Multi-Species Microbial Biofilms: Cooperation, Competition, and Spatial Dynamics

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1 Introduction

Multi-species biofilms are structured microbial communities in which cells engage in both cooperative (e.g. nutrient cross-feeding and public good production) and competitive interactions (e.g. resource competition or antagonistic inhibition) [1]. Classical theory predicts that "cheater" strains exploiting public goods will collapse cooperation, yet recent work has revealed more nuanced outcomes: the presence of cheaters can under certain conditions stabilize coexistence rather than driving collapse [2]. Spatial organization and environmental heterogeneity in biofilms further mitigate competitive exclusion by enabling niche partitioning and local interactions [1], [3]. For example, experiments and models show that distinct microenvironments or additional competitor species can prevent any one strain from taking over, preserving diversity [3], [4]. Here we review key literature on cooperation and competition in multi-species biofilms, summarize methodological advances (laboratory co-cultures, mathematical and computational models), and propose novel research objectives (such as multi-resource trade-offs and dynamic environments) to advance understanding of biofilm social dynamics. In these systems, cells grow in dense clusters and gradients, so interactions are not well-mixed. Clonal clusters of cooperators (genetically identical neighbors) can share public goods and resist stress, whereas intermixing with different strains or species often favors antagonism or competition. For instance, *Vibrio cholerae* can form tightly packed microcolonies that shelter inner cells from predators, but if an *E. coli* strain disrupts this architecture both species become vulnerable. Likewise, spatial gradients of nutrients and signals can localize cooperation in beneficial ways: in *Pseudomonas aeruginosa* swarms, applying diffusible quorum-sensing signals maintains cooperative behavior and prevents exploitation by cheaters. In summary, the spatial structure of multi-species biofilms critically shapes whether cooperation persists or is undermined by conflict [5].

2 Key Literature Summaries

Cooperation and Cheating. Multi-species biofilms often involve the production of extracellular public goods (e.g. enzymes, polymers) that benefit neighboring cells [1]. Classical ecological theory (the "tragedy of the commons") predicts that cheater strains exploiting these goods will undermine cooperation. Consistent with this, well-mixed cultures of cooperators can be invaded by non-producers, reducing overall group productivity. However, recent models and experiments have found counterintuitive results: cheaters can sometimes stabilize coexistence rather than cause collapse [2], [3]. For example, Xenophontos *et al.* modeled a two-species system in which one species produced a degradative enzyme and the other (a "cheater") did not. They showed that the presence of the cheater limited runaway overexploitation, leading to a stable snowdrift-game equilibrium instead of cooperator extinction [2]. Empirical cross-feeding consortia studies similarly show that if a cheater is obligately dependent on cooperators, cooperators tend to prevail, whereas if the cheater can partially grow independently, it can outcompete

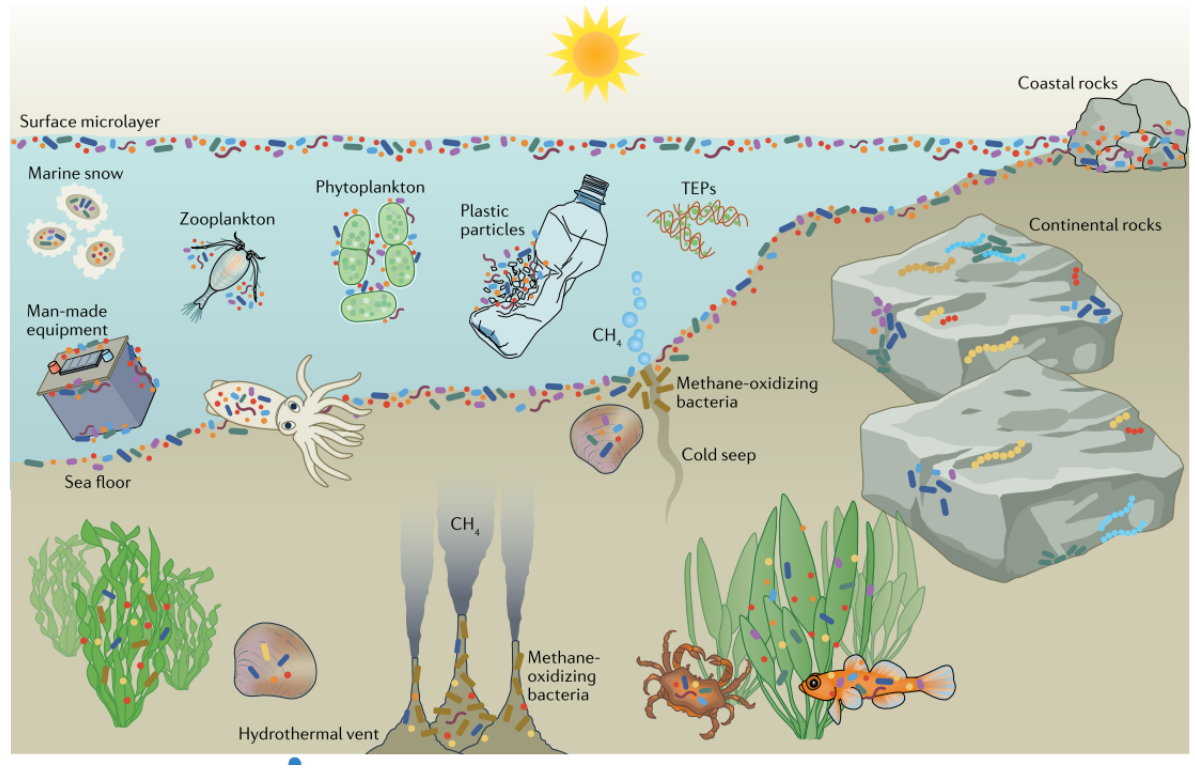

Figure 1: Marine biofilms on different substrates in the ocean [6]

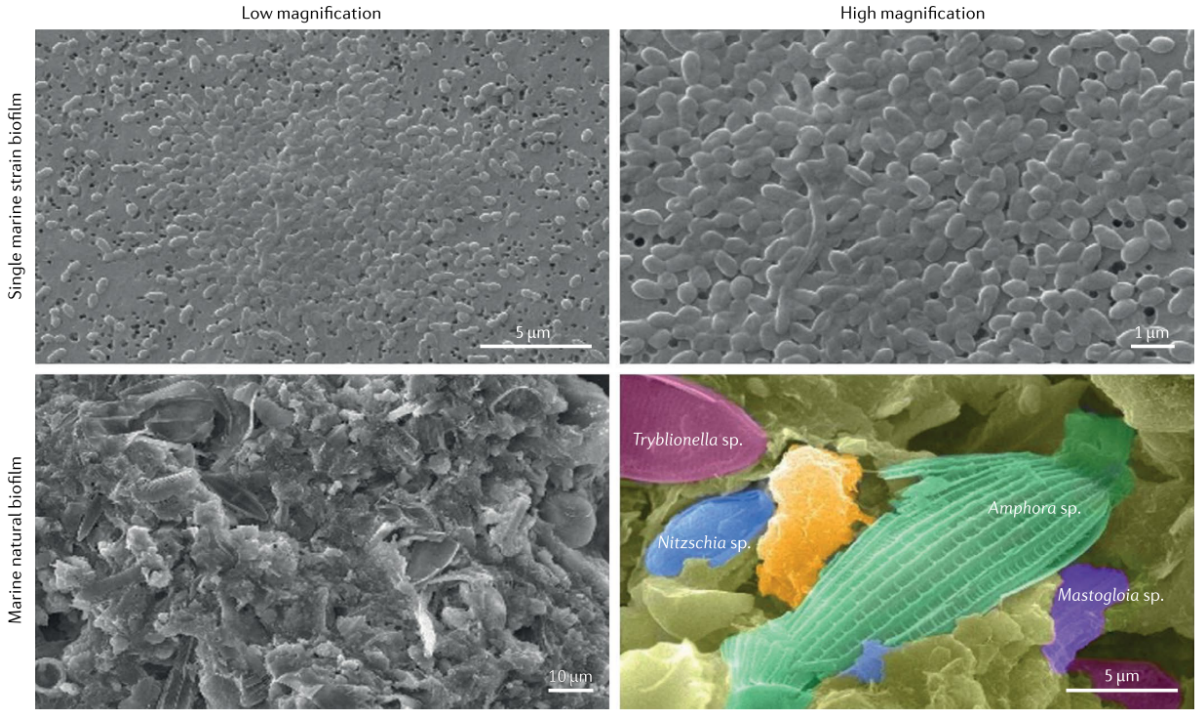

Figure 2: Composition of natural marine biofilms [6]

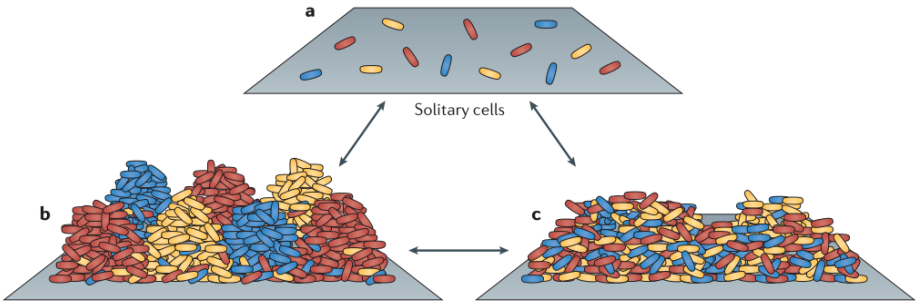

Figure 3: Spatial structuring in microbial biofilms and its influence on the evolution of social phenotypes [1]

the producer. These findings challenge the idea that mutualism always breaks down: under frequency-dependent or spatially structured conditions, cooperation can persist despite or even because of cheating [2], [3].

Nutrient Competition and Coexistence. Competition for limited resources is a fundamental driver of biofilm community structure. Classical niche theory predicts competitive exclusion when species share a single limiting substrate. In biofilms, however, resource partitioning and cross-feeding networks often permit coexistence [3]. For example, synthetic biofilm experiments demonstrate that two bacterial species can stably coexist when each specializes on a different substrate or an oxygen gradient [3]. Spatial and temporal heterogeneity also plays a role: species might each exploit different micro-niches (e.g. anoxic vs oxic zones) to avoid direct competition. Importantly, adding additional competitor species can buffer the community against domination by any one strain. In one study, introducing a background competitor prevented social cheaters from sweeping the population, enabling long-term maintenance of both cooperator and cheater phenotypes in a well-mixed community [3]. These results underscore that multi-species biofilms often evade simple exclusion through niche differentiation and complex interaction networks.

Spatial Structure and Architecture. A central theme in recent biofilm research is that spatial organization governs social dynamics [1]. Cells in a biofilm are arranged in heterogeneous microcolonies, gradients, and layers, not in a uniform mixture. This spatial structure can amplify cooperation among neighbors while limiting exploitation by distant cheaters. For instance, Nadell *et al.* showed that segregating cooperative genotypes into patches stabilizes mutualism against cheats, whereas well-mixed arrangements favor cheating or the breakdown of cooperation [1]. Spatial patterns also create protective niches: dense *V. cholerae* biofilm clusters resist predation, but intermixing with another strain collapses this protection, exposing both to predators [7]. Likewise, spatial localization of signaling and public goods can prevent cheating: Monaco *et al.* found that in structured *P. aeruginosa* colonies, perturbing quorum signals preserved the cooperating genotype rather than enabling cheaters [8]. In sum, where cells are located relative to each other (clonal patches vs mixed, core vs periphery) often determines whether cooperation persists and which species dominate [8].

3 Methodological Approaches in Recent Studies

Researchers have employed diverse experimental and modeling methods to study multi-species biofilms:

Laboratory Co-cultures: Many empirical studies use controlled two-species consortia or defined multi-species communities. Fluorescent tagging and confocal microscopy visualize species distributions, revealing clonal clusters or stratified layers. Microfluidic devices and flow cells impose defined nutrient gradients or shear flow, enabling real-time observation of competition and spatial patterning under near-natural

conditions. Experimental evolution on biofilms (serial transfer of surface communities over many generations) has revealed phenomena such as the invasion of cheater mutants and shifts in cooperation depending on nutrient limitation or added predators.

Differential Equations (ODE Models): Systems of coupled ODEs are widely used to model the population dynamics and resource concentrations of interacting species. These well-mixed models incorporate interactions like cross-feeding or inhibition. Simple ODE models have been used to analyze public-goods games: for instance, replicator-type equations can capture how a producer and a cheater species reach equilibrium or collapse depending on production costs and diffusion parameters [4]. More complex ODE frameworks include multiple substrates and metabolic trade-offs to predict multi-resource competition outcomes. While basic ODE models neglect space, they can be extended via compartmental or patch models to approximate local interactions and to identify evolutionarily stable strategies under different payoff structures.

Reaction–Diffusion and Continuum Models: To explicitly include spatial gradients, reaction–diffusion PDE models simulate biofilm biomass growth coupled to substrate diffusion [9], [10]. In these continuum models (often in 2D cross-sections), biofilm biomass is treated as a density field that expands on a surface while nutrients diffuse and are consumed. Such models reproduce nutrient-depleted zones and stratified layers (aerobic vs anaerobic) and have been applied to invasion dynamics (e.g. colonization of an established biofilm by a new species or a cheater). These models, rooted in physics and engineering, have been common in biofilm engineering (e.g. wastewater biofilms) and are increasingly adapted to ecological questions like pattern formation driven by public goods.

Particle-Based (Individual-Based) Simulations: Agent-based models explicitly represent individual cells (or small clusters) in space [11]. For example, the iDynoMiCS platform "grows" discrete bacteria on a grid that consume diffusing nutrients and mechanically push on each other, forming realistic 2D/3D biofilm structures [12]. These models can incorporate multiple species and phenotypes, cell adhesion properties, and stochastic events. By simulating, e.g., two cooperating species plus a cheater, researchers have tested how initial colonization timing and positions affect cooperator–cheater dynamics [4]. Particle-based models capture fine-grained spatial details and have produced insights on clonal sectoring, physical exclusion of cheaters, and the role of biofilm architecture (e.g. matrix stiffness) in competition. They often generate visual outputs directly comparable to microscopy, linking mechanisms to observed spatial patterns.

Evolutionary Game Theory Frameworks: Conceptual models from game theory have been applied to microbial interactions [13], [14]. Cooperative enzyme production can be framed as a public goods game, with outcomes analogous to the Prisoner's Dilemma (mutual defection leads to collapse) or the Snowdrift Game (cooperators and cheaters coexist) [4]. These game-theoretic models are sometimes implemented on spatial grids or networks, treating each microhabitat as a player interacting with neighbors. In recent years, this approach has helped explain phenomena like the Black Queen Hypothesis (loss of costly functions by some cells that rely on others) in evolutionary biofilms. Game theory has also guided experiments: for example, models predicted that restricting diffusion of benefits to cooperators (akin to kin selection) can stabilize cooperation, prompting tests of clustering or feedback strategies in microbial communities. Overall, blending game-theoretic insight with mechanistic modeling helps reveal conditions under which cooperation can evolve or be maintained.

4 Proposed Novel Research Objectives

Building on current knowledge, we propose several research directions to advance understanding of multi-species biofilm dynamics:

1. **Breakdown and Rescue of Mutualism.** Develop a biofilm model consisting of two or more mutualistic species (e.g., cross-feeders exchanging essential metabolites), and introduce a cheater or parasitic strain to perturb the system. Investigate the factors that enable mutualism to resist collapse, such as spatial segregation of partners, specific signaling mechanisms, or environmental stressors. Conversely, if the mutualistic interaction breaks down, evaluate whether targeted interventions—such as supplementation of key nutrients or the introduction of physical barriers—can restore cooperation. This objective aims to assess the robustness of cooperative biofilms and challenge the assumption that cheating inevitably leads to community collapse.

2. **Multi-Resource Competition.** Investigate communities where species compete for multiple simultaneous resources (e.g. carbon *and* nitrogen). By combining multi-substrate models and defined consortia experiments, one can test whether trade-offs (each species specializing on a different nutrient) permit stable coexistence that single-resource theory would forbid. Studies of this kind could identify conditions (e.g. oscillating resource supplies or spatial heterogeneity of nutrients) that maintain diversity, and reveal tipping points where small changes in nutrient availability lead to collapse of multispecies coexistence.

3. **Spatiotemporal Dynamics under Fluctuating Environments.** Extend models (reaction–diffusion and agent-based) to time-varying conditions, such as periodic nutrient shifts, pulsed antibiotics, or cyclic flow. Simulate how cooperators and cheaters respond: for example, do we see oscillations in dominance (analogous to predator–prey cycles) when environments fluctuate? This line of research would capture transient dynamics often missed by static studies, and could identify when biofilms are most vulnerable to interventions (e.g. timing antibiotic pulses to destabilize cheats).

4. **Symbiosis in Spatially Structured Biofilms.** Investigate how mutualistic and competitive interactions unfold within biofilms growing on surfaces under spatially heterogeneous conditions. In such environments, gradients in nutrient availability and limited diffusion of signaling molecules constrain communication to local neighborhoods. Develop models that couple cell growth with nutrient and signal transport, accounting for distinct timescales (e.g., fast diffusion vs. slow biomass accumulation). Explore how these spatial constraints influence the stability of symbiotic relationships—for instance, whether mutualists can persist when cooperation is limited to immediate neighbors, or whether local collapse spreads through the biofilm. This objective aims to link spatial structure, transport limitations, and ecological interactions to the emergence and persistence of cooperative behavior in realistic biofilm environments.

Each of these objectives integrate aspects of coexistence, mutualism, nutrient competition, and spatiotemporal complexity. Pursuing them should deepen understanding of multi-species biofilms and help develop strategies to manage biofilms in health, industry, and the environment.

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