a mathematical means for the environmental pressure to alter the fitness of i -th biological
90 entity when multiplied by x_0 .

91 By placing $\mu_{a,i}(\mathbf{E})$ in the general payoff matrix, the environmental strategy can be interpreted
92 through the evolutionary stability concepts with a caveat. Static equilibrium concepts describe
93 strategies in relation to the payoff structure. Because the environmental state is a dynamic variable,
94 the payoff matrix becomes dynamic. Consequently, the ESS classification of a strategy (e.g., S_0)
95 becomes state dependent.

Evolutionarily Stable Strategy

The concept of ESS was introduced by Smith and Price⁹ as a static equilibrium to describe strategies that, if adopted by the majority, resist invasions by rare mutants. Consider a PEG with one biological strategy with the following payoff structure, equation (1):

$$\begin{array}{c|cc}
 & S_0 & S_1 \\
 \hline
 S_0 & 0 & 0 \\
 S_1 & \mu_{a,1}(\mathbf{E}) & 0
 \end{array} \tag{1}$$

On the one hand, the biological strategy, S_1 , is always a Nash equilibrium, and its ability to invade depends entirely on the environmental state. On the other hand, S_0 is a strict Nash equilibrium and resists invasion if $\mu_{a,1}(\mathbf{E}) < 0$. S_0 disqualifies as a (strict) Nash equilibrium when $\mu_{a,1}(\mathbf{E}) > 0$ and the biological strategy can invade the environment. At a steady state, the payoff matrix becomes null, and the environmental strategy is a Nash equilibrium (not strict). These ESS classifications are independent of the implementation of $\mu_{a,1}(\mathbf{E})$.

It should be noted that the ESS classification is based on static equilibrium concepts, and a null payoff matrix does not imply the absence of selection dynamics. The selection dynamics will depend on the implementation of $\mu_{a,i}(\mathbf{E})$ and parameterization of the environmental game. The PEGs take a modular approach, and $\mu_{a,i}(\mathbf{E})$ can be set to any specific accumulation rate that depends on the environmental state. Environmental Biotechnology provides a collection of $\mu_{a,i}(\mathbf{E})$ implementations as biomass balances. The next section presents one such instance, the classic r/K game, and describes its environmental selection dynamics.

Environmental Selection Dynamics

Environmental Biotechnology is an academic discipline rooted in sanitary engineering – one of the defining public health achievements of the 20th century. Environmental Biotechnology brings to PEGs rich principles of environmental selection, integrated into biological models, empirically

validated through the design, operation, and control of tens of thousands of biological wastewater treatment processes worldwide^{10–13}. The solids residence time, θ_x , is a fundamental parameter for environmental selection¹³. In practice, engineers can set θ_x to enrich for stable microbiological communities with desirable attributes. In a medical context, θ_x may be defined by physiology, such as in the human digestive system¹⁴. This section identifies six dynamic regimes within the r/K selection game operating in parallel to the neutral selection game with *Methanosarcina* and *Methanothrix* as a canonical example of environmental succession. The analysis assumes an idealized chemostat (spatially homogeneous and no wall effects) (Supplementary Note S6). For simplicity, the energy dissipated by microorganisms is assumed to be transported out of the system by the bulk fluid (Supplementary Note S5). Parameters for *Methanosarcina* and *Methanothrix* are well-grounded in experimental data, microbial kinetics, and thermodynamics^{15–17}. Six selection dynamics are identified with Roman numerals (Regimes I–VI) and illustrated conceptually (Figure 2c), which are then identified within the parameter space of θ_x and the influent substrate concentration (S^0) (Figure 3) with full dynamics presented in Supplementary Note S6 (Extended Data Fig. 1–4).

Regime I) Washout

Monod kinetics defines the minimum solids residence time required to prevent washout, $[\theta_x^{min}]_{lim}$, for each type of microbe¹⁸ (Extended Data Fig. 1). When $\theta_x < [\theta_x^{min}]_{lim}$, $\mu_{a,i}(\mathbf{E}) < 0$ for all biological strategies, the environmental strategy is a strict Nash equilibrium. The system removes microbes faster than they can grow, and the environmental strategy resists invasions. Although $[\theta_x^{min}]_{lim}$ sets a strict condition for washout, the white region below the curve in Figure 3 ($S^0 < S^*$) also is generally known as washout.

Regimes II and IV) r/K Selection

Microbes require sufficient time and energy to invade the environmental strategy. When $[\theta_x^{min}]_{lim} < \theta_x$, θ_x sets the anticipated steady-state substrate concentration, S^* ¹⁸. If $S^0 > S^*$, $\mu_{a,i}(\mathbf{E}) > 0$. Con-

sequently, the environmental strategy disqualifies as a (strict) Nash equilibrium. Microorganisms can invade the environment, and the environment can, in turn, select biological strategies.

r/K selection is a classic microbial ecology model for ecological succession. The r-strategist represents opportunists who thrive in volatile environments (*Methanosarcina*). The K-strategist represents gleaners thriving in stable environments (*Methanothrix*). For describing competitive exclusion, S^* is the same quantity as Tilman's R^* derived for different systems^{18,19}. Those microbes with a lower S^* can completely exclude their competitors²⁰ (Supplementary Note S5 and S6). The critical solids residence time, θ_x^{crit} marks the transition between the selection dynamics. When $\theta_x > [\theta_x^{min}]_{lim}$ and $\theta_x < \theta_x^{crit}$, $S_r^* < S_K^*$ and the r-strategists can invade the environmental strategy to establish a steady presence and dominate over K-strategists (Extended Data Fig. 2). Similarly, K-strategists dominate over r-strategists when $\theta_x > [\theta_x^{min}]_{lim}$ and $\theta_x > \theta_x^{crit}$, $S_r^* > S_K^*$ (Extended Data Fig. 3).

Regime III) Environmentally Neutral Selection

Between Regimes II and IV, environmental neutrality occurs at $\theta_x = \theta_x^{crit}$, which ensures $S_r^* = S_K^*$ and $\mu_{a,1}(\mathbf{E}) = \mu_{a,2}(\mathbf{E})$ at steady state (see Supplementary Note S6). As with neutral selection in replicator dynamics³, deterministic environmental selection dynamics no longer describe the outcome of r/K selection. In principle, exclusion of microbes with identical environmental fitness can be described by neutral drift^{3,21}. In practice, however, r- and K-strategists such as *Methanosarcina* and *Methanothrix* do coexist in biological reactors due to non-ideal conditions, and their coexistence can be promoted through mechanisms such as feeding controls, cross-feeding, and spatial heterogeneity¹⁵.

Regime V) S_{min}^*

As θ_x approaches infinity, the steady-state substrate concentration asymptotically approaches S_{min}^* , which represents the minimum substrate concentration that can maintain steady-state biomass²². At S_{min}^* , the minimum energy dissipation rate for sustaining a steady-state biomass for *Methanothrix*

167 is 1.4×10^{-11} kJ cell⁻¹ y⁻¹ (Extended Data Fig. 4).

168 **Regime VI) Persistence**

169 Below S_{min}^* , microorganisms decay exponentially, which is described mathematically as fast ex-
 170 tinction²³. However, extinction can be slow enough to be relevant in terms of physical time, as
 171 microorganisms can shut down their metabolism to the bare minimum of maintenance require-
 172 ments²⁴. Deep-ocean sediment is among the few natural systems known to approximate an isolated
 173 system. Deep-ocean microorganisms may persist for thousands or millions of years by reducing
 174 the energy dissipation rate to as low as 10^{-15} kJ cell⁻¹ y⁻¹²⁴, a 4-order-of-magnitude reduction
 175 compared to *Methanotrix* at S_{min}^* . Persistence is relevant in environmental contexts where microor-
 176 ganisms buy time until invasion becomes possible, using mechanisms such as metabolic dormancy
 177 and sporulation.

178 **Significance**

179 Describing the immense diversity of this planet, represented largely by microorganisms, requires
 180 an evolutionary language^{25,26}. Earth provides life with countless environmental variations, which
 181 serve as the foundation for building immense biodiversity. Therefore, a tool is needed to ask how
 182 life invades the environment.

183 In traditional EGT, the direct interaction submatrix defines the game². Eco-evolutionary models
 184 have incorporated environmental feedback into the direct interaction matrix⁵⁻⁸. The PEGs depart
 185 from the tradition by creating the environmental game that operates in parallel to the neutral
 186 selection game. All environmental pressures were ensured to enter through the environmental
 187 game by setting the direct interaction submatrix (the game) to null (the limiting case of neutral
 188 selection).

189 The PEGs formally introduce the environment as a strategy. By placing the net accumulation
 190 rates of microorganisms in the environmental game submatrix, the PEGs recruit over a century

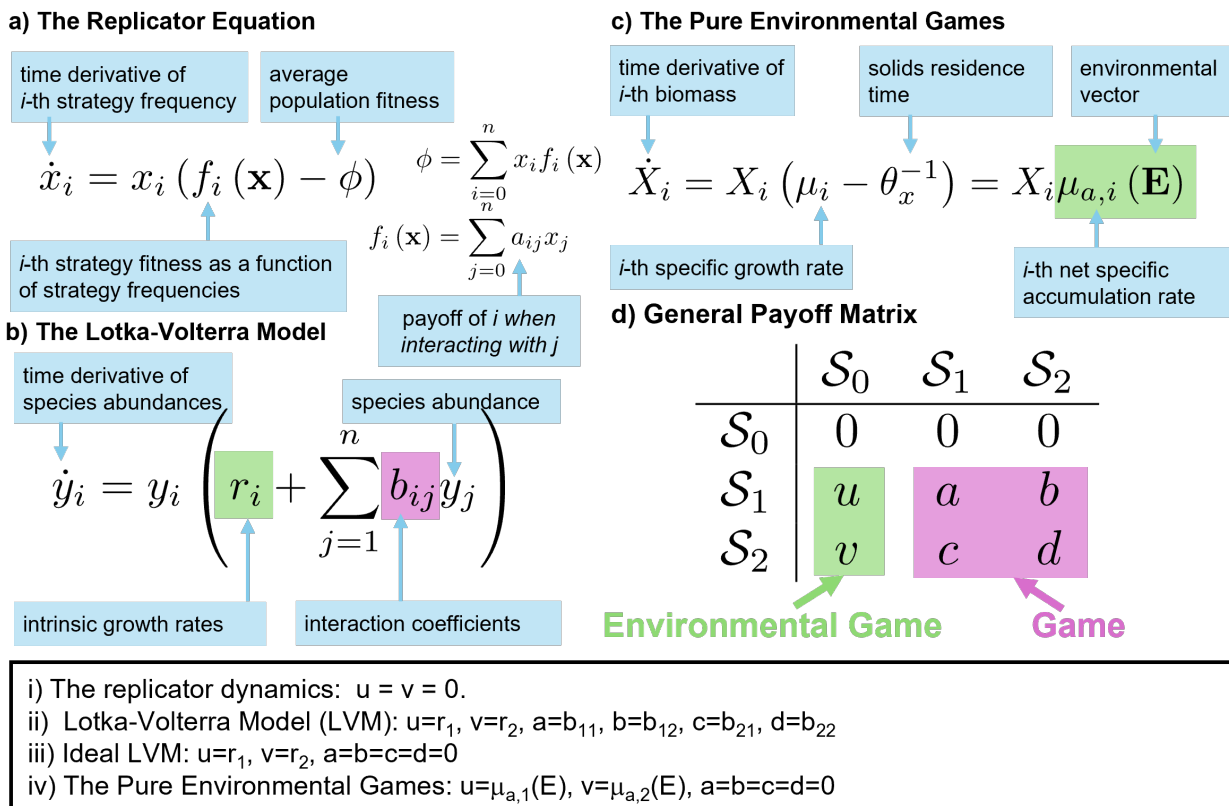
of accumulated knowledge of environmental selection from Environmental Biotechnology into evolutionary games. r/K selection was chosen as an instance of an evolutionary game because its microbial kinetics parameters can be estimated from first principles^{17,27} and are established empirically¹⁶. The r/K selection game demonstrates its environmental selection is bound by time ($[\theta_x^{min}]_{lim}$) and energy (set at S_{min}^*)^{18,22}. Within this boundary, biological strategies invade the environment and compete, not through direct interactions, but through indirect interactions mediated by the environmental strategy. Having deterministic selection continue under the neutral selection game is consistent with Tarnita and Traulsen² under $r_1 \neq r_2$. Deterministic dynamics no longer determine a unique outcome, and neutral drift can occur when $\mu_{a,1}(\mathbf{E}) = \mu_{a,2}(\mathbf{E})$.

By dynamically modeling the environmental state, the PEGs bring empirically grounded knowledge of microbial kinetics and thermodynamics into the EGT. The principles of environmental selection and ESS work elegantly together: thermodynamic feasibility has historically served as a guide for anticipating novel metabolism (e.g., anaerobic ammonium oxidation was anticipated two decades before its discovery^{28,29}), and now ESS concepts can define when these novel biological strategies can invade the environmental strategy.

Critically, the PEGs make the system boundary explicit. The PEGs describe mass exchange between the system and the surroundings using θ_x , which is also a fundamental parameter in spatially heterogeneous systems such as biofilms. According to Erwin Schrödinger, life feeds on negative entropy to maintain order³⁰. In a closed system, established when θ_x reaches infinity, exporting entropy to the surroundings is prohibited. However, deep-sea sediment microorganisms exemplify persistence by dissipating energy through interactions with the environmental strategy.

The PEGs follow a modular design principle. Analyzing the dynamic regimes within an instance of PEGs brings physical nuance to exploring traditional EGT concepts defined mathematically. The persistence regime is particularly important in microbial ecology, where extinction is not as readily observable as in macroecology. Persistence may help to explain resurgences of measles³¹ and resilience of antibiotic resistance genes³². Other instances of environmental games may be considered by modifying the payoff that biological entities receive from the environment.

218 Figure Legends



219

220 Figure 1. a) The replicator equation models the rate of change in strategy frequency $\dot{x}_i$, where the
 221 subscript i indexes strategies. The frequency increases when its fitness, f_i , exceeds the average
 222 population fitness, ϕ . f_i is determined by summing the product of the payoff coefficient, a_{ij} , by
 223 x_j . ϕ is determined by summing the products of f_i and x_i . b) The Lotka-Volterra model (LVM)
 224 models the rate of change in species abundance $\dot{y}_i$. The LVM has two parameters: intrinsic growth
 225 rate, r_i , and the interaction coefficient, b_{ij} . c) The Pure Environmental Games (PEGs) model
 226 the biomass concentration in the absolute coordinate $\dot{X}_i$. The PEGs have one coefficient, the net
 227 specific accumulation rate, $\mu_{a,i}$, which is parameterized by the environmental vector, $\mathbf{E}$. d) The
 228 general payoff matrix summarizes the relationship between three equations for the case of two
 229 biological strategies. The replicator equation models the competition of biological strategies. The
 230 first row is set to zero (i.e., $f_0 = 0$) to allow the replicator equation to track the total abundance or

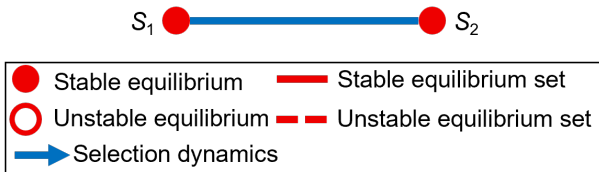
231 concentration of biological components in the absolute coordinate. The coefficients a , b , c , and
 232 d encode the payoff resulting from direct interactions between strategies. The LVM models the
 233 ecological interactions between species in the absolute coordinate. When the LVM is mapped to
 234 the replicator equation according to Hofbauer and Sigmund¹, the coefficients u and v equal the
 235 intrinsic growth rates. The ideal LVM can be derived by setting $a = b = c = d = 0$. The PEGs
 236 model systems driven by interactions of biological strategies with the environmental strategy. Mass
 237 conservation relationships from Rittmann and McCarty¹³ provide abundant instances of PEGs.
 238 Structurally, the PEGs are analogous to the ideal LVM, and u and v equal the net specific biomass
 239 accumulation rates, $\mu_{a,i}(\mathbf{E})$.

a) **Dynamic Payoff Matrix of PEGs**

	S_0	S_1	S_2
S_0	0	0	0
S_1	$\mu_{a,1}(\mathbf{E})$	0	0
S_2	$\mu_{a,2}(\mathbf{E})$	0	0

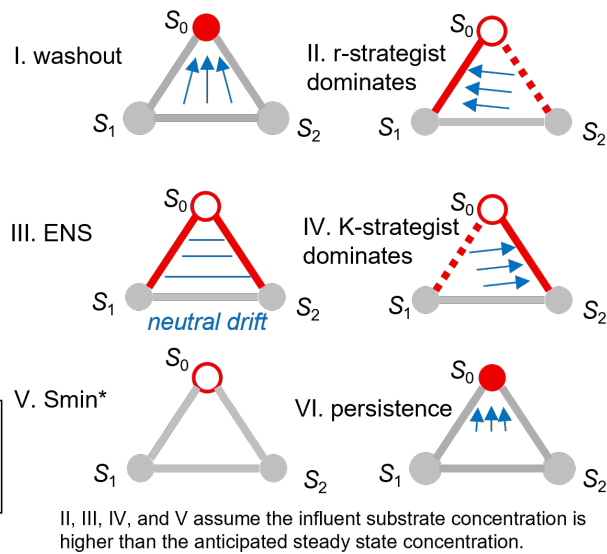
b) **Neutral Selection** between S_1 and S_2

Traditional neutral selection when $\mu_{a,1}(\mathbf{E}) = \mu_{a,2}(\mathbf{E})$



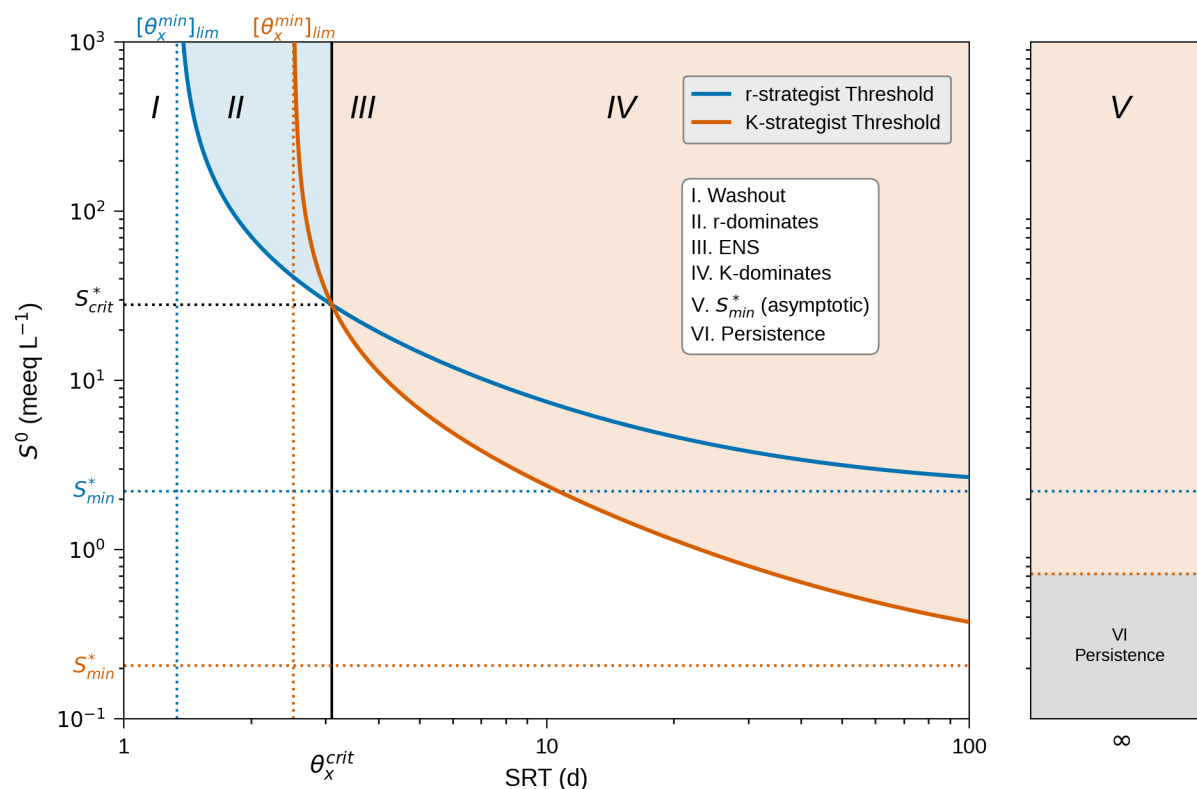
c) **Environmental Selection Dynamics**

of the r/K selection game among S_0 , S_1 , and S_2



240

241 Figure 2. a) The dynamic payoff matrix for the Pure Environmental Games (PEGs). b) The
 242 PEGs demonstrate the traditional neutral selection dynamics when $\mu_{a,1}(\mathbf{E}) = \mu_{a,2}(\mathbf{E})$. c) Six
 243 environmental regimes of the r/K environmental game that operate in parallel to neutral selection.
 244 I. In washout ($\theta_x < [\theta_x^{min}]_{lim}$), biological strategies cannot grow fast enough and cannot invade the
 245 environmental strategy. II. The r-strategist dominates by establishing a stable dynamic equilibrium.
 246 III. At $\theta_x = \theta_x^{crit}$, the anticipated steady state substrate concentrations $S_r^* = S_K^*$ (i.e., environmentally
 247 neutral selection, ENS). Thus, the r-strategist and K-strategist are environmentally neutral. IV. The
 248 K-strategist dominates. V. Under S_{min}^* , infinitesimally small biomass establishes a stable dynamic
 249 equilibrium. VI. In the extinction regime, persistence becomes an evolutionary strategy.



250

251 Figure 3. Six environmental selection regimes (Roman numerals I-VI as defined in legend) within
 252 the parameter space of the r/K environmental game. x-axis and y-axis show the solids residence time
 253 (θ_x , d) and influent substrate concentration (S^0 ; meeq L^{-1}), respectively. Blue and Vermillion lines
 254 describe the influent substrate thresholds for establishing steady state biomass. They are obtained by
 255 calculating the steady state substrate concentration for r-strategist (S_r^* ; meeq L^{-1}) and K-strategist
 256 (S_K^* ; meeq L^{-1}), respectively. The regions above S_r^* and S_K^* are color-filled to identify where
 257 respective strategists dominate: blue – r-strategist dominates and red – K-strategist dominates. The
 258 left panel and right panels describe finite θ_x and the asymptotic limit as θ_x approaches infinity,
 259 respectively. Dotted lines describe asymptotic behaviors around S_{min}^* and $[\theta_x^{min}]_{lim}$ as labelled. For
 260 *Methanothrix*, Regimes I-IV used $K_{Ac} = 6.3$ meeq L^{-1} and Regimes V-VI $K_{Ac} = 22$ meeq L^{-1} as
 261 indicated in Extended Data Table 2.

Methods

Conservation Laws

Simulations for environmental selection dynamics were performed by solving mass and energy conservation laws. A general formula for writing chemical reaction is $0 = \sum_{i=1}^{N_C} \lambda_{ip} \hat{C}_i$ where $\hat{C}_i$ is the formula for component i [M], $\lambda_{i,p}$ is the stoichiometry coefficient for component i in reaction p [M_i per reaction], and N_C is the number of components. $\lambda_{i,p}$ is negative for the reactant and positive for the product. Then, a general mass conservation law can be written as $\frac{dC_i}{dt} = \sum_{p=1}^{N_P} \lambda_{ip} \rho_p$ (E) where ρ_p is the rate of p -th process and N_P is the number of processes. For *Methanosarcina-Methanothrix* system, Extended Data Table 1 summarizes the microbiological and transport processes in the standard Gujer format. Extended Data Table 2 summarizes the microbial kinetic parameters.

During growth and decay, microorganisms dissipate energy through anabolic and catabolic reactions. The reaction stoichiometry for each reaction is written as $0 = \sum_{i=1}^{N_C} \lambda_{ip} \hat{C}_i$ where $\hat{C}_i$ is the formula for component i [M] and $\lambda_{i,p}$ is the stoichiometry coefficient for component i in a process p [M_i per reaction] when the process p is a reaction. The Gibbs free energy of reaction is written as $\Delta G_p = \Delta G_p^{o'} + RT \ln \left(\prod_i C_i^{\lambda_{ip}} \right)$ where $\Delta G_p^{o'}$ is the standard Gibbs free energy of the reaction [EM^{-1}], R is the ideal gas constant (8.314 J/K-mol), and $T = 298.15K$. The superscript "o" denotes standard condition and the prime denotes pH=7. Extended Data Table 3 summarizes the anabolic, catabolic, and endogenous decay reactions for acetoclastic methanogens in terms of eeq transferred (same for *Methanosarcina* and *Methanothrix*). Finally, the specific energy dissipation rate for the i -th microbe is calculated as $\dot{g}_{diss,i} = -\frac{1}{X_i} \sum_{p=1}^{N_P} \delta_{i,s(p)} \rho_p (\sigma_{p,ana} \Delta G_{p,ana} + \sigma_{p,cat} \Delta G_{p,cat})$ where X_i is the biomass concentration for the i -th microbe, $\sigma_{p,ana} = 1$ and $\sigma_{p,cat} = (1 - Y_i)/Y_i$ are the partitioning of the electron equivalents between anabolic and catabolic reactions per eeq_X of biomass generated, and ρ_p is the process rate of the p -th process. $\Delta G_{p,ana}$ and $\Delta G_{p,cat}$ are the Gibbs free energy of reaction for process p (kJ per eeq_{rxn}) for the reaction defined in Extended Data Table 3. $\delta_{i,s(p)}$ defines the Kronecker delta function ($\delta_{i,s(p)}=1$ when $i = s(p)$ and 0 otherwise).

288 $s(p)$ maps the process p to its associated biomass type (cin or thr), as given by the rows of the
289 energy dissipation matrix summarized in Extended Data Table 4.

290 **Numerical Methods**

291 The Lotka-Volterra model (LVM) and the replicator equation are mathematically equivalent¹.
292 All models, including LVM and conservation laws, were solved in absolute coordinates using
293 `solve_ivp` from Scipy in Python. The consistency of the numerical methods was verified by
294 solving the replicator equations directly and comparing trajectories, yielding a maximum relative
295 error of 3.4×10^{-4} . Since the simplex dynamics occur in the transformed time coordinate (τ), we
296 solve the following relationships along with the ordinary differential equations: $d\tau/dt = 1/x_0$.

297 **Code Availability**

298 All code used to generate the results and figures in this study is available at <https://github.com/amarcus1/environmental-games/tree/v1.0>. A permanent, versioned archive with a
299 DOI will be deposited via Zenodo upon acceptance and provided at that stage.
300

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306 **Author Contributions**

307 A.M. is the sole author and is responsible for all aspects of the work.

308 **Competing Interests**

309 The author declares no competing interests.

Supplementary Information for: The Environmental Games

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Extended Data Table 1: Gujer matrix for the two-species acetoclastic methanogenesis chemostat model. X_{cin} is *Methanosarcina* biomass, X_{thr} is *Methanothrix* biomass, and S_{Ac} is the acetate concentration. Model parameters are defined in Extended Data Table 2

Process	Components			Process Rate ρ (E)
	Biomass X_{cin}	Biomass X_{thr}	Aqueous S_{Ac}	
1. <i>Methanosarcina</i> Growth	+1	0	$-1/Y_{cin}$	$\mu_{m,cin} \frac{S_{Ac}}{S_{Ac} + K_{Ac,cin}} X_{cin}$
2. <i>Methanosarcina</i> Decay	-1	0	0	$b_{cin} X_{cin}$
3. <i>Methanosarcina</i> Outflow	-1	0	0	$\frac{X_{cin}}{\theta_x}$
4. <i>Methanothrix</i> Growth	0	+1	$-1/Y_{thr}$	$\mu_{m,thr} \frac{S_{Ac}}{S_{Ac} + K_{Ac,thr}} X_{thr}$
5. <i>Methanothrix</i> Decay	0	-1	0	$b_{thr} X_{thr}$
6. <i>Methanothrix</i> Outflow	0	-1	0	$\frac{X_{thr}}{\theta_x}$
7. S_{Ac} Inflow	0	0	+1	$\frac{S_{Ac}^0}{\theta_x}$
8. S_{Ac} Outflow	0	0	-1	$\frac{S_{Ac}}{\theta_x}$

Extended Data Table 2: Microbial kinetic parameters for *Methanosarcina* and *Methanothrix* for acetoclastic methanogenesis at mesophilic conditions based on previous compilations. This work uses the midpoint of the range.

Parameter (unit)	<i>Methanosarcina</i>	<i>Methanothrix</i>	References
<i>Literature range</i>			
μ_m (d ⁻¹)	0.20 – 1.4	0.09 – 0.72	17
K_{Ac} (meeq _{ac} L ⁻¹) ^a	18 – 46	1.3 – 11	16,17
Y (meeq _X meeq _{ac} ⁻¹)	0.026 – 0.14	0.023 – 0.045	17
b (d ⁻¹)	0.004 – 0.10	0.006 – 0.020	17
<i>Parameters selected for this work (midpoint)</i>			
μ_m (d ⁻¹)	0.80	0.41	
K_{Ac} (meeq _{ac} L ⁻¹)			
Regimes I to IV	32	6.3	
Regimes V to VI	32	22 ^b	
Y (meeq _X meeq _{ac} ⁻¹)	0.083	0.034	
b (d ⁻¹)	0.052	0.013	

^a Converted from mg COD L⁻¹ using 8 mg COD = 1 meeq (COD stands for chemical oxygen demand).

^b Adjusted to include thermodynamic inhibition and be consistent with S_{min}^* estimated by McCarty and Bae³³.

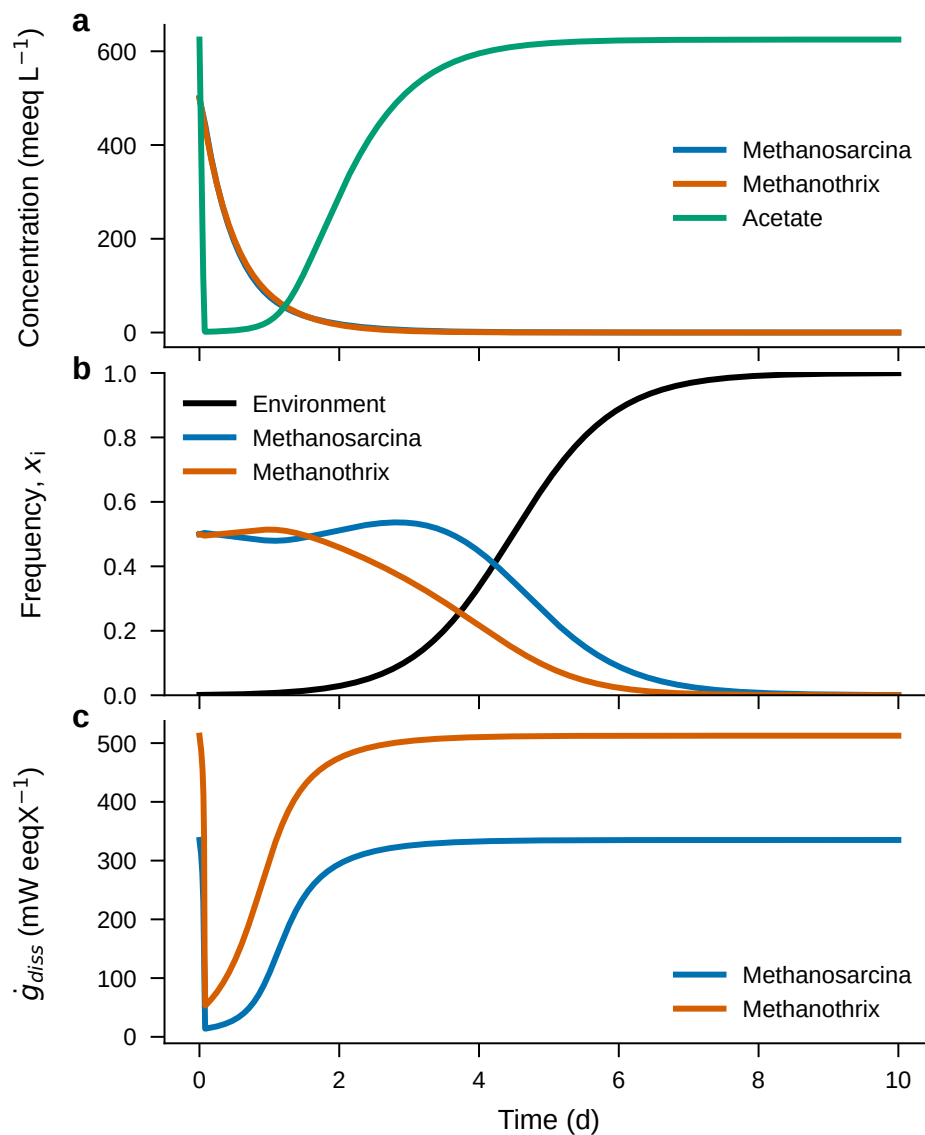
Extended Data Table 3: Reaction stoichiometry and the standard Gibbs free energy for anabolic, catabolic, and endogenous decay reactions for acetoclastic methanogenesis. All reactions are written in terms of 1 electron equivalent (eeq) transferred. Reactions are defined at pH=7, T=298.15K, and unit activity.

Reaction Name	Stoichiometry	$\Delta G^{o'}$ [kJ/eeq]
Anabolic	$0.125 \text{ CH}_3\text{COO}^- + 0.05 \text{ NH}_4^+ + 0.075 \text{ H}^+ \rightarrow 0.05 \text{ C}_5\text{H}_7\text{O}_2\text{N} + 0.15 \text{ H}_2\text{O}$	6.96
Catabolic	$0.125 \text{ CH}_3\text{COO}^- + 0.125 \text{ H}^+ \rightarrow 0.125 \text{ CH}_4 + 0.125 \text{ CO}_2$	-4.48
Endogenous Decay	$0.05 \text{ C}_5\text{H}_7\text{O}_2\text{N} + 0.15 \text{ H}_2\text{O} + 0.05 \text{ H}^+ \rightarrow 0.125 \text{ CH}_4 + 0.05 \text{ NH}_4^+ + 0.125 \text{ CO}_2$	-11.44

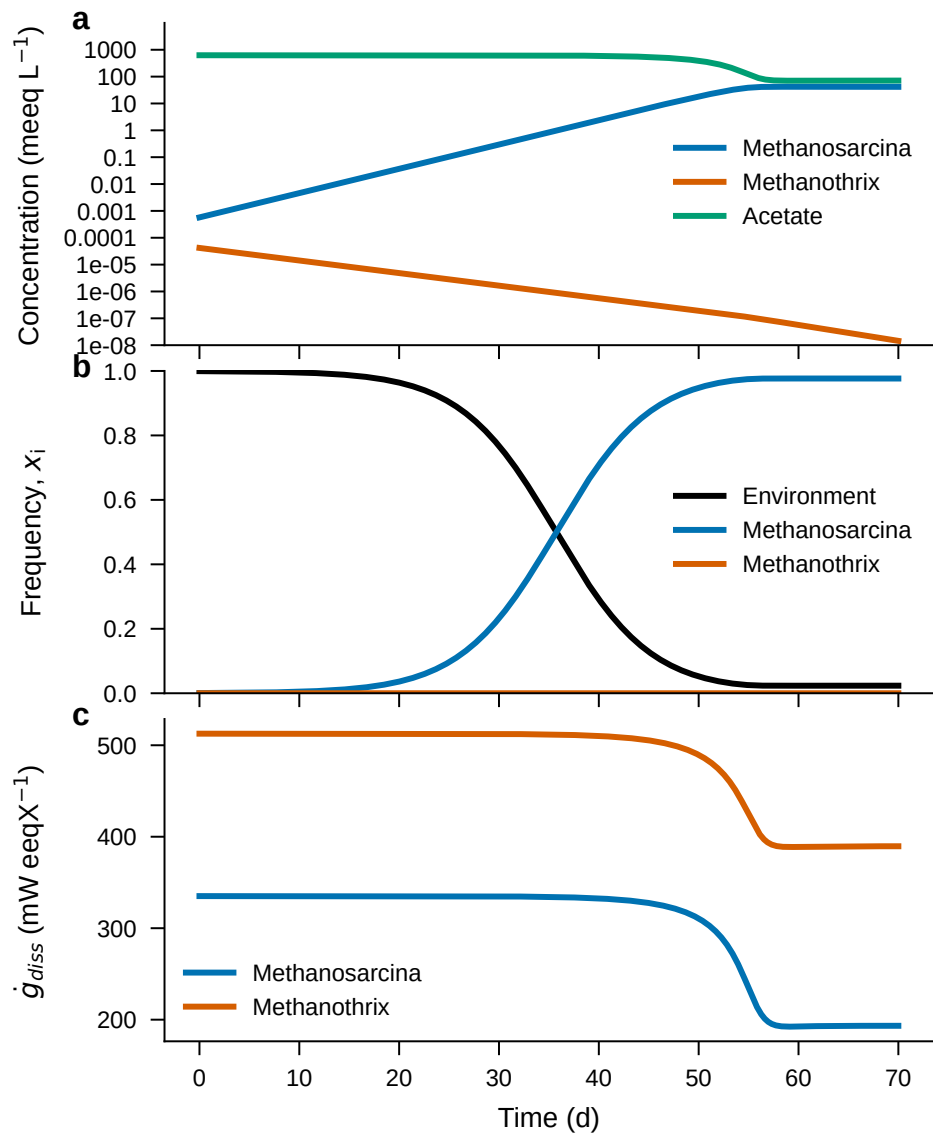
The standard Gibbs energy of formation is taken from Heijnen and Kleerebezem³⁴ for all compounds, except for biomass. Biomass is represented by $\text{C}_5\text{H}_7\text{O}_2\text{N}$ with the Gibbs free energy of formation $\Delta G_f^{o'} = 35 \text{ kJ eeq}^{-1}$, which falls near the midrange of compiled experimentally measured values Torres et al.³⁵. $\Delta G_{ana}^{o'}$ value is consistent with Chang et al.¹⁷.

Extended Data Table 4: Energy dissipation matrix for the two-species acetoclastic methanogenesis chemostat model. *Methanosarcina* (cin) and *Methanothrix* (thr) abbreviations follow Extended Data Table 1. The Gibbs free energy of reaction, ΔG (kJ per eq_{rxn}), is for reactions defined in Extended Data Table 3 after concentration corrections.

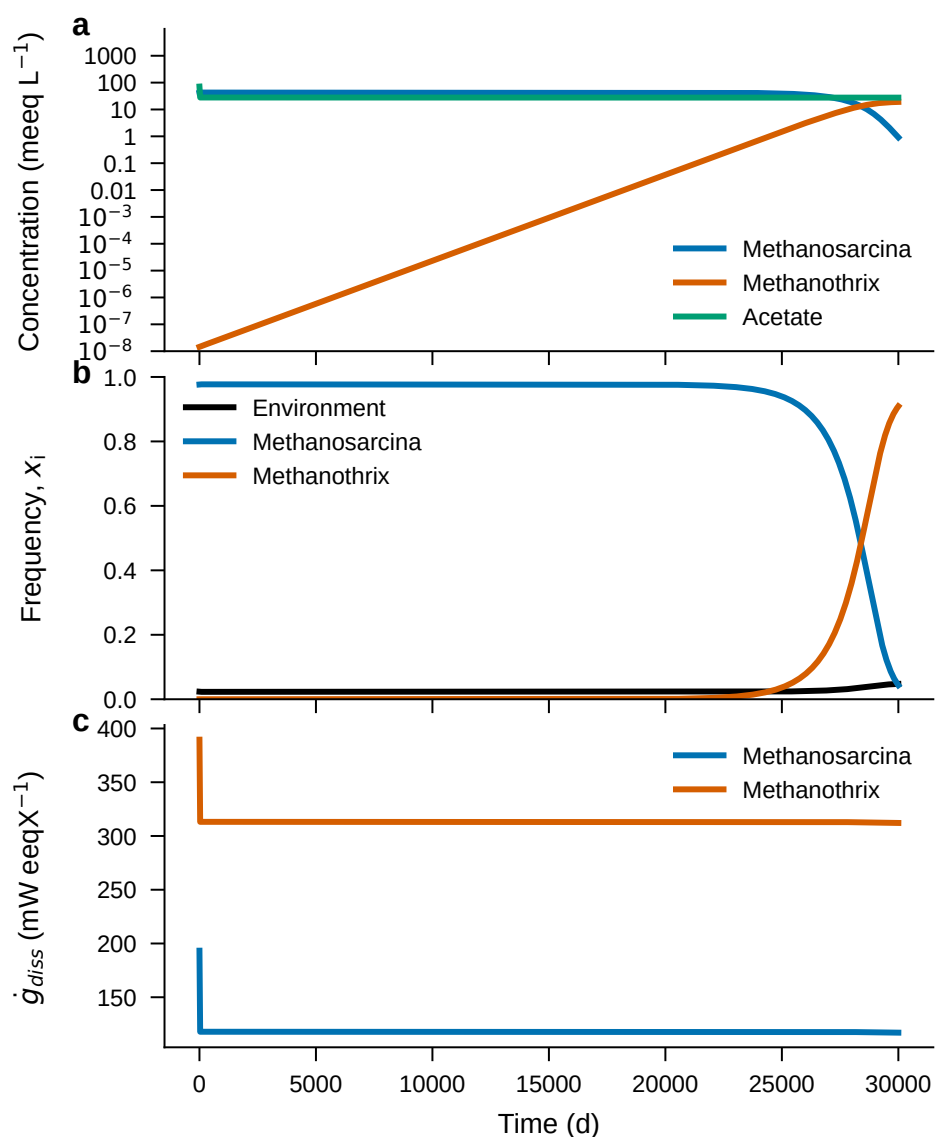
Process	Σ		ΔG		$\rho(\mathbf{E})$
	$\sigma_{p,ana}$	$\sigma_{p,cat}$	$\Delta G_{p,ana}$	$\Delta G_{p,cat}$	
1. <i>Methanosarcina</i> Growth	1	$(1 - Y_{cin})/Y_{cin}$	$\Delta G_{grow,ana,cin}$	$\Delta G_{grow,cat,cin}$	$\mu_{m,cin} \frac{S_{Ac}}{S_{Ac} + K_{Ac,cin}} X_{cin}$
2. <i>Methanosarcina</i> Decay	0	1	0	$\Delta G_{dec,cat}$	$b_{cin} X_{cin}$
3. <i>Methanothrix</i> Growth	1	$(1 - Y_{thr})/Y_{thr}$	$\Delta G_{grow,ana,thr}$	$\Delta G_{grow,cat,thr}$	$\mu_{m,thr} \frac{S_{Ac}}{S_{Ac} + K_{Ac,thr}} X_{thr}$
4. <i>Methanothrix</i> Decay	0	1	0	$\Delta G_{dec,cat}$	$b_{thr} X_{thr}$



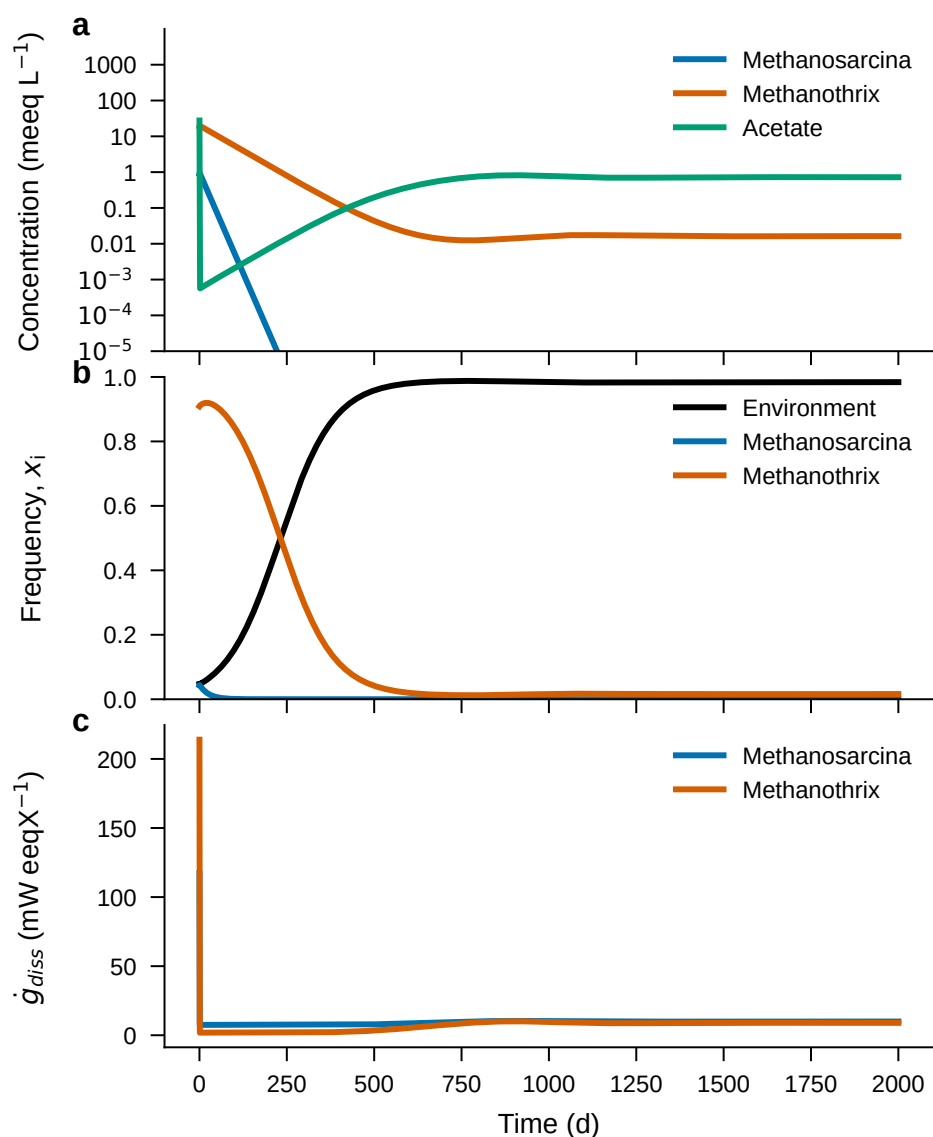
Extended Data Fig. 1 | Illustration of Washout (Regime I) in (a) absolute coordinates, (b) the simplex, and (c) specific energy dissipation rate. Parameters are summarized in Extended Data Table 2. Initial conditions are $X_{cin} = 500$ mEq L⁻¹, $X_{thr} = 500$ mEq L⁻¹, and $S_{Ac} = 625$ mEq L⁻¹.



Extended Data Fig. 2 | Illustration of r-dominates (Regime II) in (a) absolute coordinates, (b) the simplex, and (c) specific energy dissipation rate. Parameters are summarized in Extended Data Table 2. Initial conditions are $X_{cin} = 5.7 \times 10^{-4}$ mEq L⁻¹, $X_{thr} = 4.2 \times 10^{-5}$ mEq L⁻¹, and $S_{Ac}=625$ mEq L⁻¹. The y-axis is shown in the log scale for Panel a)



Extended Data Fig. 3 | Illustration of K-dominates (Regime IV) in (a) absolute coordinates, (b) the simplex, and (c) specific energy dissipation rate. Parameters are summarized in Extended Data Table 2. Initial conditions are $X_{cin} = 42$ mEq L⁻¹, $X_{thr} = 1.4 \times 10^{-8}$ mEq L⁻¹, and $S_{Ac}=71$ mEq L⁻¹. The y-axis is shown in the log scale for Panel a).



Extended Data Fig. 4 | Illustration of S_{min}^* (Regime V) in (a) absolute coordinates, (b) the simplex, and (c) specific energy dissipation rate. Parameters are summarized in Extended Data Table 2. Initial conditions are $X_{cin} = 0.034$ meeq L⁻¹, $X_{thr} = 20$ meeq L⁻¹, and $S_{Ac}=28$ meeq L⁻¹. The y-axis is shown in the log scale for Panel a).

315 References (continued)

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S1 The Grand Vision

Few topics inspire more Greatest of All Time (GOAT) debates than the rivalry among Roger Federer, Rafael Nadal, and Novak Djokovic. Federer represents a strategy of an elegant, but truly aggressive, all-court offense. Nadal represents a strategy of physical, heavy-spin baseline play. Djokovic represents a strategy of the ultimate counterpunching. Over the past two decades, these three legends have combined 66 grand slam wins. Some may argue that their competition has reached an apparent stalemate – no single playing style has a decisive advantage over the others. Who wins on any given day may depend on chance alone.

Is there a way to break the stalemate? An avid sports fan may argue that the court environment can break the stalemate: the fast grass of Wimbledon favors Federer’s strategy, the slow clay court of Roland Garros favors Nadal’s strategy, and the medium-speed hard courts of the Australian Open and the US Open favor Djokovic’s strategy. If the environment is considered, should Pete Sampras be in the conversation? Sampras represents a classic serve-and-volley strategy and dominated in the era before Wimbledon changed its grass courts. How about John McEnroe and Björn Borg, who played with wooden rackets? These examples suggest that a proper evaluation of each strategy requires an explicit consideration of the evolving environments.

This Supplementary Note develops a mathematical foundation that can handle the GOAT debate by introducing the environment as a player in a game of strategy.

The Supplementary Note is written with the recognition that the Environmental Games rest on two pillars, each coming from two separate traditions. Evolutionary Game Theory (EGT) has its historical origins in classical game theory by von Neumann and Morgenstern³³ and Nash³⁴, where players make rational decisions and mix strategies to optimize their outcomes – like how Federer may change the ratio of lob shots and passing shots depending on who he plays. Independent of the game theory tradition, Smith and Price⁹ developed the concept of evolutionarily stable strategy (ESS) to describe whether a majority in a population can adopt a strategy to resist invasion from a rare mutant. The replicator equation was developed by Taylor and Jonker³⁵ to connect the static equilibrium concepts of Price and Smith to a dynamical process. In replicator dynamics,

355 strategies are hardwired into individual phenotypes, unlike rational agents making decisions. This
356 framework is particularly well-suited for modeling microbiological systems, where microbes have
357 fixed phenotypes instead of acting like rational agents. Comprehensive treatments of EGT are given
358 by Hofbauer and Sigmund¹, Nowak³.

359 Environmental Biotechnology is an engineering discipline that applies principles from reactor
360 engineering and microbial ecology to grow microorganisms that benefit society^{11,13}. Environmental
361 Biotechnology is a forward-looking science that sets the right environmental conditions and lets
362 the beneficial microorganisms already present in the environment grow into abundance. Perry
363 McCarty laid many of the early foundations of thermodynamics and reactor engineering principles
364 governing environmental selection^{36,37}.

365 Readers will likely be familiar with at most only one of the two above-mentioned pillars.
366 Therefore, the Supplementary Note provides a detailed derivation that engineers and microbiologists
367 may find useful. A caveat is that the tennis example above is meant to illustrate the game of
368 interactions within the framework of classical game theory, where each player makes rational
369 decisions. The Environmental Games will build on the framework of replicator dynamics, where
370 each strategy is represented by distinct population frequencies.

371 The remaining sections introduce the replicator equation and EGT terminology (S2). Then,
372 the equivalence between the replicator equation and the Lotka-Volterra model (LVM) (S3) is
373 demonstrated following Hofbauer and Sigmund¹. This mathematical procedure is essential for
374 projecting the absolute coordinates of the LVM and Environmental Biotechnology models onto the
375 simplex described by the replicator equation. It also separates direct interaction coefficients and
376 intrinsic growth rates within the payoff matrix. Setting the direct interaction coefficients to zero
377 yields the idealized LVM (S4) – a baseline selection model with no direct biological interactions.
378 The Pure Environmental Games (PEGs) are derived by replacing the intrinsic growth rate with the
379 net specific accumulation rate of biomass (S5). Finally, the classical r/K selection game will be
380 used as an instance of PEGs, and six environmental selection regimes will be described (S6).

S2 Replicator Dynamics

Classical game theory provides a formal framework for studying strategic interactions among players trying to maximize their rewards. In a game with two strategies, a player can select from one of two "pure" strategies S_1 and S_2 . The payoff coefficient a_{ij} describes the payoff for strategy i interacting with j . One way to show the payoff matrix is to let the rows indexed by i represent the focal strategy and the columns indexed by j represent the opposing strategy. A payoff matrix for a general game with two strategies, with the coefficients $a_{11} = a$, $a_{12} = b$, $a_{21} = c$, $a_{22} = d$, can be shown as

$$\begin{array}{c|cc} & S_1 & S_2 \\ \hline S_1 & a & b \\ S_2 & c & d \end{array} \quad (\text{S-2})$$

where a , b , c , and d are defined on $\mathbb{R}$. In practice, a player will play a mixture of S_1 and S_2 and this mixed strategy can be described using a probability distribution: $P(S_1)$ and $P(S_2)$. By the law of total probability $P(S_1) + P(S_2) = 1$. Let us assume that the opponent plays S_1 and S_2 with probabilities $P(S_1)$ and $P(S_2)$. Then, the net payoff for the player playing S_1 is

$$E(S_1) = aP(S_1) + bP(S_2) \quad (\text{S-3})$$

and S_2 is

$$E(S_2) = cP(S_1) + dP(S_2) \quad (\text{S-4})$$

These are naive calculations assuming an arbitrary probability distribution before optimization. In classical game theory, the optimal strategies are developed by anticipating the opponent's optimal moves, which depend on how the games are played (finite versus infinite rounds). For a complete treatment of classical game theory, readers are referred to Nowak³, von Neumann and Morgenstern³³.

The replicator equation is a fundamental deterministic framework for modeling large, well-mixed populations in which individuals undergo repeated interactions. In contrast to classical

game theory, where rational players choose strategies, each individual represents a specific, fixed strategy (phenotype). If a population of size N has k_i individuals playing a strategy i , the frequency of strategy i is defined as

$$x_i = \frac{k_i}{N} \quad (\text{S-5})$$

The population frequencies for a population with $n+1$ strategies sum to one: $1 = \sum_{i=0}^n x_i$. In replicator dynamics, each strategy undergoes continuous frequency-dependent interactions to shape the population.

In replicator dynamics, the benefits or losses that each individual receives from another individual in a population are calculated in a manner that is analogous to classical game theory, but with important differences: The strategy frequency x_i replaces probability, and the fitness f_i replaces the net payoff. For a two-strategy case, the fitness of two strategies can be calculated as

$$f_1 = ax_1 + bx_2 \quad (\text{S-6})$$

411

$$f_2 = cx_1 + dx_2 \quad (\text{S-7})$$

or more generally

$$f_i = \sum_{j=0}^n a_{ij}x_j \quad (\text{S-8})$$

For non-mathematicians unfamiliar with the summation notation, notice the index j is summed over, leaving i as the matching index for both a_{ij} and f_i . While the mathematical forms between the net payoff and fitness are analogous, the interpretation differs fundamentally. In classical game theory, mixing reflects a single player's probabilistic choice, whereas in replicator dynamics, mixing reflects the population composition of individuals each committed to a fixed pure strategy.

The replicator equation is convenient for studying large mixed populations, because it has a built-in mechanism to track the outcome of repeated interactions. It models the frequency of strategy i in a population (x_i) over continuous and repeated interactions in the simplex space and

421 has the form:

$$\dot{x}_i = x_i (f_i - \phi) \quad (\text{S-9})$$

422 where ϕ is the average fitness of the population, and

$$\phi = \sum_{i=0}^n \sum_{j=0}^n x_i a_{ij} x_j \quad (\text{S-10})$$

423 Note: this work adopts a zero-indexing convention, with x_0 denoting the empty strategy, as discussed
424 later. The replicator equation is a remarkably intuitive equation that says the frequency of a strategy
425 will increase if its own fitness exceeds that of the population average.

426 The payoff matrix can be interpreted through two different classification systems: The ESS
427 classification and the EGT classification. The ESS classification describes static equilibria and
428 asks if a strategy can resist an invasion by a rare mutant by examining the payoff matrix³. Consider
429 a game of direct interactions with two strategies: S_1 and S_2 , where a payoff matrix for a 2-strategy
430 system with $a_{11} = 1, a_{12} = 0, a_{21} = 0, a_{22} = 0$ can be shown as

	S_1	S_2
S_1	1	0
S_2	0	0

(S-11)

431 A strategy is a strict Nash equilibrium (SNE) if it resists all invasions. The strategy S_k is an SNE
432 if it satisfies

$$a_{kk} > a_{ik} \quad (\text{S-12})$$

433 for all i . In this example, S_1 is an SNE because the diagonal element in the first column is larger
434 than the other elements in the same column: i.e., $a_{11} > a_{21}$ or $1 > 0$. An ESS also resists all
435 invasions from emerging mutants. An SNE implies ESS, but ESS for S_k can be established by a
436 less stringent criterion:

$$a_{kk} = a_{ik} \text{ and } a_{ki} > a_{ii} \quad (\text{S-13})$$

for all i . Although S_1 fails the second criterion (i.e., $a_{12} \neq a_{22}$ or $0 \neq 0$), it is saved by SNE already being satisfied: S_1 is both SNE and ESS.

Let's see if S_2 satisfies ESS. Because $a_{22} = a_{12}$ or $0 = 0$, the first part of ESS criteria is satisfied. However, since $a_{21} \neq a_{11}$ or $0 \neq 1$, S_2 is not an ESS. So, what is S_2 ? The answer is in the Nash equilibrium (NE). The criteria for the NE are

$$a_{kk} \geq a_{ik} \quad (\text{S-14})$$

Because $a_{22} = a_{12}$ or $0 = 0$, S_2 is an NE. An NE does not prevent invasion, but it offers a possibility for neutral drift.

SNE, ESS, and NE are static equilibrium concepts describing the ability of a resident strategy to resist invasion from a rare strategy. These concepts need to be distinguished from the following concepts:

- In EGT, dynamic equilibrium describes a state when the vector of frequencies becomes time invariant. This state can be stable or unstable.
- A related concept in Environmental Biotechnology is the steady state, which occurs when the environmental variables, such as concentrations of substrate and biomass, become time invariant.
- A thermodynamic equilibrium which occurs when the overall Gibbs free energy of reactions equals zero ($\Delta G_{rxn} = 0$).

The EGT classification or the game given by the payoff structure describes the categories of interactions demonstrated by strategies. Table S1 below shows the correspondence between the game and replicator dynamics for a two-strategy game. Traditionally, replicator dynamics assume that the intrinsic growth rates of individual strategies are equal. The game and replicator dynamics have a one-to-one correspondence when the intrinsic growth rates are equal². The PEGs explore differences in intrinsic growth rates operating in parallel to the limiting case of neutral selection,

460 revealing that deterministic outcomes remain possible even when direct interactions are absent.

461 The dynamics of frequency-based selection are discussed in Supplementary Note S4.

Supplementary Table S1: EGT classification of two-strategy games, corresponding replicator dynamics, and payoff structure conditions. The one-to-one correspondence between game and replicator dynamics holds when intrinsic growth rates are equal².

Game	Replicator Dynamics	Payoff Structure
Harmony Game	Dominance of $\mathcal{S}_1$	$a > c$ and $b > d$
Prisoner's Dilemma	Dominance of $\mathcal{S}_2$	$a < c$ and $b < d$
Stag Hunt	Bistability	$a > c$ and $b < d$
Hawk-and-Dove	Coexistence	$a < c$ and $b > d$
Neutral Selection	Neutral	$a = c$ and $b = d$

462 **S3 Transforming a Two-species Lotka-Volterra System into a**

463 **Replicator Equation**

464 **The Lotka-Volterra Systems.** The LVM from theoretical ecology also describes the game of direct
465 interactions. Instead of the relative frequency, the LVM tracks the absolute abundance of individual
466 i in a population (y_i). The LVM has the form:

$$\dot{y}_i = y_i \left(r_i + \sum_{j=1}^n b_{ij} y_j \right) \quad (\text{S-15})$$

467 r_i stands for intrinsic growth rate of species i , and b_{ij} stands for gains or losses species i experiences
468 from interacting with species j . Note that the LVM adopts a one-indexing convention. Hofbauer and
469 Sigmund¹ demonstrate that the LVM with n species is mathematically equivalent to the replicator
470 equation with $n + 1$ strategies. However, there are important physical differences between these
471 two frameworks. On the one hand, the replicator equation only has one set of coefficients in
472 the payoff matrix that represents direct player interactions. On the other hand, the LVM has two

473 sets of coefficients: the direct interaction coefficients and the intrinsic growth rates. This section
474 transforms a two-species Lotka-Volterra system to the replicator equation to demonstrate how the
475 coefficients of the Lotka-Volterra system map onto the payoff matrix.

476 Consider a two-species Lotka-Volterra system in the form:

$$\dot{y}_1 = y_1 (r_1 + b_{11}y_1 + b_{12}y_2) \quad (\text{S-16})$$

477

$$\dot{y}_2 = y_2 (r_2 + b_{21}y_1 + b_{22}y_2) \quad (\text{S-17})$$

478 The interaction terms and intrinsic growth rates for the two-species Lotka-Volterra system can be
479 represented in the matrix form as

$$B = \begin{array}{c|cc} & s_1 & s_2 \\ \hline s_1 & b_{11} & b_{12} \\ s_2 & b_{21} & b_{22} \end{array} \quad (\text{S-18})$$

480

$$r = \begin{pmatrix} r_1 \\ r_2 \end{pmatrix} \quad (\text{S-19})$$

481 where s_1 and s_2 represent species. The species abundance is defined on $\mathbb{R}^+$.

482 ***Folding Infinity onto a Simplex.*** Transforming the LVM into the replicator equation requires a
483 map between absolute quantities defined over $[0, \infty)$ and frequencies bounded within a simplex:
484 i.e., a set of points whose coordinates are non-negative and sum to one³. An intuitive approach
485 is using the total population density as the normalization factor. In Environmental Biotechnology
486 and biofilm modeling, this mapping is routinely achieved by normalizing the individual biomass
487 density by the total biomass density to define the biofilm fraction^{38,39}. This is not a desirable
488 transformation approach because the replicator equation does not track absolute quantities.

489 An effective alternative is to introduce a normalization factor embedded with a unit reference

490 value. Hofbauer and Sigmund¹ define the normalization factor D as

$$D = 1 + y_1 + y_2 \quad (\text{S-20})$$

491 The value 1 in (S-20) represents a unit value carrying the same dimension as y_i , analogous to a
492 reference concentration of 1 mol/L in chemical systems. This method maps the absolute quantities
493 to a coordinate in the simplex frequencies, x_i , as

$$x_i = y_i/D \quad (\text{S-21})$$

494 Because D carries the same physical dimension as y_i , x_i is dimensionless. Using this mapping,
495 (S-20) can be divided by D and rewritten as

$$1 = x_0 + x_1 + x_2 \quad (\text{S-22})$$

496 It should be noted that $x_0 = 1/D$ and substituting this relationship to (S-20) gives

$$y_i = Dx_i = \frac{x_i}{x_0} \quad (\text{S-23})$$

497 x_0 is defined over $(0, 1]$. When the simplex is unoccupied ($\sum_i y_i = 0$), $x_0 = 1$. x_0 diminishes as
498 pure strategies occupy the simplex. When the absolute quantity approaches infinity ($\sum_i y_i \rightarrow \infty$),
499 x_0 approaches zero. Thus, this mapping preserves the relationship between simplex coordinates
500 and absolute coordinates by dynamically tracking x_0 .

501 As noted earlier, this method increases the number of state variables from n in the Lotka-
502 Volterra system to $n+1$ in replicator dynamics. Since x_0 does not represent any species in the
503 original Lotka-Volterra system, it can be thought of provisionally as the frequency of an empty
504 strategy, $\mathcal{S}_0$. However, Supplementary Note S5 shows that x_0 represents the environment as the
505 player in the Pure Environmental Games.

506 **The Transformation.** With the empty strategy, the two-species Lotka-Volterra system can now be
 507 expressed as replicator equations. The transformation proceeds by taking logarithmic derivatives.
 508 Taking the logarithmic derivatives of (S-21)

$$\frac{\dot{x}_i}{x_i} = \frac{\dot{y}_i}{y_i} - \frac{\dot{D}}{D} \quad (\text{S-24})$$

509 Since $y_0 = 1$, (S-21) for the empty strategy is

$$\frac{\dot{x}_0}{x_0} = -\frac{\dot{D}}{D} \quad (\text{S-25})$$

510 The "dot" notation in $\dot{x}$ represents the derivative with respect to dt . By defining a new coordinate
 511 system, $1/d\tau = x_0/dt$, the average fitness ϕ is defined as

$$\phi = -\frac{x_0'}{x_0} = \frac{D'}{D} \quad (\text{S-26})$$

512 This expression can be rearranged to the replicator equation for the empty strategy:

$$x_0' = x_0 (f_0 - \phi) \quad (\text{S-27})$$

513 where it is useful to note that the fitness of the empty strategy is $f_0 = 0$. Moreover, inserting (S-16)
 514 and (S-25) into (S-24) gives

$$\frac{\dot{x}_1}{x_1} = (r_1 + b_{11}y_1 + b_{12}y_2) + \frac{\dot{x}_0}{x_0} \quad (\text{S-28})$$

515 The terms y_1 and y_2 in this equation can be eliminated by noting $y_i = x_i/x_0$ from (S-21)

$$\frac{\dot{x}_1}{x_1} = \left(r_1 + \frac{b_{11}x_1 + b_{12}x_2}{x_0} \right) + \frac{\dot{x}_0}{x_0} \quad (\text{S-29})$$

516 Multiplying the equation by x_0 gives

$$x_0 \frac{\dot{x}_1}{x_1} = (x_0 r_1 + b_{11} x_1 + b_{12} x_2) + x_0 \frac{\dot{x}_0}{x_0} \quad (\text{S-30})$$

517 Employing the same time domain transformation, $1/d\tau = x_0/dt$, gives

$$\frac{x'_1}{x_1} = (x_0 r_1 + b_{11} x_1 + b_{12} x_2) + \frac{x'_0}{x_0} \quad (\text{S-31})$$

518 where prime indicates differentiation with respect to the transformed time coordinate, $1/d\tau = x_0/dt$.

519 Noticing that the terms in the parenthesis in the fitness of species 1 and the definition of average

520 fitness in (S-25) provide the replicator equation for species 1

$$x'_1 = x_1 (f_1 - \phi) \quad (\text{S-32})$$

521 where

$$f_1 = x_0 r_1 + b_{11} x_1 + b_{12} x_2 \quad (\text{S-33})$$

522 Similarly, the replicator equation for species 2

$$x'_2 = x_2 (f_2 - \phi) \quad (\text{S-34})$$

523 where

$$f_2 = x_0 r_2 + b_{21} x_1 + b_{22} x_2 \quad (\text{S-35})$$

524 Together (S-27), (S-32), and (S-34) provide the three replicator equations from the two-species

525 Lotka-Volterra system. The average fitness can be obtained by summing these three replicator

526 equations to obtain

$$x'_0 + x'_1 + x'_2 = x_0 f_0 + x_1 f_1 + x_2 f_2 - (x_0 + x_1 + x_2) \phi \quad (\text{S-36})$$

527 Notice from (S-22) that $x_0 + x_1 + x_2 = 1$ and its derivative is zero: i.e., $x'_0 + x'_1 + x'_2 = 0$. Therefore,
528 obtain the average fitness

$$\phi = x_0 f_0 + x_1 f_1 + x_2 f_2 \quad (\text{S-37})$$

529 This concludes the transformation of the two-species Lotka-Volterra system into equivalent repli-
530 cator equations. The resulting coefficient for the replicator equation can be summarized in a payoff
531 matrix:

$$A = \begin{array}{c|ccc} & S_0 & S_1 & S_2 \\ \hline S_0 & 0 & 0 & 0 \\ S_1 & r_1 & b_{11} & b_{12} \\ S_2 & r_2 & b_{21} & b_{22} \end{array} \quad (\text{S-38})$$

532 The payoff matrix derived from the Lotka-Volterra system has several important features. First,
533 now that we are in the simplex space, S_i stands for strategies, not species. S_1 and S_2 are pure
534 strategies adopted by s_1 and s_2 . Meanwhile, S_0 is an empty strategy introduced to act as the
535 reference size of the simplex (S-20). Second, the first row contains zero because the empty strategy
536 receives no payoff. Third, the second and third columns contain the interaction terms from the
537 two-species Lotka-Volterra systems. Fourth, the first column contains the intrinsic growth rates
538 from the Lotka-Volterra system. Conceptually, each term represents the payoff a strategy receives
539 by playing against an empty strategy. When the payoff matrix operates on the strategy frequencies,
540 the term x_0 multiplies the intrinsic terms. Therefore, x_0 acts as a conversion factor to convert
541 the intrinsic growth rates of Lotka-Volterra system into an equivalent fitness value in replicator
542 dynamics. The next section shows how x_0 provides the link to incorporate the boundary condition,
543 such as the carrying capacity, in the simplex space when considering a system derived from LVM.
544 The subsequent sections demonstrate how x_0 provides the link to bring physical and chemical
545 constraints into the simplex space.

S4 The Ideal Lotka-Volterra Systems

Predator-Prey System. Theoretical ecologists use the LVM to study interactions. A classic example is the Lotka-Volterra Predator-Prey model of the form

$$\dot{y}_1 = \alpha y_1 - \beta y_1 y_2 \quad (\text{S-39})$$

$$\dot{y}_2 = \delta y_1 y_2 - \gamma y_2 \quad (\text{S-40})$$

where α is prey birth rate, γ is predator death rate, and β and δ are interaction coefficients. Using the results from transforming the two-species Lotka-Volterra systems to the replicator equation in Supplementary Note S3 (S-38), the coefficients for the Predator-Prey system can be placed in the payoff matrix as shown below:

$$A = \begin{array}{c|ccc} & S_0 & S_1 & S_2 \\ \hline S_0 & 0 & 0 & 0 \\ S_1 & \alpha & 0 & -\beta \\ S_2 & -\gamma & \delta & 0 \end{array} \quad (\text{S-41})$$

There are a few notable features. First, the first column representing the empty strategy contains the intrinsic growth terms. As stated in Supplementary Note S3, x_0 re-denominates α and γ from absolute units into simplex frequencies to alter the fitness of S_1 and S_2 , respectively. Then, because the payoff coefficients are defined on $\mathbb{R}$, the term γ carries the negative sign from the equation. Next, the interaction terms β and δ encode the predator-prey interactions. The term β carries a negative sign because predation results in the prey's loss. Finally, zero diagonal entries indicate no self-interaction.

Supplementary Figure S1a shows the model dynamics when coefficients equal $\alpha = 1.0$, $\beta = 0.1$, $\delta = 0.075$, $\gamma = 1.5$. This system has a non-trivial equilibrium at $y_1^* = \gamma/\delta = 20$ and $y_2^* = \alpha/\beta = 10$. When the system starts at an initial point away from this equilibrium ($y_1 = 25$, $y_2 = 10$ in this example), the population dynamics demonstrate an oscillatory behavior around the equilibrium.

Supplementary Figure S1b shows the model dynamics in the simplex. In the simplex, the x-axis is represented by the transformed time coordinate, τ . The simplex adds the empty strategy to track changes in absolute abundance through x_0 .

Ideal Lotka-Volterra.

Let us define the Ideal LVM in which no individuals within the population interact directly (similar to the ideal gas law in chemistry). The model is also known as the Malthusian growth model or simply the exponential growth model. We shall see in the coming sections that it becomes convenient to strip away the interaction terms while preserving the structure of the LVM. Mathematically, the LVM is represented by setting all interaction coefficients in (S-15) to zero. Consider an ideal two-species Lotka-Volterra system in the form:

$$\dot{y}_1 = y_1 r_1 \quad (\text{S-42})$$

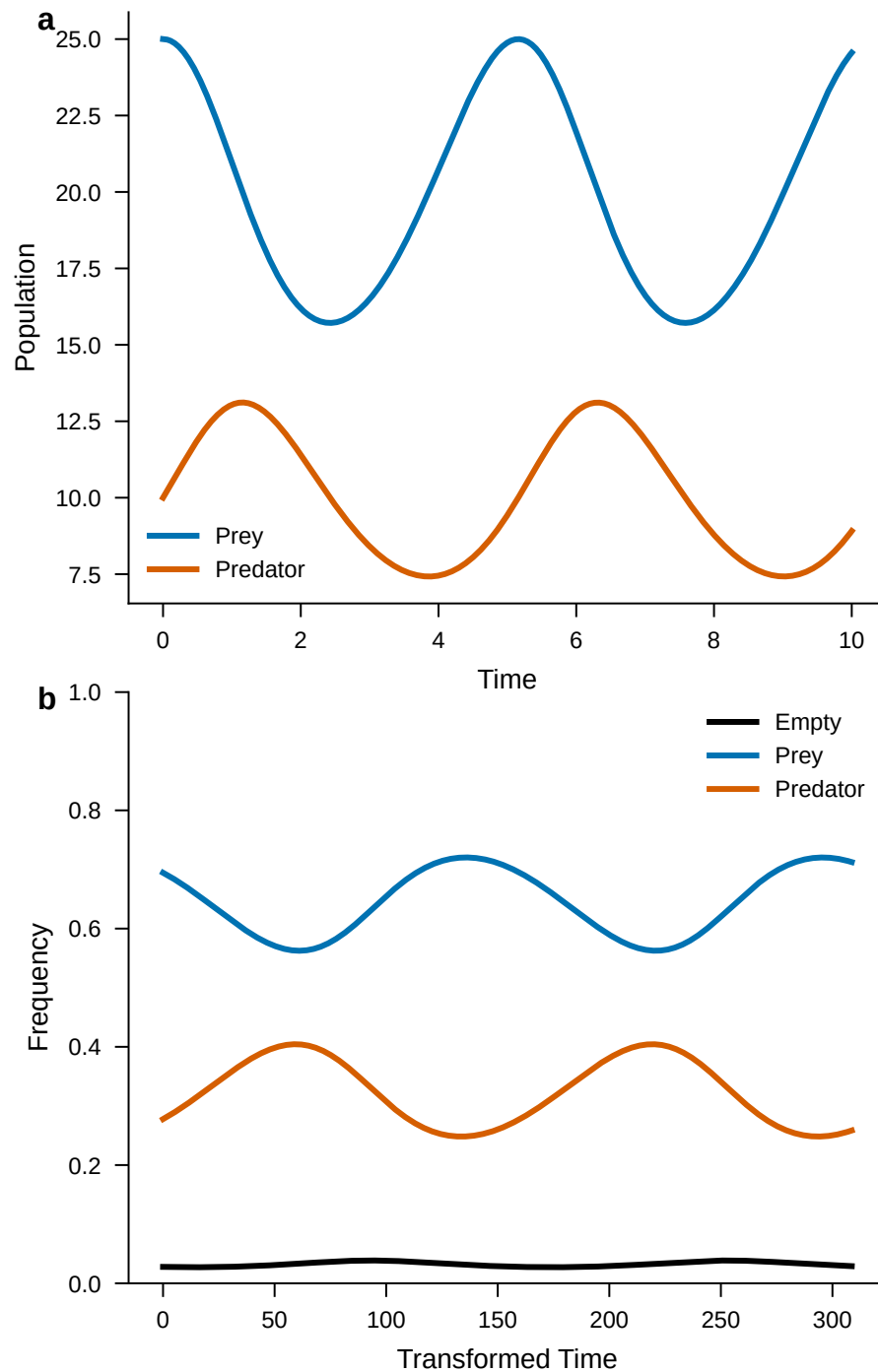
$$\dot{y}_2 = y_2 r_2 \quad (\text{S-43})$$

When this ideal Lotka-Volterra system is projected onto the simplex, the following is the payoff matrix:

$$A = \begin{array}{c|ccc} & \mathcal{S}_0 & \mathcal{S}_1 & \mathcal{S}_2 \\ \hline \mathcal{S}_0 & 0 & 0 & 0 \\ \mathcal{S}_1 & r_1 & 0 & 0 \\ \mathcal{S}_2 & r_2 & 0 & 0 \end{array} \quad (\text{S-44})$$

The ESS classification can be applied to the ideal Lotka-Volterra system to identify when the empty strategy can be invaded.

- When $r_1, r_2 > 0$, none of the strategies is an SNE because $a_{kk} \not\geq a_{ik}$ (S-12).
- When $r_1, r_2 > 0$, none of the strategies is an ESS because although $\mathcal{S}_1$ and $\mathcal{S}_2$ satisfy $a_{kk} = a_{ik}$, $a_{ki} \not\geq a_{ii}$ (S-13).
- $\mathcal{S}_1$ and $\mathcal{S}_2$ are always Nash equilibrium strategies because $a_{kk} \geq a_{ik}$ or $0 \geq 0$ (S-14),



Supplementary Figure S1: Predator-prey dynamics in a) absolute coordinates and b) simplex coordinates. Parameters: $\alpha = 1.0$, $\beta = 0.1$, $\delta = 0.075$, $\gamma = 1.5$. Initial conditions: prey = 25, predator = 10.

regardless of the signs of r_1 and r_2 .

- When $r_1, r_2 > 0$, $\mathcal{S}_0$ does not qualify for a Nash equilibrium (S-14) because $a_{00} = 0 \not> a_{10} = r_1 > 0$.

- Interestingly, when $r_1, r_2 < 0$, the empty strategy becomes an SNE because $a_{00} > a_{10}$ and a_{20} (S-12).

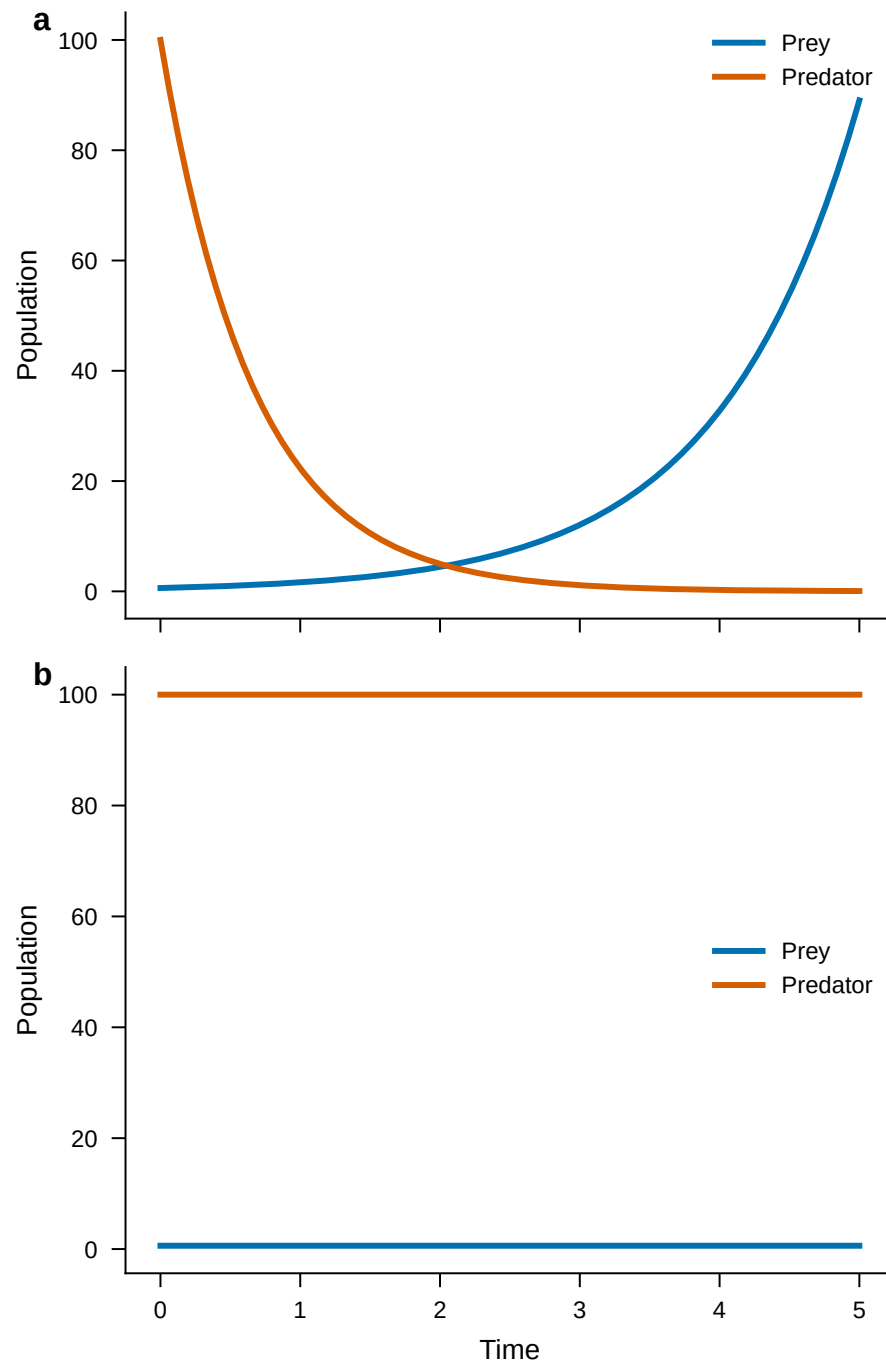
Therefore, the ideal LVM consists of a collection of Nash equilibrium strategies that can successfully establish themselves in the simplex by displacing the empty strategy when $r_1, r_2 > 0$. However, when the signs are changed to $r_1, r_2 < 0$, the empty strategy is promoted to SNE.

Supplementary Figure S2a explores the dynamics of an idealized Predator-Prey system with the interaction coefficients zeroed out, while keeping the intrinsic growth rates the same: i.e., $\alpha = 1.0$, $\beta = 0$, $\delta = 0$, $\gamma = 1.5$. The initial conditions were set at prey = 0.6, predator = 100. With no predatory interactions, the prey is free to grow exponentially while the predator's population decays exponentially. Under this Nash equilibrium, frequency-dependent dynamics no longer drive the system dynamics. Nowak³ claims that replicator dynamics no longer adequately describe biological behaviors. To drive the point that only the imposed boundary condition drives the system dynamics, Supplementary Figure S2b zeros all model parameters: $\alpha = \beta = \delta = \gamma = 0$, and consequently $r_1 = r_2 = 0$. The deterministic dynamics no longer alter the population interaction terms. Under this condition, the system is governed by stochastic processes, such as the neutral drift described by Kimura²¹, until another deterministic process replaces frequency-dependent interactions, as we shall see in Supplementary Note S5.

Without a boundary condition, the idealized predator-prey model grows exponentially without bound. This is often accomplished by replacing the constant intrinsic growth coefficient with a function of the carrying capacity, K .

$$r_i(y_i; \eta_i, K_i) = \eta_i \left(1 - \frac{y_i}{K_i} \right) \quad (\text{S-45})$$

Mathematically, the notation $r_i(y_i; \eta_i, K_i)$ reads r_i is a function of y_i parameterized by η_i and



Supplementary Figure S2: The ideal Predator-prey dynamics with initial conditions: prey = 0.6, predator = 100. a) Idealized parameters: $\alpha = 1.0$, $\beta = 0$, $\delta = 0$, $\gamma = 1.5$. b) No dynamics: $\alpha = \beta = \delta = \gamma = 0$.

608 K_i . This function allows the terms in the parentheses to approach zero as the population density
609 approaches K_i . The parameterized carrying capacity function can now replace the constant growth
610 coefficient.

$$A = \begin{array}{c|ccc} & S_0 & S_1 & S_2 \\ \hline S_0 & 0 & 0 & 0 \\ S_1 & r_1(y_1; \eta_1, K_1) & 0 & 0 \\ S_2 & r_2(y_2; \eta_2, K_2) & 0 & 0 \end{array} \quad (\text{S-46})$$

611 Supplementary Note S5 uses this parametrized implementation of the boundary condition to for-
612 mally derive the Pure Environmental Games.

613 S5 The Pure Environmental Games

614 *The Pure Environmental Game.*

615 This work defines the Pure Environmental Games as any biological system in which the absolute
616 population growth rate of the i -th member (X_i) can be expressed as

$$\frac{dX_i}{dt} = \mu_{a,i}(\mathbf{E}) X_i \quad (\text{S-47})$$

617 where

- 618 • $\mathbf{E}$ is the environmental vector specifying parameters such as pH, temperature, and concen-
619 tration of any gaseous or aqueous chemicals. For engineered systems, $\mathbf{E}$ includes control
620 parameters such as the hydraulic residence time, θ [T].
- 621 • $\mu_{a,i}(\mathbf{E})$ - the net specific accumulation rate of the i -th member [T^{-1}]. Since $\mathbf{E}$ includes
622 dynamic variables such as substrate concentration, $S(t)$, the net specific accumulation rate
623 $\mu_{a,i}(\mathbf{E})$ is implicitly time-dependent. Supplementary Note S6 will examine the behavior of
624 the dynamic payoff matrix.

625 Following the derivation of the ideal LVM in Supplementary Note S4, the term $\mu_{a,i}(\mathbf{E})$ can be

placed under the empty strategy column of the payoff matrix. The Pure Environmental Games
redefine S_0 and x_0 as

- S_0 - environmental strategy.

- x_0 - environmental frequency.

When the frequency of the environmental strategy, x_0 , multiplies $\mu_{a,i}(\mathbf{E})$, it provides a mathematical
framework to project the environmental dynamics onto the simplex, thereby exerting pressure on
biological selection.

It should be noted that the term "pure" has two separate meanings. In replicator dynamics, each
individual in a population comes with a hardwired phenotype representing a "pure" strategy. In the
Pure Environmental Games, "pure" also refers to the payoff matrix structure: the entries $a_{ij} = 0$
for all $i, j > 0$, meaning biological strategies do not directly interact with each other. Instead,
microorganisms receive payoffs from interacting with the environment, i.e., a_{i0} . Later sections
will show that S_i can represent microbial genera such as *Methanosarcina* and *Methanothrix*. The
remainder of this section will derive a specific instance of the Pure Environmental Games, known
as the idealized chemostat, from Environmental Biotechnology.

As we move from a mathematical model to a physical model, the use of internally consistent
physical units becomes essential. This section describes physical dimensions using length (L),
time (T), mass (M), and energy (E) unless specified otherwise. The notation $[\cdot]$ should be read as
"is in the units of." The masses will be denoted by subscripts M_X , M_S , and M_P for the biomass,
substrate, and product, respectively. For internal consistency, all masses will be expressed in terms
of electron equivalents: 1 electron equivalent (or 1 eeq) is defined as 1 mole of electrons. The next
section introduces all anabolic, catabolic, and decay reactions with the transfer of 1 eeq.

Monod Kinetics.

Microorganisms grow by taking up sources of energy and cellular building blocks from the
surrounding environment. The Monod kinetics⁴⁰ describes the growth rates of microorganisms
limited by the substrate – a compound serving as the primary source of energy with a symbol S

652 $[ML^{-3}]$. The single-substrate Monod kinetics assumes that only one substrate (i.e., food or electron
653 donor) is rate-limiting and has the form

$$\mu = \underbrace{\mu_m \frac{S}{S + K_S}}_{\text{growth}} - \underbrace{b}_{\text{decay}} \quad (\text{S-48})$$

654 where

- 655 • μ - net specific growth rate $[T^{-1}]$
- 656 • μ_m - maximum specific growth rate $[T^{-1}]$
- 657 • b - endogenous decay rate $[T^{-1}]$
- 658 • S - substrate concentration $[M_S L^{-3}]$
- 659 • K_S - half-maximum rate substrate concentration $[M_S L^{-3}]$

660 On the right-hand side, the first Monod term describes the generation rate of new biomass. The sec-
661 ond term is the maintenance rate of microorganisms. For modeling environmental microorganisms,
662 the Monod kinetics needs to be integrated with mass conservation relationships.

663 ***The Idealized Chemostat.***

664 Environmental Biotechnology combines conservation laws, reaction kinetics, and reaction
665 thermodynamics to model microbiological systems. The models can range from simple (e.g.,
666 reaction stoichiometry) to extraordinarily complex (e.g., 3-dimensional biofilm models). This work
667 assumes the idealized chemostat model that microbial ecologists and engineers commonly use to
668 develop microbial ecology principles and for designing wastewater treatment processes. The term
669 "idealized" means that the system is homogeneous and the microorganisms are freely suspended
670 without interactions (no flocs or biofilms). For a comprehensive treatment of the chemostats, the
671 readers are referred to¹³.

672 The chemostat, also known as a Continuous Stirred-Tank Reactor (CSTR), is a microbiological
673 reactor consisting of a continuous inflow, a continuous outflow, and a well-mixed growth chamber.

674 A combination of substrate, biomass, and product mass balance equations describes a chemostat:

$$\underbrace{V \frac{dX_a}{dt}}_{\text{accumulation}} = \underbrace{\mu_m \frac{S}{S + K_S} X_a V}_{\text{growth}} - \underbrace{b X_a V}_{\text{decay}} - \underbrace{Q X_a}_{\text{outflow}} \quad (\text{S-49})$$

675

$$\underbrace{V \frac{dS}{dt}}_{\text{accumulation}} = - \underbrace{\frac{\mu_m}{Y} \frac{S}{S + K_S} X_a V}_{\text{consumption}} + \underbrace{Q (S^0 - S)}_{\text{inflow and outflow}} \quad (\text{S-50})$$

676

$$\underbrace{V \frac{dP}{dt}}_{\text{accumulation}} = \underbrace{\mu_m \frac{(1 - Y)}{Y} \frac{S}{S + K_S} X_a V}_{\text{generation}} + \underbrace{b X_a V}_{\text{decay}} - \underbrace{Q P}_{\text{outflow}} \quad (\text{S-51})$$

677 where

678 • X_a - active biomass concentration [$M_X L^{-3}$]

679 • Y - true yield [$M_S M_X^{-1}$]

680 • S^0 - influent substrate concentration [$M_S L^{-3}$]

681 • P - effluent product concentration [$M_P L^{-3}$]

682 • V - chemostat volume [L^3]

683 • Q - hydraulic flow rate [$L^3 T^{-1}$]

684 The subscripts X and S denote biomass and substrate, respectively.

685 To transform the mass-balance relationship, we define the hydraulic residence time (HRT) as θ

686 [T], which is also the inverse of dilution rate D [T^{-1}]

$$\theta = \frac{V}{Q} = \frac{1}{D} \quad (\text{S-52})$$

687 HRT is the average time a water parcel spends in a system. Another related fundamental parameter

688 for Environmental Biotechnology is the solids residence time (SRT) or θ_x , which Rittmann and

689 McCarty¹³ formally defines as

$$\theta_x = \frac{\text{Total Biomass in the System}}{\text{Generation Rate of Biomass}} \quad (\text{S-53})$$

690 In wastewater engineering, SRT is also called the sludge age or the mean cell residence time.
 691 SRT is one of the most fundamental parameters in microbiological systems. SRT correlates with
 692 the structure and function of microbial communities in engineered and natural systems, including
 693 activated sludge⁴¹ and the human gastrointestinal tract⁴². When we apply this definition to the
 694 chemostat, we find that HRT=SRT:

$$\theta_x = \frac{VX_a}{QX_a} = \frac{V}{Q} = \theta \quad (\text{S-54})$$

695 Using (S-52) and (S-54), the equations (S-49) and (S-50) can be simplified to:

$$\frac{dX_a}{dt} = X_a \left(\mu - \frac{1}{\theta_x} \right) \quad (\text{S-55})$$

696

$$\frac{dS}{dt} = -\frac{\mu}{Y}X_a + \frac{(S^0 - S)}{\theta_x} \quad (\text{S-56})$$

697 The modified biomass balance (S-55) shows structural similarity to the replicator equation (S-9).
 698 However, there are important distinctions. In the replicator equation, the strategy frequency
 699 increases when the individual strategy fitness exceeds that of the population average fitness. In
 700 microbiological systems, microbial biomass increases when the net specific rate of microorganisms
 701 exceeds the dilution rate. In environmental dynamics, microorganisms are racing against time (i.e.,
 702 the term $1/\theta_x$), and their growth rate is limited by the substrate (S-56). In the PEGs, the term in
 703 the parentheses (S-55) represents the net accumulation rate:

$$\mu_a \left(S; S^0, \theta_x \right) = \mu - \frac{1}{\theta_x} \quad (\text{S-57})$$

704 Following the projection (Supplementary Note S3), the biomass balance can be transformed into

705 the replicator equation with the net accumulation appearing under the empty strategy.

	S_0	S_1	
S_0	0	0	(S-58)
S_1	$\mu_a(S; S^0, \theta_x)$	0	

706 It should be noted that since the original chemostat system has one active biomass component, the
 707 corresponding replicator equation has two strategies: empty strategy and active-biomass strategy.
 708 μ_a is a function of the substrate concentration S , which is a dynamic variable. Therefore, to
 709 obtain the full dynamics of this environmental game, the biomass mass balance needs to be solved
 710 together with the substrate balance with S^0 and θ_x as the boundary condition and control parameter,
 711 respectively.

712 *The Steady State.*

713 The steady-state occurs when the system dynamics stabilize, and system variables become
 714 time-invariant. For example, setting $dX_a/dt = 0$ in (S-55) gives a fundamental relationship that
 715 holds at the steady-state

$$\theta_x = \frac{1}{\mu} \quad (\text{S-59})$$

716 Rittmann and McCarty¹³ call θ_x "a fundamental descriptor of the physiological status of the system"
 717 because it equals μ^{-1} at the steady state. Supplementary Note S6 explores how θ_x acts as a "lever"
 718 for environmental selection.

719 Remarkably, at steady state, $\mu_a(S; S^0, \theta_x) = 0$, and the payoff matrix (S-58) simplifies to

	S_0	S_1	
S_0	0	0	(S-60)
S_1	0	0	

720 where all strategies, including the environmental strategy, become a Nash equilibrium. Does
 721 this mean that environmental conditions no longer exert any selective pressure across the simplex

722 boundary?

723 To answer this question, it is helpful to examine the steady-state concentrations of substrate and
724 biomass. Set the left-hand side (LHS) of (S-49) and (S-50) to zero, and divide the equations by V

$$\frac{dX_a}{dt} = \mu_m \frac{S}{S + K_S} X_a - b X_a - \frac{X_a}{\theta_x} \quad (\text{S-61})$$

725

$$\frac{dS}{dt} = -\frac{\mu_m}{Y} \frac{S}{S + K_S} X_a + \frac{(S^0 - S)}{\theta_x} \quad (\text{S-62})$$

726 Then, rearranging the biomass balance gives the steady-state substrate concentration (S^*):

$$S^* = K_S \frac{1 + b\theta_x}{\mu_m\theta_x - (1 + b\theta_x)} \quad (\text{S-63})$$

727 The steady-state active biomass concentration can be obtained by solving (S-61) for the first term
728 on the right-hand side (RHS), substituting the results in (S-62), and solving for X_a^* :

$$X_a^* = Y \left(\frac{S^0 - S^*}{1 + b\theta_x} \right) \quad (\text{S-64})$$

729 These algebraic solutions show two important features. First, the steady-state biomass concentration
730 depends on the influent substrate concentration, S^0 , set at the boundary. When $S^0 < S^*$, X_a^*
731 is negative, which is not physically possible and means that the chemostat is unable to support
732 a steady state biomass (i.e., washout). Second, when $S^0 > S^*$, S^* and X_a^* are stable dynamic
733 equilibrium points – If S and X_a deviate from S^* and X_a^* , the payoff matrix becomes non-zero and
734 drives the system back towards S^* and X_a^* .

735 This analysis differentiates a Nash equilibrium (a static equilibrium concept) from environmental
736 pressures. At the steady state, it would be incorrect to interpret an absence of environmental pressure
737 from $\mu_a(S; S^0, \theta_x) = 0$. The null payoff matrix indicates a stable dynamic equilibrium instead of
738 environmental neutrality. When the system deviates from the steady state, $\mu_a(S; S^0, \theta_x) \neq 0$ and the
739 environmental pressures re-enter the payoff matrix, restoring the system towards the steady state.

While this stability holds between the environmental strategy and biological strategies at steady state, Supplementary Note S6 explores neutrality among biological strategies under environmentally neutral selection.

Limiting Steady State Values.

There are two important limiting cases for subsequent sections. In the limit of a short θ_x , minimal substrate is consumed ($S^0 \approx S$), and very little active biomass is generated. Then, (S-64) can be rearranged to define θ_x^{min} :

$$\theta_x^{min} = \frac{K_S + S^0}{S^0 (\mu_m - b) - bK_S} \quad (\text{S-65})$$

In the limit of a very large S^0 , (S-65) simplifies to define $[\theta_x^{min}]_{lim}$, which is the absolute minimum time requirement for microbial growth¹⁸:

$$[\theta_x^{min}]_{lim} = \frac{1}{(\mu_m - b)} \quad (\text{S-66})$$

In the limit of a very large θ_x , (S-63) simplifies to define S_{min}^* , the minimum concentration capable of sustaining steady-state biomass²²:

$$S_{min}^* = K_S \frac{b}{\mu_m - b} \quad (\text{S-67})$$

S_{min}^* depends only on parameters related to microbial kinetics (i.e., K_S , μ_m , and b).

The Generalized Pure Environmental Games.

In the environment, microorganisms grow by taking up sources of energy and cellular components. In turn, they release waste products and modify the environment. A chain of microbial reactions forms a metabolic food web, in which the waste product of one microbe feeds another microbe. These metabolic interactions of microorganisms mediated through the environment can be described using the framework of multi-component reaction and transport modeling Steefel and MacQuarrie⁴³. Here, we adopt the reaction framework to describe the generalized PEGs.

759 A multi-component model treats microorganisms and chemicals as components. When chemi-
760 cal and microbiological reactions consume components, the reaction stoichiometry can be written
761 as

$$0 = \sum_{i=1}^{N_C} \lambda_{ip} \hat{C}_i \quad (\text{S-68})$$

762 where $\hat{C}_i$ is the formula for component i [M] and $\lambda_{i,p}$ is the stoichiometry coefficient for component
763 i in a process p [M_i per reaction] when the process p is a reaction. N_C is the number of components.
764 $\lambda_{i,p}$ is negative for the reactant and positive for the product. For example, the chemostat example
765 has consuming substrates and generating products. This growth reaction can be represented as

$$0 = \lambda_S S + \lambda_X X_a \quad (\text{S-69})$$

766 Then the true yield is

$$Y = \left| \frac{\lambda_X}{\lambda_S} \right| \quad (\text{S-70})$$

767 The conservation law for all components undergoing chemical and microbial reactions can be
768 written as

$$\frac{dC_i}{dt} = \sum_{p=1}^{N_P} \lambda_{ip} \rho_p(\mathbf{E}) \quad (\text{S-71})$$

769 where ρ_p is the rate of p -th process and N_P is the number of processes.

770 The conservation law is consistent with the generalized PEGs if it can be rewritten in the form

$$\frac{dX_i}{dt} = \left(\sum_{p=1}^{N_P} \lambda_{ip} \eta_p(\mathbf{E}) \right) X_i \quad (\text{S-72})$$

771 where η_p is the specific rate of p -th process and N_P is the number of processes. The process rates,
772 η_p , are positive by convention. Comparing this equation to the definition of the pure environmental
773 game (S-47) gives

$$\mu_{a,i}(\mathbf{E}) = \sum_{p=1}^{N_P} \lambda_{ip} \eta_p(\mathbf{E}) \quad (\text{S-73})$$

774 The chemostat example (S-49) illustrates this where

$$\eta_1 = \mu_m \frac{S}{S + K_S} \quad (\text{S-74})$$

775

$$\eta_2 = b \quad (\text{S-75})$$

776

$$\eta_3 = \frac{1}{\theta_x} \quad (\text{S-76})$$

777 with the stoichiometry coefficients $\lambda_{a1} = 1$, $\lambda_{a2} = -1$, and $\lambda_{a3} = -1$ Note that the first two are
778 microbiological processes, while the third is a physical transport process.

779 Table S2 summarizes the one-species chemostat model in a format commonly known as the
780 Gujer matrix. In the Gujer matrix, each row represents a process. When the process is a reaction,
781 it is described by (S-68). For transport processes, influent is positive and effluent is negative. The
782 chemostat example in this section had six processes: growth, decay, biomass outflow, substrate
783 inflow, substrate outflow, and product outflow. The middle section holds the stoichiometry coef-
784 ficients, $\lambda_{p,i}$, noting that the indices i and p are transposed relative to (S-71). The middle section
785 is also known as the stoichiometry matrix, Λ . The final column shows the process rates, ρ ($\mathbf{E}$).
786 The Gujer matrix serves as the recipe for the conservation law, which can be restored by matrix
787 multiplication.

$$\frac{dC_i}{dt} = \Lambda^T \rho (\mathbf{E}) \quad (\text{S-77})$$

Supplementary Table S2: Gujer matrix for the one-species chemostat model.

Process	Components			Process Rate ρ ($\mathbf{E}$)
	Biomass X_a	Substrate S	Product P	
1. Growth	+1	$-1/Y$	$+(1 - Y)/Y$	$\mu_m \frac{S}{S + K_S} X_a$
2. Decay	-1	0	+1	$b X_a$
3. X_a Outflow	-1	0	0	$\frac{X_a}{\theta_x}$
4. S Inflow	0	+1	0	$\frac{S_0}{\theta_x}$
5. S Outflow	0	-1	0	$\frac{S}{\theta_x}$
6. P Outflow	0	0	-1	$\frac{P}{\theta_x}$

788 **Energy Dissipation Rate.**

789 The second law of thermodynamics states that the total entropy of an isolated system must
790 increase, $\Delta S > 0$. A dilute aqueous system approximates the isothermal condition due to water's
791 high heat capacity, and the Gibbs free energy provides an equivalent statement of the second law.
792 For a biological reaction index by p , the Gibbs free energy of reaction, $\Delta G_p [EM^{-1}]$, can be written
793 as

$$\Delta G_p = \Delta G_p^{o'} + RT \ln \left(\prod_i C_i^{\lambda_{ip}} \right) \quad (S-78)$$

794 where $\Delta G_p^{o'}$ is the standard Gibbs free energy of the reaction $[EM^{-1}]$, R is the ideal gas constant
795 (8.314 J/K-mol), and T is the absolute temperature in Kelvin. Here, the superscript "o" denotes
796 standard condition and the prime denotes pH=7. ΔG_p must be less than zero for the reaction to
797 occur spontaneously. $\Delta G_p^{o'}$ is determined from the standard Gibbs free energy of formation $[EM^{-1}]$
798 for each component in the reaction multiplied by the stoichiometry

$$\Delta G_p^{o'} = \sum_{i=1}^{N_C} \lambda_{ip} \Delta G_{fi}^{o'} \quad (S-79)$$

799 For simplicity, assume that the energy dissipated by microorganisms is transported out of the system
800 by the bulk fluid. The energy dissipation rate of the system, $\dot{q}_{diss} [EL^{-3}T^{-1}]$, can be written as the
801 product of the process rate and the Gibbs free energy of the overall reaction.

$$\dot{q}_{diss} = - \sum_p \rho_p \Delta G_p \quad (S-80)$$

802 The system will perform a set of reactions that are spontaneous overall (exergonic), and the dissipa-
803 tion rate will be positive. During the synthesis of new biomass, microorganisms partition electrons
804 into cell synthesis and energy generation. When Y has the units of $[eeq_X eeq_S^{-1}]$, it is numerically
805 equivalent to the symbol f_s^0 used in Rittmann and McCarty¹³. Since the microorganisms perform
806 anabolic and catabolic reactions in parallel, it is convenient to write the overall process as the sum

807 of anabolic and catabolic reactions ($R_{p,ana}$ and $R_{p,cat}$ respectively):

$$R_p = Y R_{p,ana} + (1 - Y) R_{p,cat} \quad (\text{S-81})$$

808 Here, R_p , $R_{p,ana}$, and $R_{p,cat}$ all assume 1 mole of electrons (or 1 electron equivalent = 1 eeq)
 809 transferred. However, ρ_p are written in terms of the rate of biomass generation. Therefore, we define
 810 $\sigma_{p,ana} = 1$ and $\sigma_{p,cat} = (1 - Y)/Y$. Supplementary Table S3 summarizes the energy dissipation
 811 matrix for the one-species chemostat model. Here, both Σ and ΔG are $N_p \times 2$ matrices. For the
 812 endogenous decay processes, there is no cell synthesis and $\sigma_{p,ana} = 0$. Both $\sigma_{p,ana} = \sigma_{p,cat} = 0$ for
 813 transport, as they are not reactions. The overall reaction can be divided into anabolic and catabolic
 814 reactions

$$\dot{q}_{diss} = - \sum_p [\rho_p (\sigma_{p,ana} \Delta G_{p,ana} + \sigma_{p,cat} \Delta G_{p,cat})] \quad (\text{S-82})$$

815 Or more generally, it can be calculated via matrix operations

$$\dot{q}_{diss} = -\rho^T (\Sigma \circ \Delta G) \mathbf{1} \quad (\text{S-83})$$

816 where $\circ$ denotes element-wise (Hadamard) product. For the chemostat system, the energy dissipa-
 817 tion rate is written as

$$\begin{aligned} \dot{q}_{diss} = & -\mu_m \frac{S}{S + K_S} X_a \left(\Delta G_{grow,ana} + \frac{(1 - Y)}{Y} \Delta G_{grow,cat} \right) \\ & - b X_a \Delta G_{dec,cat} \end{aligned} \quad (\text{S-84})$$

818 Finally, the specific energy dissipation rate of microorganisms, $\dot{g}_{diss}$ [$EM_X^{-1} T^{-1}$], is defined by
 819 dividing this equation by X_a ,

$$\begin{aligned} \dot{g}_{diss} = & -\mu_m \frac{S}{S + K_S} \left(\Delta G_{grow,ana} + \frac{(1 - Y)}{Y} \Delta G_{grow,cat} \right) \\ & - b \Delta G_{dec,cat} \end{aligned} \quad (\text{S-85})$$

Supplementary Table S3: Energy dissipation matrix for the one-species chemostat model.

Process	Σ		ΔG		$\rho(\mathbf{E})$
	$\sigma_{p,ana}$	$\sigma_{p,cat}$	$\Delta G_{p,ana}$	$\Delta G_{p,cat}$	
1. Growth	1	$(1 - Y)/Y$	$\Delta G_{grow,ana}$	$\Delta G_{grow,cat}$	$\mu_m \frac{S}{S+K_S} X_a$
2. Decay	0	1	0	$\Delta G_{dec,cat}$	$b X_a$
3. X_a Outflow	0	0	0	0	$\frac{X_a}{\theta_x}$
4. S Inflow	0	0	0	0	$\frac{S^0}{\theta_x}$
5. S Outflow	0	0	0	0	$\frac{S}{\theta_x}$
6. P Outflow	0	0	0	0	$\frac{P}{\theta_x}$

S6 Environmental Selection Dynamics.

The PEGs provide a framework to describe environmental games operating in parallel to the neutral selection game. This section explores the r/K selection game as an instance of PEGs with *Methanosarcina* and *Methanothrix* (formerly *Methanosaeta*) as two classic microorganisms competing for a shared resource – acetate in this case^{15,44}. Six dynamic regimes are identified by exploring the parameter space of the r/K selection game.

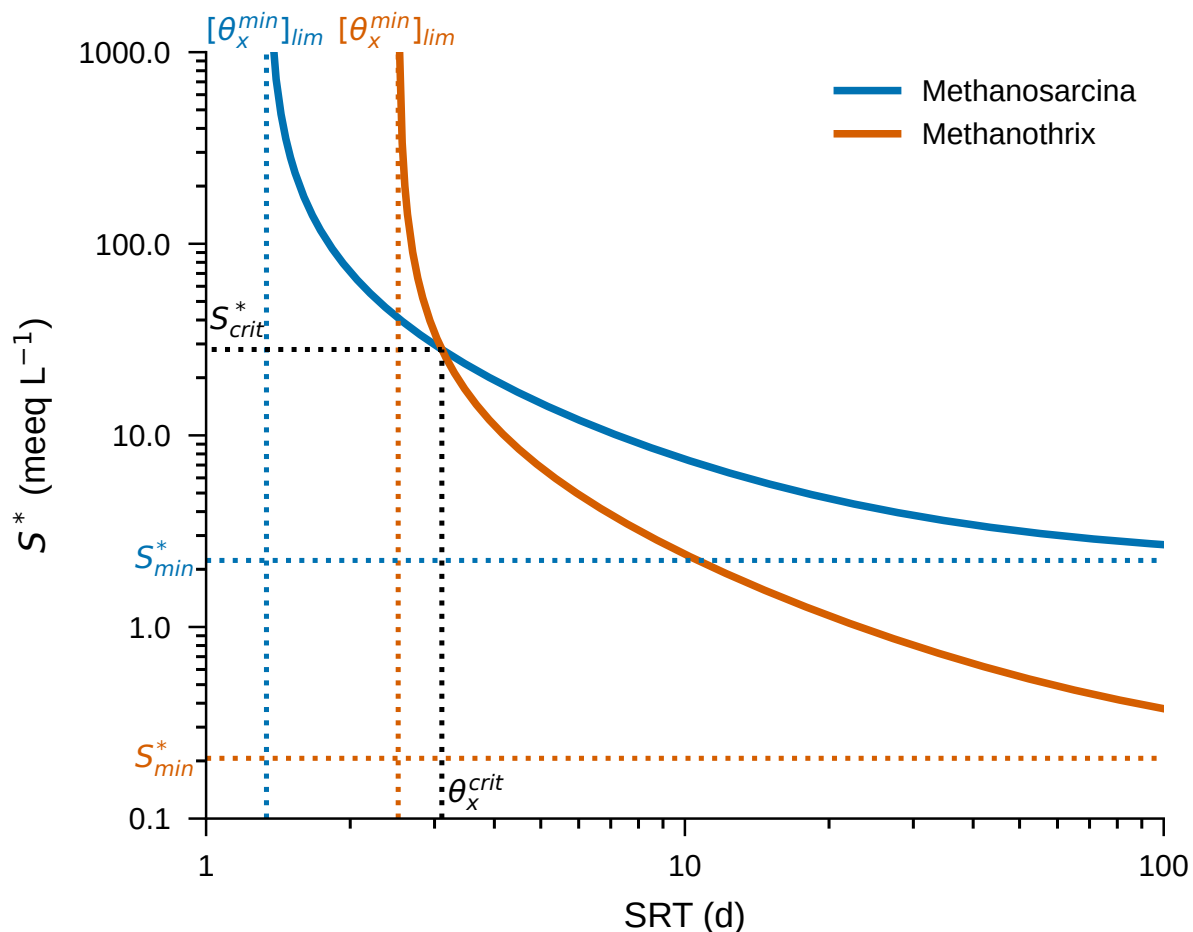
Extended Data Table 2 summarizes the microbial kinetic parameters for *Methanothrix* and *Methanosarcina*. *Methanosarcina*'s competitive advantage to grow rapidly is characterized by the higher μ_m (r-strategy). *Methanothrix*'s competitive advantage to scavenge the scarce acetate is characterized by the lower K_S (K-strategy).

Anticipated Steady State.

The steady-state substrate concentration, S^* , (S-63) was developed independently by two separate traditions. For engineers, S^* represents the effluent quality and water quality standard they must meet to avoid compliance violations and expensive penalties¹⁸. For microbial ecologists, S^* (or more commonly R^*) represents the anticipated substrate concentration microorganisms will

835 "experience" when the system reaches the steady-state and provides a basis for competitive exclu-
 836 sion^{19,20}. θ_x is the "lever" that engineers have to control water quality, and microbial ecologists
 837 have to control microbial community composition.

838 Supplementary Figure S3 summarizes the relationship between S^* and θ_x using (S-63), which
 839 represents outcomes if *Methanosarcina* and *Methanothrix* were grown in separate chemostats. As
 840 shown in (S-66), $[\theta_x^{min}]_{lim}$ is the minimum time requirement for growth. When $\theta_x > [\theta_x^{min}]_{lim}$, the
 841 methanogens are able to grow and S^* increases with θ_x : $[\theta_x^{min}]_{lim} = 1.3$ d for *Methanosarcina* and
 842 $[\theta_x^{min}]_{lim} = 2.5$ d for *Methanothrix*, respectively.



Supplementary Figure S3: The relationship between the anticipated steady-state substrate concentration, S^* , and the solids residence time, θ_x , if *Methanosarcina* and *Methanothrix* were grown in separate chemostats. Parameters are summarized in Table 2.

$$[\theta_x^{min}]_{lim, cin} = \frac{1}{\mu_{m, cin} - b_{cin}} = \frac{1}{0.80 - 0.052} = 1.3 \text{ d} \quad (\text{Methanosarcina}) \quad (\text{S-86})$$

$$[\theta_x^{min}]_{lim, thr} = \frac{1}{\mu_{m, thr} - b_{thr}} = \frac{1}{0.41 - 0.013} = 2.5 \text{ d} \quad (\text{Methanothrix}) \quad (\text{S-87})$$

843 Furthermore, S^* is a monotonically decreasing function of θ_x and approaches the value of S_{min}^* as
844 defined in (S-67).

$$\begin{aligned} S_{min, cin}^* &= K_{S, cin} \frac{b_{cin}}{\mu_{m, cin} - b_{cin}} \\ &= 32 \cdot \frac{0.052}{0.80 - 0.052} = 2.2 \text{ meeq L}^{-1} \quad (\text{Methanosarcina}) \quad (\text{S-88}) \end{aligned}$$

$$\begin{aligned} S_{min, thr}^* &= K_{S, thr} \frac{b_{thr}}{\mu_{m, thr} - b_{thr}} \\ &= 6.3 \cdot \frac{0.013}{0.41 - 0.013} = 0.21 \text{ meeq L}^{-1} \quad (\text{Methanothrix}) \quad (\text{S-89}) \end{aligned}$$

845 The $S_{min, thr}^*$ of 0.2 meeq L⁻¹ or (1.7 mgCOD L⁻¹) is several factors lower than a range of S_{min}^*
846 estimates 5.8 to 31.3 mgCOD L⁻¹ obtained by McCarty and Bae⁴⁵ likely because of thermodynamic
847 inhibition. Since McCarty and Bae⁴⁵ estimates K_{Ac} between 106 to 577 mgCOD L⁻¹ (or 13 to
848 72 meeq L⁻¹) for the same digesters, this work considers thermodynamic inhibition by an upward
849 correction of $K_{S, thr}$ from 6.3 to 22 meeq L⁻¹ only for Regime V and VI (Extended Data Table 2).
850 Therefore, the updated $S_{min, thr}^*$ value for *Methanothrix* is

$$\begin{aligned} S_{min, thr}^* &= K_{S, thr} \frac{b_{thr}}{\mu_{m, thr} - b_{thr}} \\ &= 22 \cdot \frac{0.013}{0.41 - 0.013} = 0.72 \text{ meeq L}^{-1} \quad (\text{Methanothrix}) \quad (\text{S-90}) \end{aligned}$$

851 For calculating the specific energy dissipation by *Methanosarcina* and *Methanothrix*, ΔG_{ana} needs
852 to be calculated at the steady state. Extended Data Table 3 summarizes the reaction stoichiometry
853 for the anabolic reaction (cell synthesis), catabolic reaction (energy generation), and decay reactions

854 for acetoclastic methanogens. Assuming pH=7, pCO₂ = 0.5 atm, [NH₄]=0.01 mmol/L, and unit
855 activity for biomass and water, ΔG value can be expressed as a function of S_{Ac}

$$\begin{aligned}\Delta G_{grow,ana}^* &= \Delta G_{grow,ana}^{o'} + RT \ln \left(\frac{[C_5H_7O_2N]^{0.05} [H_2O]^{0.15}}{[CH_3COO^-]^{0.125} [NH_4^+]^{0.05}} \right) \\ &= 6.96 + RT \ln \left(\frac{1}{[S_{Ac}^* \cdot \frac{1}{8}]^{0.125} [0.01]^{0.05}} \right)\end{aligned}$$

856 Then, at S_{min}^{*} the energy values for ΔG_{grow,ana} are

$$\begin{aligned}\Delta G_{grow,ana,cin}^* &= \Delta G_{grow,ana}^{o'} + RT \ln \left(\frac{1}{[S_{min,cin}^*]^{0.125} [0.01]^{0.05}} \right) \\ &= 6.96 + \frac{8.314 \cdot 298.15}{1000} \ln \left(\frac{1}{[0.0022 \cdot \frac{1}{8}]^{0.125} [0.01]^{0.05}} \right)\end{aligned}\tag{S-91}$$

$$\begin{aligned}&= 10.1 \text{ kJ eeq}^{-1} \quad (\text{Methanosarcina}) \\ &\tag{S-92}\end{aligned}$$

$$\begin{aligned}\Delta G_{grow,ana,thr}^* &= \Delta G_{grow,ana}^{o'} + RT \ln \left(\frac{1}{[S_{min,thr}^*]^{0.125} [0.01]^{0.05}} \right) \\ &= 6.96 + \frac{8.314 \cdot 298.15}{1000} \ln \left(\frac{1}{[0.00072 \cdot \frac{1}{8}]^{0.125} [0.01]^{0.05}} \right)\end{aligned}\tag{S-93}$$

$$\begin{aligned}&= 10.4 \text{ kJ eeq}^{-1} \quad (\text{Methanothrix}) \\ &\tag{S-94}\end{aligned}$$

857 Similarly, ΔG_{grow,cat} at S_{min}^{*} are

$$\begin{aligned}\Delta G_{grow,cat}^* &= \Delta G_{grow,cat}^{o'} + RT \ln \left(\frac{[pCH_4]^{0.125} [pCO_2]^{0.125}}{[CH_3COO^-]^{0.125}} \right) \\ &= -4.48 + RT \ln \left(\frac{(0.5)^{0.125} (0.5)^{0.125}}{[S_{Ac}^* \cdot \frac{1}{8}]^{0.125}} \right)\end{aligned}$$

858

$$\begin{aligned}\Delta G_{grow,cat,cin}^* &= \Delta G_{grow,cat}^{o'} + RT \ln \left(\frac{(0.5)^{0.125} (0.5)^{0.125}}{[S_{min,cin}^* \cdot \frac{1}{8}]^{0.125}} \right) \\ &= -4.48 + \frac{8.314 \cdot 298.15}{1000} \ln \left(\frac{(0.5)^{0.125} (0.5)^{0.125}}{[0.0022 \cdot \frac{1}{8}]^{0.125}} \right)\end{aligned}\quad (S-95)$$

$$= -2.4 \text{ kJ eeq}^{-1} \quad (\text{Methanosarcina}) \quad (S-96)$$

$$\begin{aligned}\Delta G_{grow,cat,thr}^* &= \Delta G_{grow,cat}^{o'} + RT \ln \left(\frac{(0.5)^{0.125} (0.5)^{0.125}}{[S_{min,thr}^* \cdot \frac{1}{8}]^{0.125}} \right) \\ &= -4.48 + \frac{8.314 \cdot 298.15}{1000} \ln \left(\frac{(0.5)^{0.125} (0.5)^{0.125}}{[0.00072 \cdot \frac{1}{8}]^{0.125}} \right)\end{aligned}\quad (S-97)$$

$$= -2.0 \text{ kJ eeq}^{-1} \quad (\text{Methanothrix}) \quad (S-98)$$

859 Finally, $\Delta G_{dec,cat}$ at S_{min}^* for both *Methanosarcina* and *Methanothrix* are

$$\begin{aligned}\Delta G_{dec,cat}^* &= \Delta G_{dec,cat}^{o'} + RT \ln \left(\frac{[p_{CH_4}]^{0.125} [NH_4^+]^{0.05} [p_{CO_2}]^{0.125}}{[C_5H_7O_2N]^{0.05} [H_2O]^{0.15}} \right) \\ &= -11.44 + RT \ln \left(\frac{(0.5)^{0.125} (0.01)^{0.05} (0.5)^{0.125}}{1} \right) \\ &= -12.4 \text{ kJ eeq}^{-1}\end{aligned}$$

860 Then, the anticipated specific energy dissipation rate can be calculated at S_{min}^* using (S-85).

$$\begin{aligned}\dot{g}_{diss,cin} &= -\mu_{m,cin} \frac{S_{min,cin}^*}{S_{min,cin}^* + K_{Ac,cin}} \left(\Delta G_{grow,ana,cin}^* + \frac{(1 - Y_{cin})}{Y_{cin}} \Delta G_{grow,cat,cin}^* \right) \\ &\quad - b_{cin} \Delta G_{dec,cat}^* \\ &= -0.80 \cdot \frac{2.2}{2.2 + 32} \left(10.1 + \frac{0.917}{0.083} (-2.4) \right) - 0.052 (-12.4) \\ &= 1.5 \frac{\text{kJ}}{\text{eeq}_X} \cdot \frac{1 \text{ d}}{86400 \text{ s}} \frac{1000 \text{ J}}{1 \text{ kJ}} \frac{1000 \text{ mW}}{1 \text{ W}} \\ &= 17 \frac{\text{mW}}{\text{eeq}_X} \quad (\text{Methanosarcina})\end{aligned}\quad (S-99)$$

$$\begin{aligned}
 \dot{g}_{diss,thr} &= -\mu_{m,thr} \frac{S_{min,thr}^*}{S_{min,thr}^* + K_{Ac,thr}} \left(\Delta G_{grow,ana,thr}^* + \frac{(1 - Y_{thr})}{Y_{thr}} \Delta G_{grow,cat,thr}^* \right) \\
 &\quad - b_{thr} \Delta G_{dec,cat}^* \\
 &= -0.41 \cdot \frac{0.72}{0.72 + 22} \left(10.4 + \frac{0.966}{0.034} (-2.0) \right) - 0.013 (-12.4) \\
 &= 0.8 \frac{\text{kJ}}{\text{eeq}_X \cdot \text{d}} \frac{1 \text{ d}}{86400 \text{ s}} \frac{1000 \text{ J}}{1 \text{ kJ}} \frac{1000 \text{ mW}}{1 \text{ W}} \\
 &= 9 \frac{\text{mW}}{\text{eeq}_X} \quad (\text{Methanothrix})
 \end{aligned} \tag{S-100}$$

861 Therefore, the specific energy dissipation rates of maintaining one electron equivalent of *Methanosarcina*
 862 and *Methanothrix* are 17 and 9 mW eeq_X⁻¹ at S_{min}^* , respectively.

863 In Supplementary Figure S3, there is a point where the lines for *Methanothrix* and *Methanosarcina*
 864 cross. This can be called the critical point where the dominant community member shifts, as the
 865 next subsection shows. At the critical point, *Methanosarcina*'s ability to grow rapidly is exactly
 866 neutralized by *Methanothrix*'s ability to scavenge acetate. For this *Methanothrix*-*Methanosarcina*
 867 system, the critical point occurs at $\theta_x^{crit} = 3.1 \text{ d}$ with $S_{crit}^* = 28 \text{ meeq L}^{-1}$.

868 Dynamics of *Methanosarcina*-*Methanothrix* System.

869 The anticipated steady states and critical points established in the previous subsection provide
 870 anchors for interpreting system dynamics. A system of equations – (S-101), (S-102), and (S-103)
 871 – describes the *Methanosarcina*-*Methanothrix* system

$$\frac{dX_{cin}}{dt} = X_{cin} \left(\mu_{m,cin} \frac{S_{Ac}}{S_{Ac} + K_{Ac,cin}} - b_{cin} - \frac{1}{\theta_x} \right) \tag{S-101}$$

872

$$\frac{dX_{thr}}{dt} = X_{thr} \left(\mu_{m,thr} \frac{S_{Ac}}{S_{Ac} + K_{Ac,thr}} - b_{thr} - \frac{1}{\theta_x} \right) \tag{S-102}$$

873

$$\begin{aligned} \frac{dS_{Ac}}{dt} = & -\frac{\mu_{m,cin}}{Y_{cin}} \frac{S_{Ac}}{S_{Ac} + K_{Ac,cin}} X_{cin} \\ & -\frac{\mu_{m,thr}}{Y_{thr}} \frac{S_{Ac}}{S_{Ac} + K_{Ac,thr}} X_{thr} \\ & + \frac{S_{Ac}^0 - S_{Ac}}{\theta_x} \end{aligned} \quad (S-103)$$

874 Describing the energy dissipation rate requires integration of reaction stoichiometry with reac-
875 tion kinetics. Table 3 summarizes the anabolic, catabolic, and decay reactions for acetoclastic
876 methanogens. All acetoclastic methanogens share the same ΔG values for the anabolic, catabolic,
877 and endogenous decay reactions. However, the coefficient Y_i combines ΔG values in different
878 proportions. Therefore, the energy dissipation rate for the chemostat following (S-83) is

$$\begin{aligned} \dot{q}_{diss} = & -\mu_{m,cin} \frac{S_{Ac}}{S_{Ac} + K_{Ac,cin}} X_{cin} \left(\Delta G_{grow,ana} + \frac{(1 - Y_{cin})}{Y_{cin}} \Delta G_{grow,cat} \right) \\ & - b_{cin} X_{cin} \Delta G_{dec,cat} \\ & -\mu_{m,thr} \frac{S_{Ac}}{S_{Ac} + K_{Ac,thr}} X_{thr} \left(\Delta G_{grow,ana} + \frac{(1 - Y_{thr})}{Y_{thr}} \Delta G_{grow,cat} \right) \\ & - b_{thr} X_{thr} \Delta G_{dec,cat} \end{aligned} \quad (S-104)$$

879 Extended Data Table 1 summarizes the Gujer matrix for the *Methanosarcina*-*Methanothrix* chemo-
880 stat model.

881 **Washout** ($\theta_x < [\theta_x^{min}]_{lim}$) (**Regime I**)

882 Consider an idealized chemostat with a highly favorable $S_{Ac}^0 = 625$ meeq L⁻¹ and initial $X_{cin} =$
883 500 meeq L⁻¹ and $X_{thr} = 500$ meeq L⁻¹. Extended Data Fig. 1 shows the *Methanosarcina*-
884 *Methanothrix* dynamics in the absolute coordinate with $\theta_x = 0.5$ d $< [\theta_x^{min}]_{lim,cin}$ and $[\theta_x^{min}]_{lim,thr}$.
885 Initially, the acetate concentration sharply decreases from $S_{Ac} = 625$ to 1.6 meeq L⁻¹ within 2.5
886 h due to the high concentration of *Methanosarcina* and *Methanothrix* (Extended Data Fig. 1a).
887 Correspondingly, $\dot{q}_{diss,cin}$ drops from 340 to 14 mW eq_X⁻¹ and $\dot{q}_{diss,thr}$ from 510 to 55 mW eq_X⁻¹.
888 The energy dissipation rate for *Methanothrix* is higher than *Methanosarcina* because it requires
889 a higher proportion of catabolic reaction per unit of biomass (i.e., $1 - Y_{thr} > 1 - Y_{cin}$). The

890 decrease in biomass concentration is more gradual as the physical biomass removal lags behind
 891 microbiological acetate consumption. During the first day ($t < 1$ d), the biomass concentration for
 892 *Methanosarcina* decreases from 500 to 76 meeq L⁻¹ and *Methanothrix* decreases from 500 to 82
 893 meeq L⁻¹. *Methanothrix* fares marginally better as a K-strategist by scavenging acetate at a low
 894 concentration. After $t = 1$ d, both populations are washed out, and S_{Ac} returns to the influent level
 895 by Day 8. In tandem, the energy dissipation rates return to the highest levels ($\dot{g}_{diss,cin} = 340$ mW
 896 eeq_X^{-1} and $\dot{g}_{diss,thr} = 510$ mW eeq_X^{-1}), but are not sufficient to let microorganisms grow fast enough
 897 (Extended Data Fig. 1c).

898 Extended Data Fig. 1b shows the same dynamics projected onto the simplex. Initially, the
 899 simplex is fully occupied by an equal amount of *Methanosarcina* and *Methanothrix* strategies.
 900 During the first day ($t < 1$ d), *Methanothrix* gains in simplex frequency from 0.5 to 0.52, while
 901 *Methanosarcina* loses in simplex frequency from 0.5 to 0.48. Thereafter, *Methanothrix* is washed
 902 out, but between Days 1 and 3 *Methanosarcina* momentarily gains in simplex frequency from 0.48
 903 to 0.53 by taking advantage of the elevated S_{Ac} as an r-strategist. However, this resistance proves
 904 futile. Both methanogen strategies are washed out, yielding the simplex to the environmental
 905 strategy.

906 Why does the environmental strategy resist invasion? At time zero, the net-specific accumula-
 907 tion rates for *Methanosarcina* and *Methanothrix* can be calculated by examining the terms in the

908 parentheses for (S-101) and (S-102) at $t = 0$ d:

$$\begin{aligned}\mu_{a,cin}(\mathbf{E}) &= \mu_{m,cin} \frac{S_{Ac}}{S_{Ac} + K_{Ac,cin}} - b_{cin} - \frac{1}{\theta_x} \\ &= 0.80 \cdot \frac{625}{625 + 32} - 0.052 - \frac{1}{0.5} \\ &= 0.761 - 0.052 - 2.0 \\ &= -1.3 \text{ d}^{-1} \quad (\text{Methanosarcina})\end{aligned}\tag{S-105}$$

$$\begin{aligned}\mu_{a,thr}(\mathbf{E}) &= \mu_{m,thr} \frac{S_{Ac}}{S_{Ac} + K_{Ac,thr}} - b_{thr} - \frac{1}{\theta_x} \\ &= 0.41 \cdot \frac{625}{625 + 6.3} - 0.013 - \frac{1}{0.5} \\ &= 0.406 - 0.013 - 2.0 \\ &= -1.6 \text{ d}^{-1} \quad (\text{Methanothrix})\end{aligned}\tag{S-106}$$

909 Using these values, the dynamic payoff matrix at $t=0$ (S-107) reveals that the environmental strategy
910 is SNE (Supplementary Notes S2 and S4). This washout case ($\theta_x < [\theta_x^{min}]_{lim,thr}$), formally
911 described by Lawrence and McCarty¹⁸, prohibits microorganisms from growing in a chemostat.
912 This work shows that this condition is equivalent to the scenario where the environmental strategy
913 is an SNE. If environmental conditions do not provide enough time for microbes to grow, life cannot
914 emerge.

	<i>Environment</i>	<i>Methanosarcina</i>	<i>Methanothrix</i>
<i>Environment</i>	0	0	0
<i>Methanosarcina</i>	-1.3	0	0
<i>Methanothrix</i>	-1.6	0	0

(S-107)

915 **r-dominates** ($[\theta_x^{min}]_{lim,cin} < \theta_x < \theta_x^{crit}$) (**Regime II**)

916 The washout (Regime I) example illustrates that time can prohibit microorganisms from invading
917 the environment. This example increases $\theta_x = 2$ d, such that $[\theta_x^{min}]_{lim,cin} < \theta_x < \theta_x^{crit}$ to illustrate
918 Regime II: r-dominates. The final state of the Regime I simulation was taken to be the initial

condition: $X_{cin} = 5.7 \times 10^{-4}$ meeq L⁻¹ and $X_{thr} = 4.2 \times 10^{-5}$ meeq L⁻¹. The influent acetate is kept at $S_{Ac}^0 = 625$ meeq L⁻¹.

Extended Data Fig. 2a shows the *Methanosarcina*-*Methanothrix* dynamics in the absolute coordinate. At $t = 0$ d, the net specific accumulation rates for *Methanosarcina* and *Methanothrix* according to (S-101) and (S-102) are

$$\begin{aligned}\mu_{a,cin}(\mathbf{E}) &= \mu_{m,cin} \frac{S_{Ac}}{S_{Ac} + K_{Ac,cin}} - b_{cin} - \frac{1}{\theta_x} \\ &= 0.80 \cdot \frac{625}{625 + 32} - 0.052 - \frac{1}{2} \\ &= 0.761 - 0.052 - 0.5 \\ &= 0.21 \text{ d}^{-1} \quad (\text{Methanosarcina})\end{aligned}\tag{S-108}$$

$$\begin{aligned}\mu_{a,thr}(\mathbf{E}) &= \mu_{m,thr} \frac{S_{Ac}}{S_{Ac} + K_{Ac,thr}} - b_{thr} - \frac{1}{\theta_x} \\ &= 0.41 \cdot \frac{625}{625 + 6.3} - 0.013 - \frac{1}{2} \\ &= 0.406 - 0.013 - 0.5 \\ &= -0.11 \text{ d}^{-1} \quad (\text{Methanothrix})\end{aligned}\tag{S-109}$$

924

	Environment	<i>Methanosarcina</i>	<i>Methanothrix</i>
Environment	0	0	0
<i>Methanosarcina</i>	+0.21	0	0
<i>Methanothrix</i>	-0.11	0	0

(S-110)

Using these values, the dynamic payoff matrix at $t=0$ (S-110) reveals that the positive net accumulation rate of *Methanosarcina*, $\mu_{a,cin}(\mathbf{E}) = +0.21$, disqualifies the environmental strategy from being SNE and ESS. On the other hand, *Methanosarcina* receives positive payoff from the environment to gain abundance from $X_{cin} = 5.7 \times 10^{-4}$ meeq L⁻¹ at $t = 0$ d to the steady-state level of $X_{cin} = 42$ meeq L⁻¹ by $t = 40$ d. In tandem, the steady-state acetate concentration drops to $S_{Ac} = 71$ meeq L⁻¹ and *Methanothrix* is being washed out ($X_{thr} = 1.4 \times 10^{-8}$ meeq L⁻¹ at $t = 40$), unable to receive positive payoff from the environment. Correspondingly, $\dot{g}_{diss,cin}$ drops from 340

932 to 190 mW eeq_X^{-1} and $\dot{g}_{diss,thr}$ from 510 to 390 mW eeq_X^{-1} (Extended Data Fig. 2c). Therefore,
 933 *Methanosarcina* can accumulate and sustain at the same or lower energy dissipation rates given
 934 sufficient time at $\theta_x = 2$ d. These observations are corroborated by displacement of *Methanothrix*
 935 by *Methanosarcina* in the simplex (Extended Data Fig. 2b). Using these values, the net specific
 936 accumulation rates for the dynamic payoff matrix at $t = 40$ d are

$$\begin{aligned}\mu_{a,cin}(\mathbf{E}) &= \mu_{m,cin} \frac{S_{Ac}}{S_{Ac} + K_{Ac,cin}} - b_{cin} - \frac{1}{\theta_x} \\ &= 0.80 \cdot \frac{71}{71 + 32} - 0.052 - \frac{1}{2} \\ &= 0.551 - 0.052 - 0.5 \\ &= 0.00 \text{ d}^{-1} \quad (\text{Methanosarcina})\end{aligned}\tag{S-111}$$

$$\begin{aligned}\mu_{a,thr}(\mathbf{E}) &= \mu_{m,thr} \frac{S_{Ac}}{S_{Ac} + K_{Ac,thr}} - b_{thr} - \frac{1}{\theta_x} \\ &= 0.41 \cdot \frac{71}{71 + 6.3} - 0.013 - \frac{1}{2} \\ &= 0.376 - 0.013 - 0.5 \\ &= -0.14 \text{ d}^{-1} \quad (\text{Methanothrix})\end{aligned}\tag{S-112}$$

937 At the steady-state, $\mu_{a,cin}(\mathbf{E}) = 0.00$ (S-113). Therefore, using these values, the dynamic payoff
 938 matrix reveals that the environmental strategy is promoted to a Nash equilibrium at the steady
 939 state. While both *Methanosarcina* and environmental strategies are Nash equilibrium strategies,
 940 they have deterministic outcomes because environmental dynamics drive these strategies towards
 941 a steady state. Therefore, the steady-state is not environmentally neutral.

	<i>Environment</i>	<i>Methanosarcina</i>	<i>Methanothrix</i>
<i>Environment</i>	0	0	0
<i>Methanosarcina</i>	0.00	0	0
<i>Methanothrix</i>	-0.14	0	0

(S-113)

942 **K-dominates** ($\theta_x > \theta_x^{crit}$) (**Regime IV**)

943 This example uses the final state of Regime II as the initial condition: $X_{cin} = 42 \text{ meeq L}^{-1}$,
 944 $X_{thr} = 1.4 \times 10^{-8} \text{ meeq L}^{-1}$, $S_{Ac} = 71 \text{ meeq L}^{-1}$. As stated earlier, this *Methanosarcina*-
 945 *Methanothrix* system has a $\theta_x^{crit} = 3.107 \text{ d}$ (four significant digits are shown here for illustrative
 946 purposes). This example adds a tiny $\delta = 0.01 \text{ d}$ to this critical value and sets $\theta_x = 3.107 + 0.01 =$
 947 3.117 d to illustrate the profound effects of a minuscule deviation.

948 Extended Data Fig. 3a shows the *Methanosarcina*-*Methanothrix* dynamics in the absolute
 949 coordinates. Upon changing the θ_x , the system takes about 100 days to reach a new stable state of
 950 $S_{Ac} = 27.9 \text{ meeq L}^{-1}$. In tandem, $\dot{g}_{diss,cin}$ drops from 190 to 120 mW eq_X^{-1} and $\dot{g}_{diss,thr}$ from 390
 951 to 320 mW eq_X^{-1} (Extended Data Fig. 3c). The net specific accumulation rates for *Methanosarcina*
 952 and *Methanothrix* are negligibly small:

$$\begin{aligned}\mu_{a,cin}(\mathbf{E}) &= \mu_{m,cin} \frac{S_{Ac}}{S_{Ac} + K_{Ac,cin}} - b_{cin} - \frac{1}{\theta_x} \\ &= 0.80 \cdot \frac{27.9}{27.9 + 32} - 0.052 - \frac{1}{3.117} \\ &= 0.3726 - 0.052 - 0.3208 \\ &= -0.0002 \text{ d}^{-1} \quad (\text{Methanosarcina})\end{aligned}\tag{S-114}$$

$$\begin{aligned}\mu_{a,thr}(\mathbf{E}) &= \mu_{m,thr} \frac{S_{Ac}}{S_{Ac} + K_{Ac,thr}} - b_{thr} - \frac{1}{\theta_x} \\ &= 0.41 \cdot \frac{27.9}{27.9 + 6.3} - 0.013 - \frac{1}{3.117} \\ &= 0.3345 - 0.013 - 0.3208 \\ &= +0.0007 \text{ d}^{-1} \quad (\text{Methanothrix})\end{aligned}\tag{S-115}$$

953 Placing these μ_a values at $t = 100 \text{ d}$ in the dynamic payoff gives a false appearance that the system

954 is at an environmentally neutral selection (see Regime III. Environmental Neutrality).

	<i>Environment</i>	<i>Methanosarcina</i>	<i>Methanothrix</i>	
<i>Environment</i>	0	0	0	(S-116)
<i>Methanosarcina</i>	-0.0002	0	0	
<i>Methanothrix</i>	+0.0007	0	0	

955 For the first 20,000 days, or 55 years, there is no apparent change in X_{cin} and S_{Ac} . However, quietly
956 *Methanothrix* grows exponentially from 1.4×10^{-8} meeq L⁻¹ to $X_{thr} = 0.078$ meeq L⁻¹. Exponential
957 growth continues beyond this point, as *Methanothrix* becomes the dominant methanogen, displacing
958 *Methanosarcina* by 30,000 days. Therefore, despite having a higher specific energy dissipation
959 rate, *Methanothrix* can come to dominance by having a lower K_S . It takes a total of 82 years for
960 *Methanothrix* to reach steady state. Extended Data Fig. 3b shows that this exponential growth of
961 *Methanothrix* has continued for the entire 82 years!

962 This example illustrates that in an idealized chemostat system, a minuscule competitive advan-
963 tage derived from having $\theta_x = \theta_x^{crit} + \delta$ can compound over the years and allow rare species to
964 become dominant. While maintaining an idealized chemostat with a tiny delta is not practical for
965 engineered systems, 82 years is only a fraction of a second on an evolutionary scale.

966 **Environmentally Neutral Selection ($\theta_x = \theta_x^{crit}$) (Regime III)**

967 Even a tiny deviation from θ_x^{crit} allows the r/K strategies to govern environmental dynamics in
968 an idealized chemostat. However, when $\theta_x = \theta_x^{crit}$ (which is not practical to simulate numerically),
969 environmentally neutral selection challenges this idea. At $\theta_x^{crit} = 3.107$ d with $S_{crit}^* = 28.07$ meeq
970 L⁻¹ (four significant digits are shown here for illustrative purposes), the net specific accumulation

971 rates of *Methanosarcina* and *Methanothrix* both equal zero:

$$\begin{aligned}\mu_{a,cin}(\mathbf{E}) &= \mu_{m,cin} \frac{S_{Ac}}{S_{Ac} + K_{Ac,cin}} - b_{cin} - \frac{1}{\theta_x} \\ &= 0.80 \cdot \frac{28.07}{28.07 + 32} - 0.052 - \frac{1}{3.107} \\ &= 0.374 - 0.052 - 0.322 \\ &= 0.00 \text{ d}^{-1} \quad (\text{Methanosarcina})\end{aligned}\tag{S-117}$$

$$\begin{aligned}\mu_{a,thr}(\mathbf{E}) &= \mu_{m,thr} \frac{S_{Ac}}{S_{Ac} + K_{Ac,thr}} - b_{thr} - \frac{1}{\theta_x} \\ &= 0.41 \cdot \frac{28.07}{28.07 + 6.3} - 0.013 - \frac{1}{3.107} \\ &= 0.335 - 0.013 - 0.322 \\ &= 0.00 \text{ d}^{-1} \quad (\text{Methanothrix})\end{aligned}\tag{S-118}$$

972 When these values are placed in the dynamic payoff matrix, all entries are zero (S-119).

	<i>Environment</i>	<i>Methanosarcina</i>	<i>Methanothrix</i>
<i>Environment</i>	0	0	0
<i>Methanosarcina</i>	0.00	0	0
<i>Methanothrix</i>	0.00	0	0

(S-119)

973 Therefore, *Methanosarcina* and *Methanothrix* have equal fitness. Following Nowak³, who calls
974 frequency-neutral interactions between Nash equilibrium strategies neutral selection, we define the
975 analogous condition in the environmental game as environmentally neutral selection with caveats:
976 In the traditional game, the condition $a = c$ and $b = d$ defines neutral selection. In the r/K selection
977 game, $\theta_x = \theta_x^{crit}$ and $S^0 > S^*$ ensure an environmentally neutral selection, while equal fitness is an
978 outcome of the steady state.

979 While θ_x^{crit} is a useful theoretical construct, the probability of a system operating at exactly this
980 point in an idealized chemostat system is vanishingly small. Near θ_x^{crit} , deterministic environmental
981 selection cannot predict the outcome in an idealized system such as the chemostat. Mechanistic

coexistence mechanisms – such as spatial heterogeneity in flocs⁴⁶ and time-varying environments in sequencing batch reactors¹⁵ – may obscure this boundary in non-idealized systems. In the absence of such mechanisms, stochastic drift in the spirit of Kimura^{21, 47} determines which species ultimately prevails.

S_{min}^* (Regime V)

This example uses the final state of Regime IV as the initial condition: $X_{cin} = 0.034$ meeq L⁻¹, $X_{thr} = 20$ meeq L⁻¹, and $S_{Ac} = 28$ meeq L⁻¹. The influent is kept at $S_{Ac}^0 = 625$ meeq L⁻¹. The solids residence time is adjusted to $\theta_x = 100,000$ d or 274 years to simulate severe mass-transfer resistance for the chemostat.

Extended Data Fig. 4a shows the *Methanosarcina-Methanothrix* dynamics in the absolute coordinates. Upon startup, S_{Ac} immediately drops from 28 to 0.0021 meeq L⁻¹ due to severe mass transfer resistance. During this 1000 d period, Extended Data Fig. 4b shows that *Methanothrix* had been decaying. Thereafter, the system slowly relaxes to the final state of $X_{thr} = 0.0163$ meeq L⁻¹ and $S_{Ac} = 0.72$ meeq L⁻¹, while *Methanosarcina* washes out.

The final $X_{thr} = 0.0163$ meeq L⁻¹ is consistent with the $S_{min} = 0.72$ meeq L⁻¹ (S-89) which is realized at a large extreme of θ_x . At this limit, the final biomass concentration can be calculated by replacing S with S_{min}^* , the steady-state biomass equation (S-64)

$$X_{a,thr}^* = Y \left(\frac{S^0 - S_{min}^*}{1 + b\theta_x} \right) \quad (\text{S-120})$$

For example, the predicted steady-state biomass concentration for *Methanothrix* with $\theta_x = 100,000$ d and $S_{Ac}^0 = 625$ meeq L⁻¹

$$X_{a,thr}^* = 0.034 \left(\frac{625 - 0.72}{1 + 0.013 \times 100,000} \right) = 0.016 \frac{\text{meeq}}{\text{L}} \quad (\text{S-121})$$

which is equal to the dynamic simulation final state. In fact, the steady-state biomass concentration will approach zero as θ_x approaches infinity, meaning the steady-state biomass approaches a vanishingly small but nonzero concentration.

Persistence. (Regime VI)

$\dot{g}_{diss,thr} = 8.9 \text{ mW eeq}_X^{-1}$ represents the true minimum amount of energy dissipation required for *Methanothrix* to invade the environment (Extended Data Fig. 4c). Using $2.8 \times 10^{-13} \text{ g per cell}^{13}$ and 113 g of cell per 20 eeq_X according to the cellular formula $\text{C}_5\text{H}_7\text{O}_2\text{N}$ in Extended Data Table 3, this equates to $1.4 \times 10^{-11} \text{ kJ cell}^{-1} \text{ y}^{-1}$:

$$\begin{aligned} 8.9 \frac{\text{mW}}{\text{eeq}_X} \cdot \frac{20 \text{ eeq}_X}{113 \text{ g}} \cdot \frac{2.8 \times 10^{-13} \text{ g}}{\text{cell}} \cdot \frac{1 \text{ W}}{1000 \text{ mW}} \cdot \frac{1 \text{ J/s}}{1 \text{ W}} \cdot \frac{31,556,926 \text{ s}}{\text{y}} \cdot \frac{1 \text{ kJ}}{1000 \text{ J}} \\ = 1.4 \times 10^{-11} \text{ kJ cell}^{-1} \text{ y}^{-1} \end{aligned} \quad (\text{S-122})$$

However, Lever et al.²⁴ reports a minimum energy dissipation of $1 \times 10^{-15} \text{ kJ cell}^{-1} \text{ y}^{-1}$ at the extreme starvation condition in the deep sediment column, which is about 4 orders of magnitude lower than at this S_{min}^* . Under extreme starvation, cells break down all machinery that is not essential for bare minimum survival. Under these conditions, microorganisms persist through net decay over thousands and millions of years, gradually yielding the ground to the environmental strategy. This is markedly different from S_{min}^* , which sets a minimum energy dissipation requirement to maintain steady-state biomass and invade the environment. Microorganisms have developed strategies such as dormancy, sporulation, and other persistence mechanisms to survive unfavorable growth conditions under conditions where $S < S_{min}^*$.

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