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The demographic determinants of human microbiome health

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Abstract

The human microbiome is a vast reservoir of microbial diversity and increasingly recognized to play a fundamental role in human health. In polymicrobial communities, the presence of one species can modulate the demography (growth and distribution) of other species. These demographic impacts generate feedbacks in multi-species interactions, which can be magnified in spatially structured populations (e.g., host-associated communities). Here we argue that demographic feedbacks between species are central to microbiome development, shaping whether and how potential metabolic interactions come to be realized between expanding lineages of bacteria. Understanding how demographic feedbacks tune metabolic interactions and in turn shape microbiome structure and function is now a key challenge to our abilities to better manage microbiome health.

Keywords

microbiome; multispecies communities; metabolic interactions; demography; spatial organization

The human microbiome: an ecological network of metabolic interactions

The human body is home to an extraordinary diversity of microbes, which are increasingly suggested to play pivotal roles in human health. Human microbiome (see Glossary) sequencing projects have revealed intriguing correlations between specific patterns of microbial diversity and multiple aspects of host health, including autoimmune disorders

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[1,2], diabetes [3], obesity [4,5], and even psychiatric conditions [6]. The establishment of microbial causal roles (particularly in obesity) is gathering pace thanks to experimental manipulations of germ-free mice (e.g., [7]), however the causal mechanisms frequently remain obscure.

A major challenge to unraveling the mechanisms of microbiome functioning is the necessity to combine molecular and ecological approaches to the study of highly complex assemblies of billions of interconnected bacterial cells. Systems biological approaches are beginning to make important headway by building and analyzing complex computational models of metabolic interactions within microbial communities [8-10], however these approaches typically make strongly simplifying assumptions on the spatio-temporal dynamics of constituent species, reducing their population biology to a simple 'presence/absence' dichotomy. This simplification (shared by ecological approaches to microbial community assembly [11]) allows a mapping of potential metabolic interactions among species, but fails to predict the extent to which any interaction will be realized. To address this issue, we propose a spatially-explicit population dynamic framework of microbiome development, to understand when and how potential metabolic interactions come to be realised via demographic feedbacks (reciprocal impacts on growth and distribution) between expanding lineages of bacteria.

Metabolic interactions and demographic feedbacks within a minimal microbiome

To develop a complete mechanistic understanding of a microbial community, it is key to understand how the presence of one species modulates the growth of each of the other species, and how these coupled demographics together shape the functional and spatial structuring or architecture of the community. Microbes constantly modify their environment through the secretion and excretion of both functional exo-products [12,13] and metabolic by-products [14,15] setting the stage for complex interspecific interactions. Of particular interest are 'cross-feeding' interactions, where species use metabolic by-products of others as energy or nutrient resources. Some cross-feeding relationships are characterized as mutualistic (enhancing both species' growth rates [14,16]), however the exchange of metabolites can also promote exploitation where one species gains at the expense of another [17].

If we reduce the complexity of species interactions to a simple menu of discrete impacts on interacting species (positive +, negative -, or neutral 0), then for a two-species community there are six distinct patterns of potential ecological interaction [(0,0), (0,+), (0,-), (+,+), (+,-), (-,-)]. However, there is a combinatorial explosion in potential ecological complexity with increasing community diversity [18]. Given the high dimensionality of interactions within the human microbiome, there is a pressing need to create tractable model systems, as a necessary step towards understanding more complex multispecies dynamics. Here we suggest that in order to understand more diverse and complex microbial communities (such as the human microbiome), we first need to develop a thorough mechanistic understanding of coupled metabolic and demographic dynamics in defined 'minimal' microbiomes.

Previous studies on defined two-species communities highlight that these are capable of considerable ecological complexity [19-22]. Recent theoretical work has suggested that a single mechanism of interspecific metabolic exchange among two species can generate a diverse array of ecological relationships, spanning mutualism, competition, and exploitation; and that such diversity can arise by simply changing the properties of the metabolite that is exchanged (Figure 1A; [19]). A relevant empirical example of this diversity of outcomes is the interaction between the human oral commensal bacterium *Streptococcus gordonii* (*Sg*) and the pathogenic oral bacterium *Aggregatibacter actinomycetemcomitans* (*Aa*). Co-culture experiments of *Sg* and *Aa* in well-mixed liquid cultures have highlighted that the *Sg*–*Aa* interaction (mediated by *Sg* metabolic by-products lactate and H₂O₂) can readily move between mutualism and competition, depending on environmental conditions (Figure 1B) [23]. In aerobic conditions, *Aa* consumes lactate and relieves *Sg* of H₂O₂ toxicity, generating a marginally mutualistic relationship. However, under anaerobic conditions *Aa* cannot grow on lactate, and therefore competitive interactions (mediated by shared consumption of glucose) dominate (Figure 1B). In contrast, when grown in a structured *in vivo* model infection system, *Aa* gains significantly from the association while *Sg* neither benefits nor is harmed [23]. A key outstanding question is whether this stronger benefit to *Aa* is due solely to the many biochemical differences between the *in vitro* and *in vivo* growth environment, or whether there is a significant contribution from the effect of growing in a spatially structured environment.

Demography matters in spatially structured communities

Empirical work on microbial cross-feeding has shown that spatial structure plays an important role in shaping species interactions (e.g., [21,22,24-26]). From a modeling perspective, studying the role of metabolic interactions and demographic feedbacks in shaping the dynamics of spatially structured microbial communities is a challenging task, in part due to the computational challenge of studying mechanistically explicit models over space and time. In the past few years, there has been a rising interest in developing *in silico* models of microbial communities (for recent reviews see [27,28]). For instance, population-level models have been extended to study the dynamics and stability of the gut microbiota [29,30]. Metagenomic data combined with metabolic network analysis has recently been used to provide new insights into the correlation between species co-occurrence and predicted potential metabolic interactions in the gut microbiome [9]. Multispecies stoichiometric metabolic models [31-33] have also proven useful to predict potential species interactions. While these models provide valuable information, they do not explicitly consider spatial structure nor the effect of the chemico-physical environment.

Novel approaches, however, are emerging to address this gap. Recently, Harcombe *et al.* developed a novel computational approach that incorporates spatial structure into stoichiometric models of multispecies communities [34]. Specifically, they showed that their computational framework is able to predict the spatio-temporal dynamics of two- and three-species cross-feeding engineered microbial communities from the genome-scale metabolic network information of each individual species. Moreover, in recent years there has been considerable effort to develop individual-based models (IBMs) that simulate the growth of spatially extended microbial communities, such as biofilms [35,36]. Spatially explicit IBMs

provide an excellent framework to investigate the demographic consequences of species interactions [20,21,26,37]. Recent computational studies on two metabolically interacting species have illustrated that when species provide mutual benefits (growth in a mutualism media, Figure 2), specific patterns of lineage mixing are promoted and the resulting pattern of species mixing is highly robust in the face of variation in initial demographic conditions (initial species frequency and spatial distribution) [20,21,26,34]. In contrast, when competitive interactions dominate, lineages tend to segregate and system behavior is highly contingent on initial conditions (Figure 2, [20,21,26]). Together, these studies support the idea that demographic feedbacks between neighbouring lineages are magnified in spatially structured populations.

In a microbiome context, in addition to interacting with other microbial species, microbes also interact with their host. This raises many questions, such as what is the effect of the host in shaping these interactions, and how the host manipulates its own microbiota and selects for beneficial symbionts instead of harmful symbionts. Using an IBM of a host–gut microbiome, Schluter and Foster showed that host epithelial secretions of nutrients and antimicrobials play a critical role in modulating the microbiota [38]. They suggest that the selective effects imposed by the host at the epithelial surface are stronger than the selective effects of nutrients or antibiotics in the lumen contents, because of basic demographic asymmetries; cells favoured at the epithelial surface are more likely to replicate and found lineages growing out towards the lumen, whereas cells favoured in the lumen are much less likely to be founder cells for future generations, due to the elevated risk of being sloughed off [38].

Metabolically mediated demographic interactions and host health

Previous theoretical and experimental work have focused on the links between microbial metabolic interactions, spatial patterning, and ecological functioning among constituent microbial species [20-22,37,39,40]. However these microbe–microbe interactions are in turn central to the ability of microbial communities to perform services for the host, both positive, such as the production of nutrients [14] and protection against pathogens [41], and negative, such as enhanced virulence [23,42,43]. Here we discuss how a better understanding of the coupled effect of demographic and metabolic feedbacks can foster our understanding of services by the microbiome and how this can lead to potential new treatments.

Phenotypic antibiotic resistance through spatial structuring and partner shading

In a microbiome context, of particular interest is to understand the emergent resistance properties of microbial communities to antibiotic assault, and how antibiotics will affect microbiome services to the host. Generally, the success of antibiotic-resistant pathogens is due to genetic mutations that confer protection against the antibiotic. However, resistance can also be achieved without mutations, known as non-inherited [44] or phenotypic [45] antibiotic resistance. Unlike genetic resistance, phenotypic resistance is transient and environment-dependent, and includes mechanisms such as the dormancy of persister cells in biofilms [46], or reduced viral adsorption rate in bacterial resistance to phage [45]. Recently, another potential mechanism of phenotypic antibiotic resistance has been suggested. Connell

et al. provide evidence for the key role played by the spatial relationships of species in conferring cross-species phenotypic antibiotic resistance [47]. Using a two-species microbial community constituted of genetically resistant beta-lactamase producing *Pseudomonas aeruginosa* (*Pa*), and a susceptible *Staphylococcus aureus* (*Sa*), they showed that *Pa* protected the susceptible *Sa* from beta-lactam antibiotics, and this protection was significantly enhanced due to their pre-defined spatial arrangement (*Sa* was confined within a shell of *Pa*) [47]. Although in this study the species spatial arrangement was imposed *a priori*, this raises the interesting question of whether antibiotic protection could arise via spatial structuring of emergent species. And if so, can we manipulate microbe–microbe functional and spatial relationships to favourably influence microbiome services to the host? Our prediction is that mutualistic biofilms, with a high degree of species mixing, are more resistant to narrow-spectrum antimicrobial clearance due to partner shading. The rationale for this prediction is the following: under competitive interactions the two species segregate (Figure 2), rendering the susceptible strain isolated and vulnerable to clearance. In contrast, under mutualistic interactions, the two species interdigitate (Figure 2) and thus increase the average distance between the bulk fluid (maximal antimicrobial density) and target cells.

In addition to cross-species phenotypic resistance via partner shading, other mechanisms of cross-species antibiotic resistance have been suggested (see [48] for a recent review). For example, it was recently shown that co-culture of *Salmonella enterica* serovar Typhimurium with *Escherichia coli* enhances *S. typhimurium* tolerance to antibiotics [49]. When grown in co-culture, *S. typhimurium* can sense indole, a metabolite produced by *E. coli* but not produced by *S. typhimurium*, and this induces *S. typhimurium* antibiotic tolerance [49]. Enhanced tolerance of *S. typhimurium* to antibiotics when grown with *E. coli* had also been observed previously, but this time, tolerance was due to the exploitation of beta-lactamase producing *E. coli* [50].

Another important phenotypic mechanism of antibiotic resistance is the ‘inoculum effect’, described as an enhanced antibiotic resistance (i.e., higher minimum inhibitory concentration or MIC) with increasing inoculum density [51]. This density-dependent antibiotic resistance can have important implications for community-mediated resistance [48]. Our prediction here is that the inoculum effect will also contribute to enhanced resistance in mutualistic communities (as these are by definition more productive and achieve higher densities than competitive communities, see legend Figure 1A). Furthermore, a synergy between the inoculum effect and the partner shading effect could further increase resistance to narrow-spectrum antimicrobial clearance as increasing density and mixing combine to limit control. Hence, integrating structural and functional relationships into the broader theme of community-mediated resistance could shed light into the mechanisms underlying drug resistance.

Promoting microbiome health by managing polymicrobial interactions

Theoretical and experimental work on spatially explicit ‘minimal’ microbiomes can shed light on the causal mechanisms linking environmental inputs (e.g., nutrients or drugs) with microbiome structure and function as well as host health. Measuring host health (and disease) as a function of defined multispecies interactions can be approached experimentally

using *in vivo* model systems [23,42,52], but also using simpler *in vitro* proxies, such as community productivity, antibiotic resistance, and robustness (i.e., invasion resistance). The main goal is then to identify the metabolic and demographic consequences of varying key environmental drivers (such as nutrients, drugs, flow rates, and initial mixing) for the structure and functioning of two-species communities (Figure 3). Knowing how the community responds to these environmental changes would present us with novel points of intervention, potentially allowing the tuning of species numbers (densities and frequencies), behaviour, and emergent structuring of communities.

If we can influence human microbiome health via manipulation of their nutrient, drug, and mixing parameters, then what are the goals? For some microbial communities, the medical priority will be to prevent the establishment of a known pathogenic species (as in the case of *Sg* and *Aa* interactions where the priority is to prevent *Aa* establishment, [23]), but for other communities, the objective may be to encourage the growth of specific species or sets of species that are associated with human health (e.g., *Bifidobacteria* [53,54] or *Bacteroides thetaiotomicron* [55], suggested to reduce the occurrence of metabolic disorders associated with obesity and/or diabetes).

Moreover, virulence often emerges as a property of multi-species interactions, in particular mutualistic ones. For example, it has been demonstrated theoretically that mutualistic interactions among pathogens (e.g., HIV and tuberculosis [56]) are particularly dangerous due to the compounding effect of within-host demographic feedback [57], highlighting the importance of identifying effective control strategies to reduce mutualistic interdependency involving pathogens.

More generally, the existence of strong demographic feedbacks between species implies that treatment strategies cannot always focus on the behaviour of target species in isolation, but rather on managing the properties of polymicrobial interactions. These interactions open the door towards potential control strategies aiming to maximise (when beneficial – top row, Figure 4) or minimise (when harmful – bottom row, Figure 4) positive metabolic interactions between species.

Concluding remarks: challenges and opportunities for progress

Microbiomes are immensely diverse and complex. While focusing on a two-species ‘minimal’ microbiome model is a vast simplification of real systems, a ‘minimal’ microbiome already gives rise to a diverse and complex network of potential ecological scenarios of microbe–microbe (Figure 1) and microbiome–host interactions (Figure 4). Here we suggest that a thorough mechanistic understanding of a two-species spatially-extended microbiome is therefore a necessary step towards understanding more diverse microbial communities and developing a better management of the human microbiome health. Furthermore, this approach is more easily testable experimentally (both *in vitro* and *in vivo*). A major challenge, however, is to integrate the findings of a ‘minimal’ microbiome approach into our understanding of diverse, natural microbiomes. A potential approach to this challenge is to group microbial species by functional traits rather than by phylogenetic similarity [58,59]. Grounded in ecological theory, the idea is that species with similar

metabolisms and pathways would be grouped into the same functional group [59]. Using this functional classification approach provides a powerful complexity-reducing filter, and could allow testing the predictions of a ‘minimal’ microbiome model in natural microbial communities.

Another major challenge to furthering our understanding of microbiome dynamics is the ability to link scales from molecules to cells and communities (Box 1). But this is an exciting time to work at this interface, as new technologies are emerging that allow us to unravel the spatio-temporal dynamics of microbial communities [60]. For example, it is now possible to confine microbial cells in diffusive pico-litre scale traps to study their social behaviour [61], visualize single microbial cells in space and in real time [e.g., confocal laser scanning microscopy and fluorescence in situ hybridization (FISH)], as well as spatially map the molecular environment of microbial communities (e.g., scanning electrochemical microscopy [62,63] and nano-scale secondary-ion mass spectrometry (NanoSIMS) [64]). Major progress has been made by combining some of these techniques. For example, the coupling of FISH with SIMS or NanoSIMS (FISH-SIMS or FISH-NanoSIMS, respectively) has bridged the gap between species identity and metabolic function [65,66]. Using FISH-NanoSIMS, a recent study revealed the important role played by methanotrophic archaea in mediating the spatial distribution and extent of nitrogen fixation in methane seep sediments [67]. These approaches are particularly relevant when one wants to unravel the potential roles played by behavioural and regulatory interactions in the spatial and functional interactions of species. For instance, recent empirical work in a mouse model suggests that *Sg*–*Aa* synergistic interaction is enhanced by *Aa*'s ability to sense H₂O₂ and respond by modulating its own spatial positioning via dispersal. This ensures that the benefits of *Aa* cross-feeding on lactate are maximized while the costs incurred by the toxic effect of H₂O₂ are minimized [43]. This example illustrates the important role played by bacterial regulation (phenotypic plasticity) in shaping species spatial organization and enhancing bacterial virulence.

From a modeling perspective, IBMs of biofilms have been of particular relevance to investigate questions at this interface as IBMs use a bottom-up approach where the community structure and dynamics arise as an emergent property of the interactions between individual cells [68]. In particular, these models have emphasized the importance of looking at structuring of surface attached communities as an emergent property of collective bacterial behavior and demography [20,21,26,37,69].

Coupling experimental techniques with spatially explicit IBMs of microbe–microbe interactions or host–microbe interactions is a challenging but promising research direction. But, in a time where multi-‘omics’ approaches can produce a vast amount of data at an unprecedented pace, integrating experiments with theory can be essential to effectively exploit and interpret this information.

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Glossary

Competition	ecological interaction where all species are negatively affected by association due to, for example, consumption of shared and limiting resources.
Cross-feeding	act of using the extracellular metabolic by-products of other organisms for growth.
Demography	the study of population structure, emerging from patterns of births, deaths, migration, and development.
Demographic feedback	an interaction where one species influences the demographic processes (births, deaths, and movement) of another species, and vice-versa.
Exo-product	molecule secreted extracellularly and usually costly to produce (e.g., exopolysaccharide or exoenzyme).
Exploitation	ecological interaction where one species benefits from association at the expense of the other species.
Individual-based models (IBMs)	computational models tracking the dynamics of individual 'agents', often used to explore emergent properties of the population aggregate behavior.
Microbiome	the set of microorganisms sharing a particular habitat, e.g. the human lower intestine.
Mutualism	mutually beneficial ecological interaction between species.

Box 1**Outstanding questions**

- How do different mechanisms of metabolic interaction translate into functional relationships among species (competition, exploitation, and mutualism)?
- How do functional relationships among species dictate patterns of spatial mixing?
- Can we reverse-engineer functional and metabolic relationships from an analysis of spatial mixing patterns?
- What are the implications of polymicrobial spatial and functional relationships for community performance (e.g., productivity, robustness to environmental perturbations)?
- How do metabolic interactions and demographic feedbacks between species shape community-mediated resistance to antibiotics?
- How do hosts tune metabolic and demographic interactions within host-associated communities to promote microbiome health?
- Can we promote microbiome beneficial services to their host through interventions targeting microbe–microbe metabolic and demographic interactions?

Highlights

- Metabolic interactions drive demographic feedbacks between species.
- Demographic feedbacks strengthen or attenuate underlying metabolic interactions.
- Demography is a key determinant of microbiome development and functioning.
- Treatment strategies must consider the dynamical properties of species interactions.

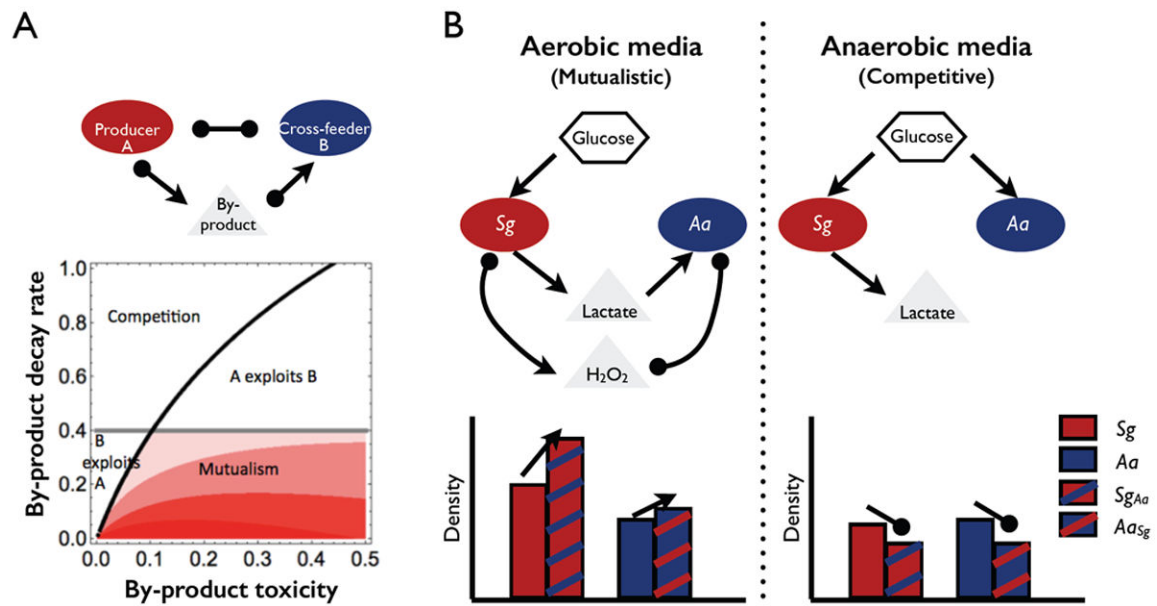


Figure 1.

Metabolic interactions within a two-species community. (A) A single mechanism of metabolic exchange can generate diverse functional relationships in liquid (well-mixed) cultures. Figure adapted from [19] © 2012 by The University of Chicago. Mutualism: density of A in co-culture (A_B) is larger than when alone (A), and B in co-culture (B_A) is larger than alone (i.e., $A_B > A$ & $B_A > B$). Red-scale indicates strength of mutualism ($A_B + B_A - (A + B)$). Competition: $A_B < A$ & $B_A < B$. A exploits B: $A_B > A$ & $B_A < B$. B exploits A: $A_B < A$ & $B_A > B$. (B) *Streptococcus gordonii* (Sg) and *Aggregatibacter actinomycetemcomitans* (Aa) engage in multiple forms of metabolic interactions. Schematic model of Sg–Aa metabolic exchange under aerobic (left) and anaerobic (right) conditions [63]. Open arrows represent a positive effect, whereas oval arrows represent a negative effect upon the population or metabolite they are pointing toward. Sg and Aa form mutualistic or competitive interactions in liquid culture, dependent on oxygenation. Redrawn from [23].

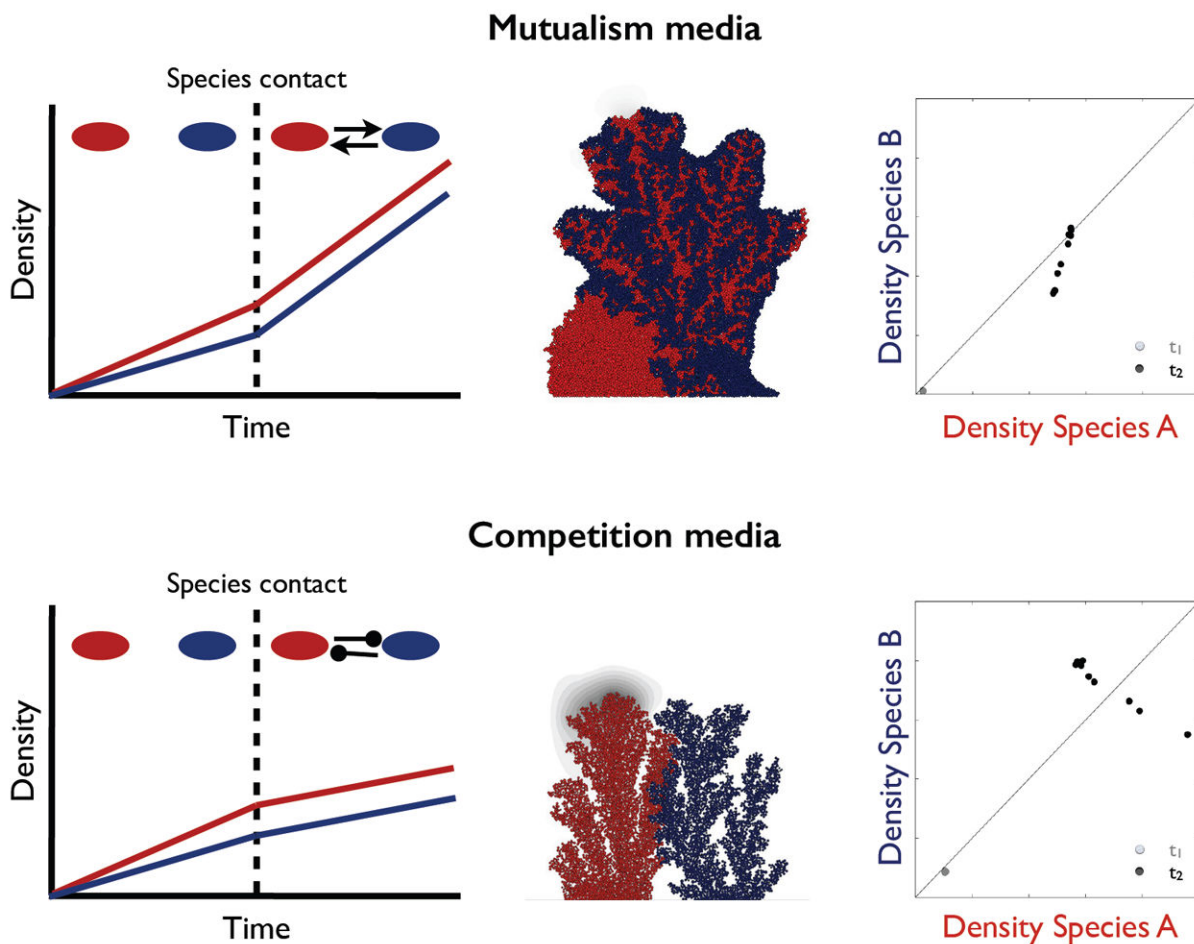
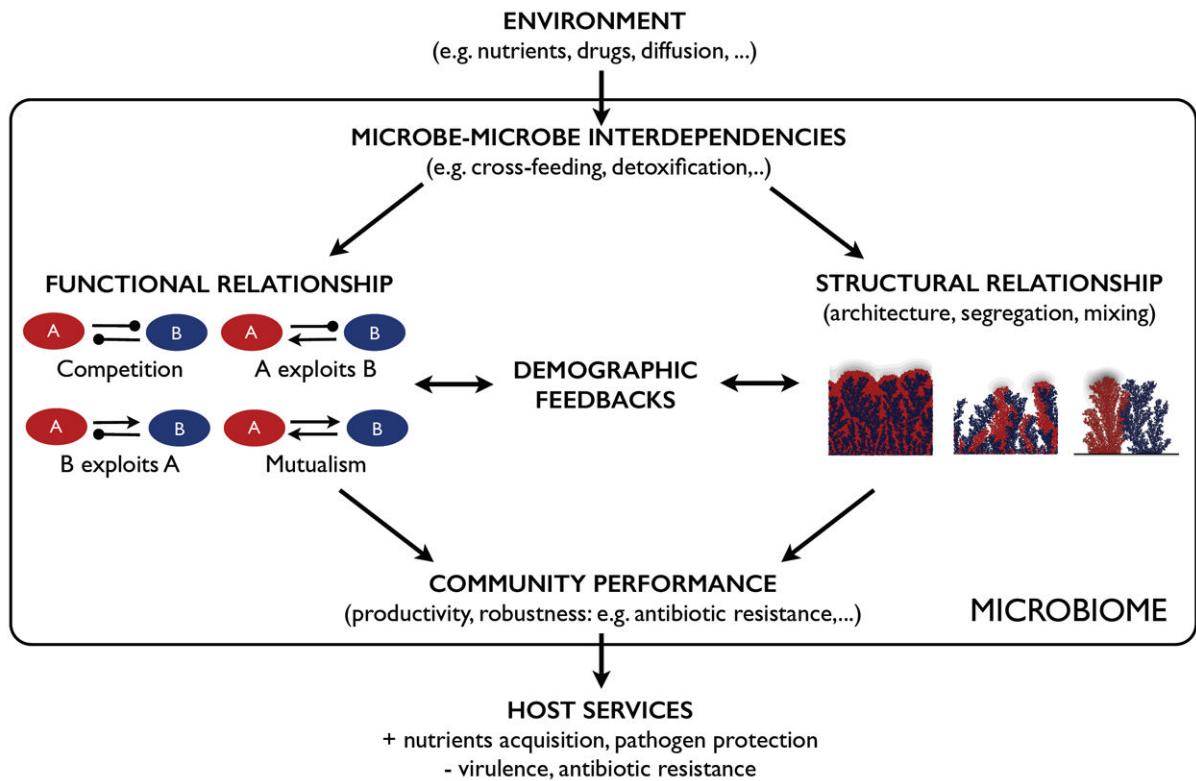


Figure 2.

Distinct metabolic interactions produce distinct emergent functional and spatial relationships. Top panel, 'mutualism medium' - the red species produces and blue species consumes a by-product toxic to the red species. The two species tend to mix and we observe a positive correlation between the two species densities across replicate communities (right plot, $n = 9$, t_1 and t_2 represent initial and final timepoints). Bottom panel, 'competition medium' - no metabolic exchange and competition for nutrients and space. The two species segregate and we observe a negative correlation between the two species densities (right plot). Both simulations were initiated with an identical segregated inoculum. Biofilm images and scatter plots are adapted from [20].

**Figure 3.**

Schematic illustrating an integrative approach that aims at bridging the gap between cell and community properties to build an integrated mechanistic account of the functioning of the microbiome. Demographic feedbacks create links between structural and functional relationships. For example, mutualistic functional relationships will tend to increase lineage mixing (Figure 2), which in turn will enhance mutualistic functional relationships (Figure 2). Conversely, competitive functional relationships promote lineage segregation, which in turn attenuates competition (see [20]).

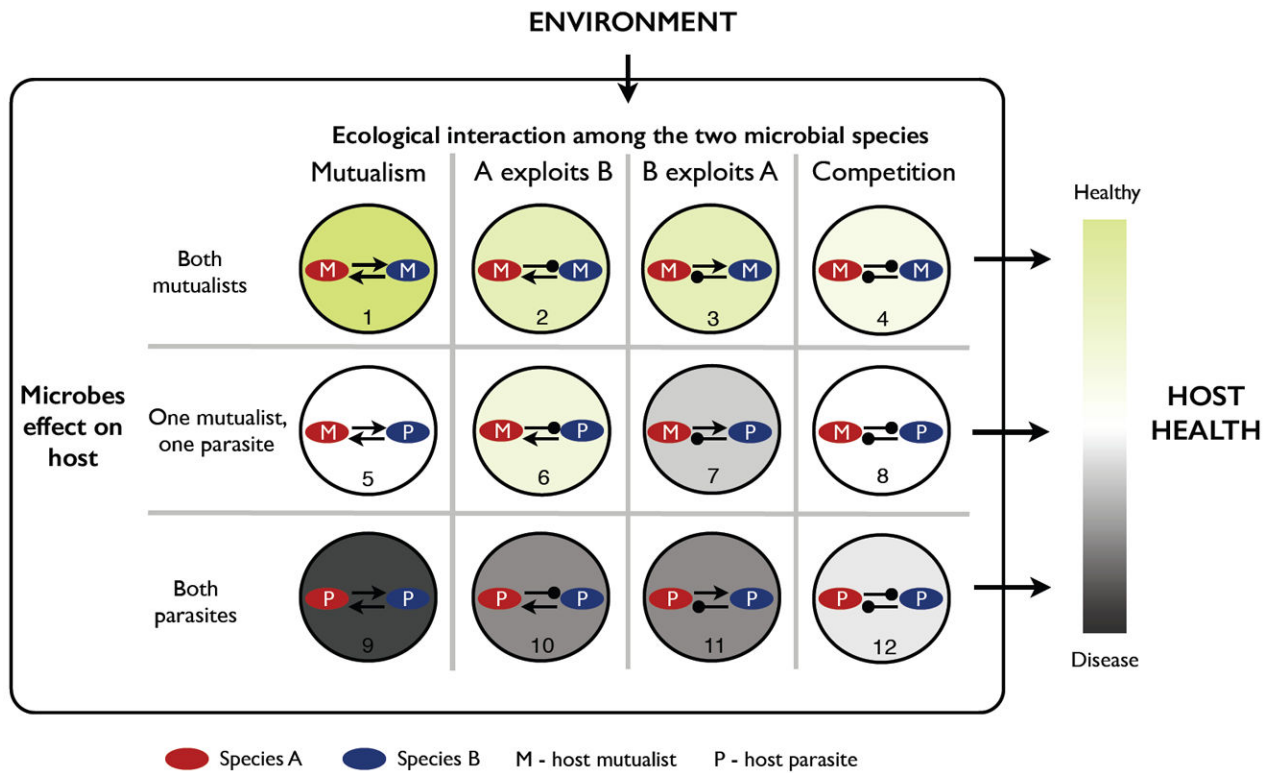


Figure 4.

Schematic illustrating the diversity of potential ecological scenarios occurring between a host and a 'minimal' two-species community. To promote health and prevent disease, we aim at maximizing mutualism between host mutualists (1) and minimizing mutualism between host parasites (9). What we want to maximize crucially depends on the species that are present (i.e., mutualist or parasite) and the nature of their interaction (mutualism, exploitation, or competition), which is contingent on their environment. For instance, let's focus on our minimal *Sg*–*Aa* microbiome model. When under anaerobic conditions, *Sg* and *Aa* are engaged in a competitive interaction. *Sg* is a better competitor, so it outcompetes *Aa* and thus protects the host. When under aerobic conditions, *Sg* and *Aa* are engaged in a mutualistic interaction. Although *Sg* is usually a commensal to the host, the harm to the host by *Aa* reduces the host fitness, thus this potential scenario should be minimized. For simplicity, the color shading illustrates potential symbiont effects on host health, assuming symmetric symbiont interactions, but the outcome will depend on the strength and symmetry of the interaction.