

Reciprocal Negative Feedbacks Stabilize an Emergent Polymorphism in a Cross-Feeding Microbial Consortium

1. Introduction

Pseudomonas putida presents a well-defined biological problem, as this diversity is conditional on a cross-feeding interaction with a partner species, *Acinetobacter johnsonii* (Al-Tameemi and Rodríguez-Verdugo, 2024). To formally dissect this problem, we apply the theoretical framework of Lehmann and Mullon, 2025 using a modular approach. This deconstructs any stable adaptive polymorphism into three necessary functional components: a trade-off (S-Condition), disruptive selection (H-Condition), and stabilizing feedback (I-Condition).

Two of these components are well-explained by established mechanisms in this system. The S-Condition (the trade-off) is established by a Phenotype level Production module, where a single, large-effect mutation in the *fleQ* gene creates an intrinsic resource allocation trade-off between motility and metabolic uptake efficiency, giving rise to the high-yield harvester morph (Al-Tameemi and Rodríguez-Verdugo, 2024). The H-Condition (disruptive selection) is generated by a Phenotype level Regulation module. The partner species, *A. johnsonii*, acts as a niche constructor by creating a heterogeneous landscape of benzoate patches, which favors the distinct specialist strategies of the forager and harvester while selecting against intermediates (Christensen et al., 2002; Hansen et al., 2007). This deconstruction reveals that the I-Condition (stabilizing feedback) is the core of the unresolved puzzle. Reciprocal invasion assays in well-mixed liquid cultures failed to detect the negative frequency-dependent selection required for coexistence, explicitly ruling out a simple, spatially-independent mechanism (Al-Tameemi and Rodríguez-Verdugo, 2024). The framework thus guides the formulation of three distinct, spatially-explicit hypotheses for this unknown stabilizing mechanism.

1.1. Hypothesis 1: Stabilization via Indirect Reciprocal Negative Feedback

The stabilizing feedback ($I < 0$) required for coexistence is hypothesized to be an emergent property of two distinct, reciprocal negative interactions between the morphs, each corresponding to a different regulatory module from the theoretical framework.

First, the motile forager population acts through a Phenotype level Regulation (e_z) module. By globally depleting the primary resource, foragers impose a negative, frequency-dependent fitness cost on the sessile harvesters, which depend on high local resource concentrations (Christensen et al., 2002).

Conversely, the harvester population acts through a Fitness level Regulation (e_p) module. Through localized, high-density metabolism, harvesters create anoxic microenvironments, imposing a dynamic of competitive "suffocation" that is inhibitory to the motile foragers (Xavier and Foster, 2007).

Together, these independent regulatory modules are predicted to create the reciprocal negative feedback loop necessary to stabilize the polymorphism. As one morph becomes more common, it disproportionately harms the other, preventing competitive exclusion.

1.2. Hypothesis 2: Stabilization via Hybrid Niche Construction

The stabilizing feedback ($I < 0$) is hypothesized to emerge from a Hybrid/Mixed Module, where coexistence is an emergent property of niche construction by the harvester morph and a trait-mediated response by the forager morph.

The mechanism unfolds in three steps. First, dense harvester clusters construct an anoxic micro-niche through their collective metabolism, which acts as the primary regulating factor e_p . Second, the forager's ancestral production factor z is its programmed "oxygen-starvation dispersal response", which causes it to be actively expelled from these anoxic zones. Finally, the harvester morph, having lost this ancestral response due to mutations affecting motility and surface properties such as *fleQ* or *wapH*, can tolerate and persist within the very niche it creates.

This interaction generates stabilizing feedback by creating a protected spatial refuge for the harvesters. The forager's own phenotype leads to its self-exclusion from harvester-dominated territory, shielding the harvesters from direct competition. Furthermore, this mechanism is predicted to have a secondary, reinforcing effect on disruptive selection ($H > 0$). By hardening the boundaries between the anoxic harvester niche and the oxic forager niche, it further penalizes intermediate strategies, thus strengthening the fitness valley that favors the two specialized morphs.

1.3. Hypothesis 3: Stabilization via Coupled Niche Construction and Pleiotropy

We hypothesize that the polymorphism is maintained by a Coupled Module, where disruptive selection ($H > 0$) and stabilizing feedback ($I < 0$) are the pleiotropic effects of a single biological process: the formation of dense biofilms by the harvester morph. This process is driven by the *fleQ* master regulator, which inversely controls motility and the production of biofilm matrix components. The pleiotropic nature of this regulator is predicted to satisfy two distinct conditions for adaptive polymorphism simultaneously:

Disruptive Selection ($H > 0$): The formation of a physically distinct biofilm constitutes niche construction. This acts as a Phenotype (Regulation) e_z module, creating a spatially heterogeneous landscape. It generates strong disruptive selection by favoring the high-yield harvester strategy within the biofilm niche and the motile forager strategy outside of it, while selecting against intermediate phenotypes.

Stabilizing Feedback ($I < 0$): Concurrently, the same biofilm structure provides stabilizing feedback. The high cell density and matrix create localized anoxic zones that inhibit the oxygen-sensitive foragers. This acts as a Payoff (Regulation) e_π module, where interference from the harvester's constructed niche reduces the forager's ability to grow and compete, thus preventing the forager from invading. In this scenario, a single pleiotropic genetic switch (*fleQ*) drives one process (biofilm formation) that generates both disruptive and stabilizing forces. This hypothesis is consistent with the experimental observation that the polymorphism is only maintained in a spatially structured context.

2. Aim

To begin dissecting these possibilities, the present study develops a computational model to formally test the first and most parsimonious of these hypotheses: Indirect Reciprocal Negative Feedback. This initial model is designed to evaluate whether the stable polymorphism can be explained solely by foundational ecological interactions global resource depletion by one morph and localized niche construction (anoxia) by the other. By establishing a baseline model for this core mechanism, we can determine the sufficiency of these simple feedbacks in maintaining coexistence. The latter two hypotheses, which invoke more complex trait-mediated behaviors and biofilm dynamics, are therefore presented as distinct, plausible alternatives whose investigation would require future, specialized models.

3. Methods: An Agent-Based Model of Polymorphism

3.1. Model Rationale and Focus

A key feature of this model is its starting point with two discrete, coexisting morphotypes, the motile "forager" and the non-motile "harvester." This design choice is a direct reflection of the specific biological system under investigation and represents a departure from classic adaptive dynamics models, which typically analyze the gradual emergence of polymorphism from a single ancestral lineage (Lehmann and Mullon, 2025). The *Pseudomonas putida* system provides a biological "shortcut" to this process; the experimental literature demonstrates that a single, large-effect mutation in the *fleQ* gene is sufficient to create the non-motile, high-yield "harvester" morph from the ancestral "forager" type (Al-Tameemi and Rodríguez-Verdugo, 2024). Consequently, our model is not designed to investigate the origin of this polymorphism (the S and H conditions) but to focus entirely on the ecological conditions required for its stable maintenance, which is governed by the stabilizing feedback of the I-condition $I < 0$.

Because the experimental literature indicates this stabilizing mechanism is spatially dependent and is not detectable in well-mixed cultures (Al-Tameemi and Rodríguez-Verdugo, 2024), a two dimensional, spatially explicit agent based model (ABM) was constructed. The model simulates the growth, movement, and interaction of individual "Forager" and "Harvester" morphotypes of *P. putida* competing for diffusing benzoate and oxygen. The environment is shaped by the partner species, *Acinetobacter johnsonii*, which is abstracted in the model as the fixed, benzoate-producing source points (Christensen et al., 2002; Hansen et al., 2007).

3.1.1. Model Environment

The simulation environment consists of a 100×100 grid with periodic boundary conditions. Benzoate is produced at a constant rate, p_{bzn} , from a fixed lattice of source points. Oxygen is supplied uniformly across the grid at a constant influx rate, p_{O_2} . Both substrates are subject to diffusion and a uniform washout rate, w . Diffusion is modeled at each time step using a 2D convolution with a five-point Laplacian kernel, governed by diffusion coefficients D_{bzn} for benzoate and D_{O_2} for oxygen.

3.1.2. Agent Behaviors

Each agent is characterized by its spatial position and biomass. The model progresses in discrete time steps, during which the following agent behaviors are executed in sequence:

Substrate Uptake: A decoupled uptake mechanism was implemented. The potential uptake rate, U , for each substrate was calculated for each agent based on local substrate concentration $[S]$ using Michaelis-Menten kinetics:

$$U = V_{max} \frac{[S]}{K_m + [S]}.$$

Scenario-specific efficiency multipliers, μ_{bzn} for foragers and μ_{O2} for harvesters, were applied to the potential uptake rates. The growth of an agent is determined by the single limiting substrate,

$$U_{lim} = \min(U_{bzn}, U_{O2}).$$

However, the amount of each substrate removed from the environment is based on its full potential uptake rate (U_{bzn} and U_{O2}), simulating wasteful consumption. For harvesters, oxygen consumption is distributed over a 3×3 grid cell area centered on the agent.

Growth and Death: The biomass of each agent, B , is updated based on the limiting uptake rate, substrate-specific yield coefficients Y_f for foragers, Y_h for harvesters), and maintenance costs c_{maint} for both $c_{motility}$ for foragers only). The change in biomass is calculated as

$$\Delta B = (U_{lim} \cdot Y) - c_{maint} - c_{motility}.$$

Agents with a biomass of zero or less are removed from the simulation.

Division: Agents with a biomass greater than or equal to two undergo division. The parent agent's biomass is reset to one, and a new daughter agent with a biomass of one is placed in a randomly selected adjacent grid cell. A global population limit, N_{max} is enforced.

Movement: Foragers exhibit a "smart flee" dispersal behavior. If the local oxygen concentration falls below a defined threshold, θ_{O2} the agent relocates to a randomly selected grid cell where the oxygen concentration is above this threshold. If no such sites are sufficiently available, it relocates to a random position on the grid. In oxygen-sufficient conditions, foragers perform a random walk to an adjacent cell. Harvesters are non-motile.

All simulations were initiated with 50 forager and 50 harvester agents, each with a biomass of 1.0, placed at random positions on the grid. Resource grids were initially set to zero.

Model parameters, listed in full in Supplementary Table 1, were selected and calibrated to ensure stable coexistence in the control scenario, consistent with the dynamics observed in the experimental system.

4. Results

4.1. Competitive Balance is Sensitive to Specialist Efficiency

To establish the baseline competitive dynamics of the system, we first simulated three scenarios in which the metabolic efficiencies of the specialists were altered. Under the control parameters, the model produced a stable coexistence between the two morphs, characterized by harvesters forming dense clusters at resource source points and foragers occupying the periphery (Fig. 1A-B). This balance, however, was sensitive to the strength of the trade-off. A significant increase in the harvester's oxygen uptake efficiency led to the competitive exclusion of the forager (Fig. 1C-D). Conversely, a large increase in the forager's benzoate uptake efficiency resulted in the collapse of the harvester population (Fig. 1E-F). These results confirm that a balanced trade-off is required for coexistence and motivate a direct test of the underlying stabilizing mechanism.

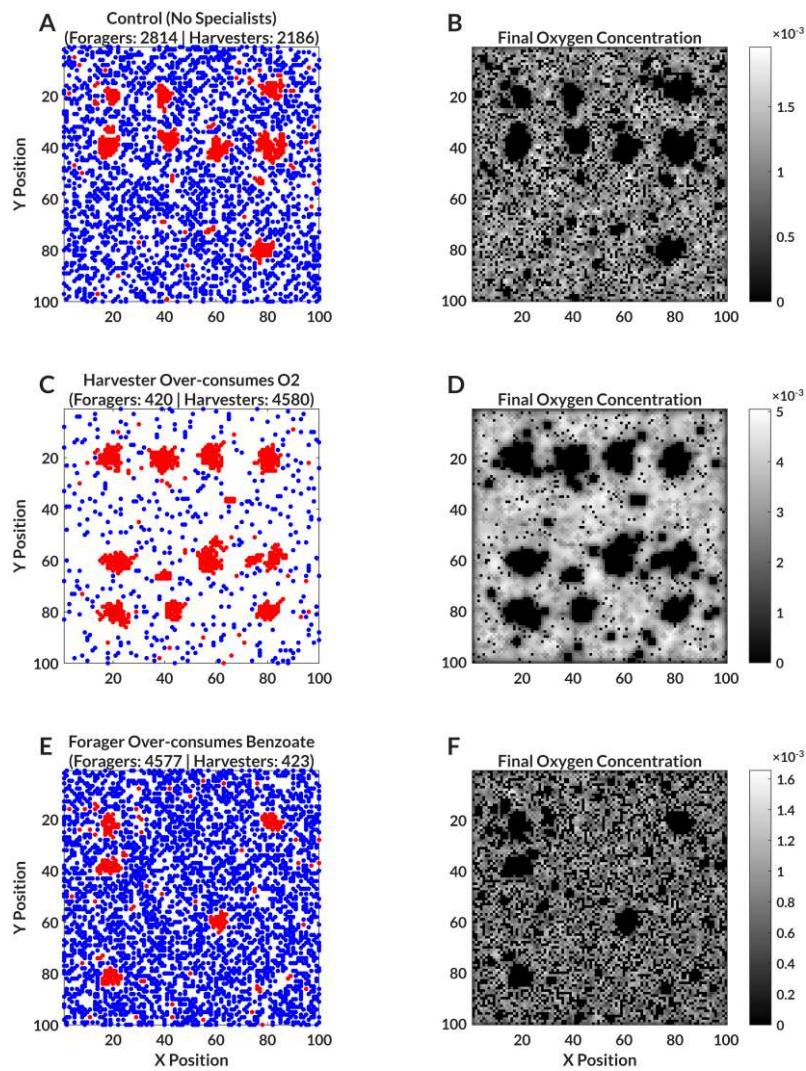


Figure 1 | Effect of Specialist Strategies on Community Structure and Environment.

Final states from the agent-based model comparing three metabolic scenarios. Left panels (A, C, E) show the final spatial distribution of Forager (blue) and Harvester (red) agents. Right panels (B, D, F) show the corresponding final oxygen concentration grid, where white indicates high concentration. Scenarios are: **(A-B)** Control ($\mu_{bzn} = 1.0$, $\mu_{O_2} = 2.5$); **(C-D)** Harvester Specialist ($\mu_{O_2} = 10.0$); and **(E-F)** Forager Specialist ($\mu_{bzn} = 15.0$).

4.2. In Silico Invasion Analysis Reveals Stabilizing Feedback

To formally test for the presence of the stabilizing feedback ($I < 0$) proposed in Hypothesis 1, we performed an *in silico* invasion analysis. The analysis revealed a strong negative correlation between the initial frequency of the forager morph and its relative fitness (Fig. 2). The forager exhibited a positive selection rate when rare, indicating it could successfully invade a resident population of harvesters. As the forager's frequency increased, its relative fitness declined, becoming negative when it was the common type. The negative slope of the linear regression, which intersects the equal fitness line, is the definitive signature of negative frequency-dependent selection. This result demonstrates that the reciprocal negative feedbacks implemented in our spatial model are sufficient to generate the stabilizing mechanism required for stable coexistence.

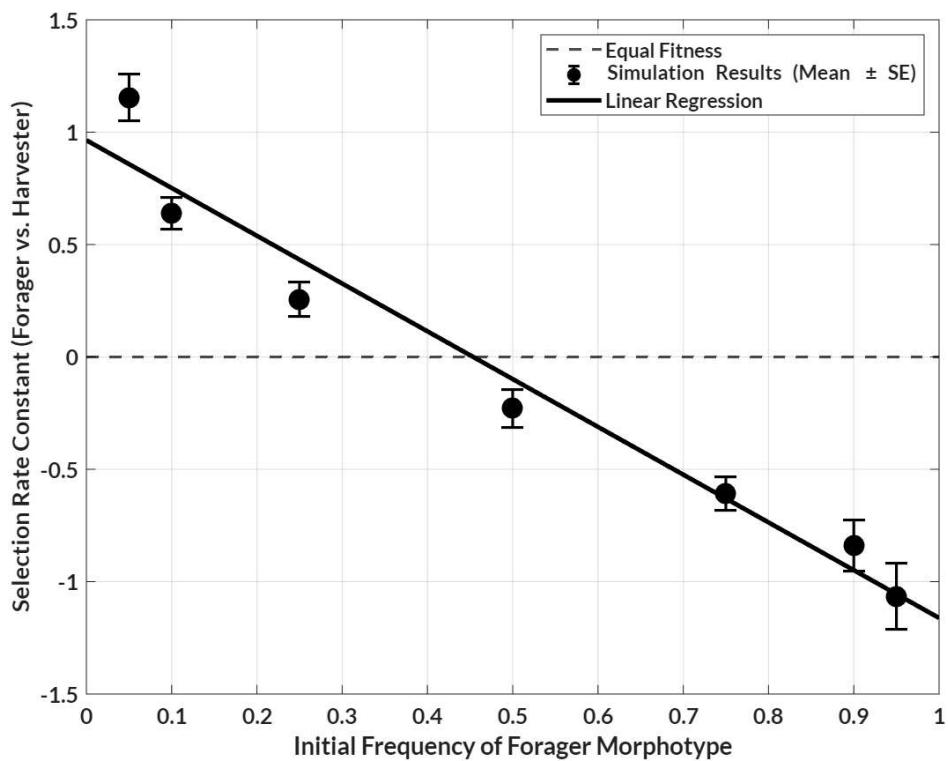


Figure 2 | In Silico Invasion Analysis Demonstrates Negative Frequency-Dependent Selection.

The relative fitness of the forager morph (selection rate constant, y-axis) is plotted as a function of its initial frequency in the population (x-axis). A positive selection rate indicates a fitness advantage for the forager. Each point represents the mean of 10 replicate agent-based simulations (\pm SE). The solid line shows the linear regression of the data, and the dashed line indicates equal fitness between the two morphs. The negative slope, which crosses the equal fitness line, is the definitive signature of negative frequency-dependent selection that allows for stable coexistence.

5. Discussion

Our agent-based model demonstrates that the reciprocal negative feedback proposed in Hypothesis 1 are a sufficient mechanism to explain the stable coexistence of the forager and harvester morphs of *P. putida*. The *in silico* invasion analysis successfully reproduced the negative frequency-dependent selection required for a stable polymorphism. This is a critical result, as this stabilizing feedback was absent in previous well-mixed experimental assays. This finding suggests that simple, foundational ecological interactions, when expressed in a spatially explicit context, can generate the necessary conditions for maintaining an adaptive polymorphism as described by our theoretical framework.

A point of theoretical precision is warranted here. While our model's demonstration of negative frequency-dependent selection (Fig. 2) is the definitive signature of a stable coexistence, it is imprecise to label this outcome itself as $I < 0$. The selection interaction coefficient, $I(x^*) = \frac{\partial^2 p}{\partial x \partial y} |_{x=y=x^*}$, is a specific, local property of the fitness function evaluated at the monomorphic singular strategy from which branching originates. Our model, by starting with two established morphotypes, does not simulate this branching event but rather the subsequent ecological dynamics. The result in Figure 2 is therefore more accurately described as a demonstration of system-level negative frequency-dependent selection. This selection is the consequence of such stabilizing forces, not a direct measurement of the local coefficient I itself.

Our model offers a resolution to the central puzzle motivating this work, which was the observation of a stable polymorphism conditional on the presence of *A. johnsonii* that could not be explained by simple frequency-dependence. The model shows how spatial structure is the key by synthesizing two independently observed phenomena into a single, cohesive mechanism. The global benzoate depletion by motile foragers, which harms sessile harvesters, is a known dynamic in biofilms, while the localized anoxia created by dense cell clusters, which harms motile foragers, has also been experimentally demonstrated.

While this model provides a robust, proof-of-concept explanation, it is important to acknowledge its simplifying assumptions. The partner species, *Acinetobacter johnsonii*, was abstracted as a static environmental feature rather than a dynamic population, and the simulation is two-dimensional, whereas real biofilms have complex three-dimensional structures. Furthermore, our model's support for Hypothesis 1 does not preclude the action of the alternative mechanisms proposed in Hypotheses 2 and 3. These limitations highlight clear avenues for future research. The model makes testable predictions, such that future experimental work could quantify the predicted resource gradients in co-culture or could be designed to decouple the feedback loops, for instance, by negating the effects of anoxia in a hyperoxic environment. Future computational studies should also expand upon this model to explicitly test Hypotheses 2 and 3.

In conclusion, this study provides a plausible and mechanistic resolution to a key puzzle in experimental evolution. More broadly, our work underscores the critical role of spatial structure and reciprocal niche construction in maintaining microbial diversity. It demonstrates how simple, local interactions can generate the complex, system-level feedbacks required to stabilize polymorphisms that arise from single, large-effect mutations. This provides a key insight into how the vast functional diversity observed in natural microbial communities can be maintained.

6. Supplementary Info

Supplementary Table 1 | Core Model Parameters

Parameter	Symbol	Description	Value
Agent Properties			
Forager Yield	Y_f	Biomass produced per unit of resource consumed by a forager.	0.50
Harvester Yield	Y_h	Biomass produced per unit of resource consumed by a harvester.	0.55
Motility Cost	$c_{motility}$	Biomass cost per time step for forager movement.	1e-4
Maintenance Cost	c_{maint}	General biomass cost per time step for all agents.	1e-4
Resource Dynamics			
Benzoate Diffusion	D_{bzn}	The diffusion coefficient for benzoate across the grid.	0.1
Oxygen Diffusion	D_{O_2}	The diffusion coefficient for oxygen across the grid.	0.2
Benzoate Production	p_{bzn}	Rate of benzoate production at source points per time step.	3.0
Oxygen Influx	p_{O_2}	Rate of uniform oxygen influx across the grid per time step.	4e-4
Washout Rate	w	Proportion of all resources removed from the grid per time step.	5e-2
Uptake Kinetics			
Max Uptake Rate	V_{max}	The maximum potential substrate uptake rate (unmodified).	1.0
Oxygen Half-Saturation	K_{m,O_2}	Oxygen concentration at which uptake is half of V_{max}	1e-2
Benzoate Half-Saturation	$K_{m,bzn}$	Benzoate concentration at which uptake is half of V_{max}	1e-2
Forager Benzoate Multiplier	μ_{bzn}	Uptake efficiency multiplier	1.0

		for benzoate, specific to foragers.	
Harvester O ₂ Multiplier	μ_{O_2}	Uptake efficiency multiplier for oxygen, specific to harvesters.	2.5
Behavior & Simulation			
Oxygen Flee Threshold	θ_{O_2}	Oxygen concentration below which foragers initiate dispersal.	5e-4
Max Population	N_{max}	The maximum number of agents allowed in the simulation.	5000
Grid Size	-	The dimensions of the square simulation grid.	100x100
Simulation Duration	-	The maximum number of time steps for each simulation run.	1500

7. References

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