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Synthetic ecology of microbes: mathematical models and applications

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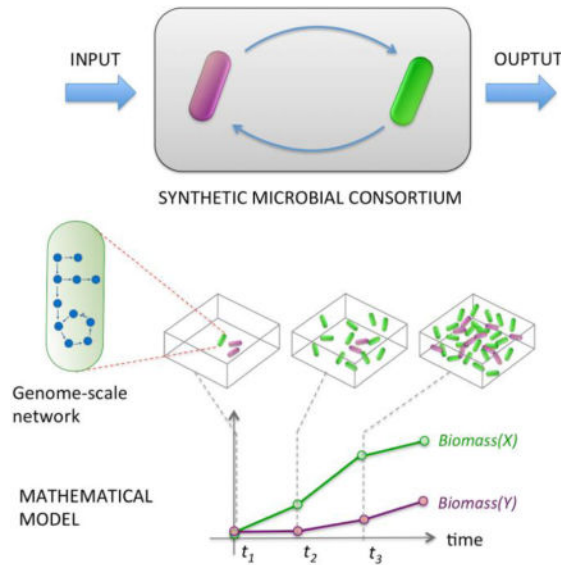
Abstract

As the indispensable role of natural microbial communities in many aspects of life on Earth is uncovered, the bottom-up engineering of synthetic microbial consortia with novel functions is becoming an attractive alternative to engineering single-species systems. Here, we summarize recent work on synthetic microbial communities with a particular emphasis on open challenges and opportunities in environmental sustainability and human health. We next provide a critical overview of mathematical approaches, ranging from phenomenological to mechanistic, which can be used to decipher the principles that govern the function, dynamics and evolution of microbial ecosystems. Finally, we present our outlook on key aspects of microbial ecosystems and synthetic ecology that require further developments, including the need for more efficient algorithms and a better integration of empirical methods and model-driven analysis, the importance of improving gene function annotation, and the value of a standardized library of well-characterized organisms to be used as building blocks of synthetic communities.

Graphical Abstract

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Keywords

Microbial communities; Synthetic Biology; Systems Biology; Metabolic network modeling; Metabolic engineering

Introduction

Synthetic ecology of microbes is concerned with the design, construction and understanding of engineered microbial consortia¹. It is a young, fast-developing research area, clearly distinct from synthetic biology, though related to it in a number of ways. Why would one want to engineer new microbial communities? How would engineered communities differ from natural ones? And how could one hope to design a community with desired properties, other than by tinkering with their intracellular circuits, or by mixing different species, based on experience and intuition? Here, we delve into these questions by discussing several examples of prior work in this area, and by presenting an overview of the growing landscape of mathematical approaches aimed at understanding the function, dynamics and evolution of microbial ecosystems, and at enabling the rational design of new microbial consortia.

The idea of designing microbial consortia is inspired by the ubiquitous presence of microbial communities on our planet, and the key role that these communities play in many aspects of human life. Microbial communities are implicated in biogeochemical cycles² and human health³, and have been enlisted for a wide array of biotechnological applications, ranging from the ancient arts of brewing and cheese-making⁴ to recent efforts towards the overproduction of biofuels and chemicals^{5; 6} and wastewater treatment^{7; 8}. Engineering novel microbial communities may involve inducing the coexistence of unusual combinations of wild-type organisms, or constructing ecosystems of genetically modified species, thus creating a continuum of possible strategies between synthetic biology and ecology.

One of the appeals of synthetic ecology is that it may enable us to perform novel tasks by understanding and embracing – rather than avoiding - properties that are inherent in the natural microbial world, such as diversity, competition for resources, division of labor and obligate interdependence. A community of organisms may perform tasks that no individual species could possibly perform on its own (i.e., from a functional perspective, a community is more than the simple sum of its parts ⁹). Moreover, relative to monocultures, engineered communities may achieve increased stability and resilience ¹⁰. The emergence of community-level properties is a result of interactions among different species. Inter-species interactions are one of the primary factors shaping the structure, function and dynamics of microbial communities and are believed to play a key role in the emergence of biodiversity ^{11; 12}. Interactions among species can be mediated by a complex web of diffusible chemical signaling molecules and/or metabolites ^{13; 14; 15} or by direct contact with neighboring microorganisms ^{16; 17; 18}. These interactions can be obligatory or non-obligatory, beneficial or deleterious.

Beneficial interactions often involve cases where one or more species feed on products of other community members ^{19; 20; 21}. In obligatory cooperative (i.e., syntrophic) interactions, individual species cannot survive in absence of their partners. For example, in a cross-feeding interaction, both species could secrete nutrients essential for the growth of their partner ^{22; 23; 24}. Alternatively, one species could rely on the waste product of the other while maintaining a favorable thermodynamic condition in return ^{25; 26; 27; 28}. For example, in the absence of a suitable electron acceptor, methanogens can provide thermodynamically favorable growth condition for sulfate-reducing bacteria by scavenging hydrogen in the environment while using the fermentation by-products (e.g., acetate, formate) produced by sulfate-reducers ^{27; 28}. Whether obligatory beneficial interactions are abundant in natural communities is still an open question, potentially relevant for understanding unculturability of the vast majority of microorganisms in the laboratory in monocultures ²⁹. A common hypothesis is that unculturability of many species is due to their dependence on other microbial species for nutrients or growth factors ³⁰.

Negative interactions are ubiquitous in nature. In addition to the competition for the same limiting resource, these interactions include growth inhibition effects of signaling molecules like bacteriocins, and active killing through a wide range of antibiotic mechanisms ^{31; 32; 33}. Moreover, community dynamics can be heavily affected by parasitic interactions (e.g., between bacteria and their bacteriophage) ^{34; 35} where the parasite benefits while the host is negatively affected.

A broad spectrum of applications have driven the desire to build new communities, ranging from the conceptual challenge of characterizing small synthetic systems as a gateway towards understanding the more complex natural ones to the interest in achieving a specific biotechnological task (e.g., treatment of human diseases, overproduction of biochemicals and bioremediation of contaminated environments). Here, we first review some examples of such efforts and next focus on how mathematical modeling can complement these empirical efforts to better understand ecological principles underpinning the function and dynamics of microbial communities. We also present an overview of the existing challenges and future

perspectives in synthetic ecology with a particular focus on the role of mathematical modeling.

Using synthetic ecology to understand natural microbial communities

Natural microbial communities often contain tens to thousands of microbial species³⁶. This makes it challenging to experimentally characterize the identity of community members, their function and interactions. A bottom-up approach to address these limitations is to design synthetic microbial consortia that could serve as simplified models of their natural counterparts, while affording enhanced tractability and controllability. These synthetic systems would allow one to explore a number of key ecological and evolutionary questions such as the impact of interactions and environmental factors on the emergence, evolution and maintenance of coexistence. Synthetic microbial consortia can be established by co-culturing wild-type species in a growth medium similar to their natural habitat, or they can be constructed by using targeted genetic perturbations or design of environmental conditions that induce new interspecies interactions (e.g., through metabolic exchange, antibiotic secretion, or quorum sensing) (see Fig. 1). Here, we review some examples of such efforts.

Artificial consortia with wild-type species grown in a medium resembling their natural habitat have been established to gain a deeper understanding of the community properties. An example is co-culturing methanogens and sulfate-reducing bacteria to better understand methane production and mutualistic interactions in subsurface anaerobic environments as noted earlier^{25; 27; 28}. They have been also used to elucidate the biodiversity-function and biodiversity-stability relationships in natural microbial communities^{37; 38; 39; 40}. For example, Von Canstein et al³⁷ found that increasing the diversity of microbial species in a biofilm improves the mercury removal efficiency in a changing environment. Assessing the stability of synthetic bacterial communities of different diversities (ranging from one to 12 members) showed that the biomass of more diverse communities are stabilized against (i.e., less affected by) abiotic perturbations such as addition of heavy metals, NaCl and warming⁴⁰. While the majority of studies focused on the impact of richness (the number of species), Wittebolle et al⁴¹ examined the impact of initial community evenness (relative abundance of species) using eighteen different denitrifying bacterial species from four different phyla and microbial microcosms. This study demonstrated that the initial evenness is a key determinant of the functional stability of the community.

For cases where establishing a consortium is not possible with wild-type species, a common strategy is to implement defined genetic perturbations, which can create new inter-species interactions through metabolite exchange or antibiotic production^{22; 23; 42; 43; 44; 45; 46; 47}. For example, by using 14 knockout strains of *Escherichia coli*, each lacking a gene responsible for the production of an essential amino acid, Mee et al²³ constructed communities of increased complexity (from two- to 14-member) in order to assess the impact of synthetic cross-talk between the mutants on population dynamics and stability (see Fig. 1B). In another study, Harcombe⁴³ used a synthetic two-species system composed of *Salmonella enterica* and an *Escherichia coli* mutant unable to synthesize an essential amino acid to elucidate the mechanisms and evolutionary origins of cooperation between unrelated species. This study concluded that cooperation can evolve under two conditions, namely the

presence of a preexisting reciprocation mechanism and the preferential availability of reciprocation to cooperative phenotypes.

Using synthetic genetic circuits to induce new interactions through quorum sensing has been also widely used to establish synthetic microbial communities^{48; 49; 50; 51; 52; 53; 54}. For example, in order to assess experimentally the relation between the parameters of Hamilton's rule (a mathematical model for the emergence and maintenance of cooperation⁵⁵) and the quantities that govern the behavior of a microbial ecosystem, Chuang et al⁵⁰ engineered two producer and non-producer populations of *E. coli*, where producers synthesize the growth-enhancing Rhl autoinducer molecule as the common good. This autoinducer activates the expression of an antibiotic resistance gene in both producers and non-producers. This study showed that the nonlinearity of the growth benefit as a function of the common good tends to limit the predictive accuracy of the Hamilton's rule⁵⁰. In another study, a population-driven synthetic quorum sensing switch was engineered to enable the dispersal of a second cell type into an existing colonizer biofilm, the subsequent formation of a robust two-species biofilm and finally the displacement of the initial colonizers⁵³. Rather than using genetic circuits that modulate gene expression in independent cells through quorum sensing, Chen et al⁵⁴ recently took a different strategy by constructing a dual feedback oscillator genetic circuit distributed across two nonisogenic populations of *E. coli*. The consortium consists of an "activator" and a "repressor" *E. coli* strain, each implementing half of a dual-relaxation oscillator and communicating through two orthogonal signaling molecules. Emergent population-level oscillations were observed only when the two organisms are cultured together⁵⁴.

In addition to genetic perturbations, it is known that appropriate design of environmental conditions can induce or significantly alter the dynamics and stability of microbial interactions^{56; 57; 58}. For example, by using a microfluidic device controlling the spatial structure and chemical communication Kim et al⁵⁹ reported the realization of a stable syntrophic consortium of three different species of wild-type soil bacteria, where each species performs a unique function essential for the survival of the entire community. In another study, Zuroff et al⁵⁷ showed that by fine tuning the oxygen transport rate a stabilized mutualism between the obligate anaerobic *Clostridium phytofermentans* and yeast can be established in which yeast protects *C. phytofermentans* from oxygen inhibition in return for soluble carbohydrates released from the degradation of lignocellulosic material. Another study reported on the impact of antibiotic levels as a key environmental factor in shaping a wide range of synthetic interactions including extinction, mutualism and commensalism between two *E. coli* populations⁵⁸.

Applications of synthetic ecology in biomedicine, metabolic engineering and environmental sciences

The construction of synthetic microbial communities has been pursued for a number of practical applications including human health, the production of chemicals, bioenergy, foods and drugs, and the mitigation of harmful human-induced environmental damage. These efforts have been so far mostly driven by experience and intuition, and by knowledge of the

metabolic capabilities and environmental interactions of different organisms. In the following, we briefly review some of these application areas.

Human health and disease

Microbial communities that live on or within our body have a marked, though still poorly understood, effect on the physiology and health of the human host and seem to reliably perform crucial tasks for us. For example, the human gut microbiota enable the breakdown of otherwise indigestible polysaccharides and are essential for the development and homeostasis of the immune system in the gut and for resistance against pathogenic bacteria^{60; 61}. Specific shifts in taxonomic composition (states of “dysbiosis”) of the human microbiota are known to be associated with an increasing number of diseases³. Previous studies have reported strong associations between the composition of the gut microbiota and several complex diseases such as obesity and atherosclerosis, diabetes and inflammatory bowel disease^{62; 63; 64; 65; 66; 67}. On the other hand, diet, environment and age are also known to influence the composition and structure of the gut microbiota^{64; 68; 69}. Similarly to the gut, the oral cavity is the home for one of the most complex microbial communities in the human body, forming highly structured biofilms in the form of dental plaques^{70; 71}. These communities are responsible for two major categories of diseases including dental caries (tooth decay) and periodontitis (inflammatory and infectious gum disease)^{9; 72; 73; 74}.

While there is a flourishing industry proposing ways to enhance the health-promoting effects of certain human-associated microbes, the systematic validated use of synthetic microbial communities to cure disease is still at the very early stages of investigation, with a recognized large potential impact⁷⁵. For example, the recent advent of gut-on-a-chip technology⁷⁶ lays the foundation to construct such synthetic communities in order to facilitate the study of intestinal physiology, digestive diseases and drug development. Another area where synthetic ecology can contribute is constructing synthetic microbial consortia helping to shift an imbalanced microbiota in the human body to the healthy state. A classical example is treating *Clostridium difficile* infection by using fecal microbiota transplant, where fecal bacteria from a healthy individual are transplanted into a recipient with *C. difficile* infection. This method has been reported to be more effective than using antibiotics (see⁷⁷ for a review). An alternative to using natural communities from healthy individuals could be to design efficient synthetic consortia for the targeted treatment of a wide of range of other microbiota-associated diseases.

Consortia-based cell factories

Recent advances in synthetic biology have allowed researchers to engineer single-species microbial cell factories for the enhanced production of chemicals and energy^{78; 79; 80}. However, the efficient microbial conversion of complex biological feedstock to desired products typically requires multiple different functionalities. This poses a challenge in engineering monocultures as optimizing a single species for one trait usually comes at the expense of other traits due to the existence of tradeoffs in the performance limits of different functional traits^{6; 81; 82}. These difficulties have attracted researchers to the challenge of designing synthetic consortia-based microbial cell factories with task-specialized species. This “division of labor” would allow the community as a whole to perform multiple

functions (e.g., utilizing multiple resources) in parallel or serially thereby leading to enhanced productivity and stability^{6; 10}. Another advantage of using consortia-based cell factories is its inherent compartmentalization, which facilitates the decrease in cross-reactions and side products⁵. An example of a complex overproduction task that can be addressed using multi-species microbial cell factories is the conversion of lignocellulosic biomass to biofuels, which involves the hydrolysis of lignocellulose to soluble sugars and subsequently the conversion of these sugars to biofuels or any other product of interest. There is no known microorganism capable of performing all these tasks. A possible solution is to construct a synthetic consortium composed of a lignocellulose degrader and another microorganism to ferment the released sugars to the products of interest (see Fig. 2 and Table 1). An example of such approach, among others^{57; 83; 84; 85; 86}, is a synthetic consortia composed of the fungus *Trichoderma reesei* secreting cellulase to hydrolyze lignocellulosic feedstock into soluble saccharides and *Escherichia coli*, which converts these saccharides into the products of interest such as isobutanol⁸⁷.

Another challenge in the conversion of lignocellulosic biomass to biofuels is that there is no microorganism that can ferment all pentoses and hexoses produced from the hydrolysis of cellulose. Wild-type microorganisms either use these sugars sequentially (e.g., first glucose and then xylose) or are in principle incapable of utilizing pentoses (such as *Saccharomyces cerevisiae*). A possible resolution of this issue for the latter is using synthetic biology techniques to “knock in” the genes enabling the degradation of otherwise non-degradable sugars, e.g., introducing the genes of xylose consumption pathways into *S. cerevisiae*⁸⁸. However, these approaches suffer from the preferential use of sugars, which leads to a decreased productivity^{89; 90}. A recently pursued alternative strategy to avoid all these limitations is the design of synthetic consortia where each strain exclusively uses only one sugar^{91; 92; 93; 94}. For example, Xia et al⁹⁴ reported the engineering of a consortium composed of three substrate-selective *E. coli* mutants each capable of metabolizing only glucose, xylose or arabinose by removing the genes responsible for the metabolism of the other two sugars. This consortium was capable of simultaneously consuming the mixture of all three sugars. In another study, both steps of lignocellulose degradation and the conversion of mixture of sugars to a product of interest were integrated by using a co-culture consisting of *Clostridium thermocellum* and *Clostridium thermolacticum*⁸⁵. While both species have multiple de-polymerization enzymes enabling them to degrade different forms of cellulose, the former is efficient in catabolizing glucose and the latter is proficient in pentose degradation. This allowed the enhanced production of ethanol from cellulose by the co-culture of both species⁸⁵.

Another common limitation associated with using synthetic biology techniques to assemble novel metabolic pathways converting a desired feedstock to a product of interest is that different parts of these typically long pathways often require specialized environments or compartments for optimal operation. A recent study proposed the resolution of this issue by a division of labor strategy where the long conversion pathway is divided among multiple community members⁹⁵. In this study, the synthetic pathway for the production of precursors of anti-cancer drug paclitaxel was divided into two modules one expressed in *S. cerevisiae* and the other in *E. coli*. Neither of these two organisms can produce the paclitaxel

precursors on their own, however, each provides the best host environment for part of the pathway they are harboring. The stable co-culture established by mutualistic interactions between these two organisms (where taxadiene, a metabolic intermediate produced by *E. coli*, is used and functionalized by yeast) enabled the enhanced production of a number of different paclitaxel precursors⁹⁵. A few other studies took a similar strategy to achieve higher production yields of products of interest using two genetically modified strains of *E. coli*^{96;97}. For example, Saini et al⁹⁶ reported on the enhanced production yield of *n*-butanol from glucose upon the distribution of the *n*-butanol production pathway across two different *E. coli* strains, one producing butyrate from glucose and the other producing *n*-butanol from butyrate.

Microbial consortia for environmental applications

The importance of using microbial communities for the bioremediation of contaminated environments has been known for years as these environments contain a mixture of multiple different organic wastes and metals, which cannot be degraded and/or removed by a single microorganism. Synthetic microbial consortia have been used as an alternative to naturally occurring communities to improve and accelerate the biodegradation of pollutants^{98; 99; 100; 101; 102}. For example, a co-culture of a genetically engineered *Escherichia coli* and a wild-type *Ochrobactrum* sp. was established in a laboratory-scale bioreactor to degrade methyl parathion, a highly toxic pesticide commonly used for agriculture crop protection¹⁰⁰. The engineered *E. coli* strain overproduces methyl parathion hydrolase converting methyl parathion into *p*-nitrophenol, which is a toxic intermediate and serves as the sole carbon, nitrogen and energy source for and degraded by *Ochrobactrum* sp. More recently a synthetic consortium composed of three fungal strains *Aspergillus lentulus*, *Aspergillus terreus* and *Rhizopus oryzae* for the simultaneous removal of multiple metals and dyes was reported¹⁰³. Even though the detailed mechanism of inter-species interactions was not explored in this study, it was shown that these fungal species stably work in concert by distribution of tasks among different specialized members, where *Aspergillus lentulus* removes Cu²⁺ and Acid Blue 161, *Aspergillus terreus* removes Cr⁶⁺ and *Rhizopus oryzae* removes Pigment Orange 34. This synthetic community was reported to be more efficient in the removal of these metals and dyes compared to their monoculture counterparts¹⁰³.

Bioelectrochemical systems such as microbial fuel cells have been used to simultaneously degrade complex organic matter in contaminated environments and to produce electric power, chemicals and biofuels^{104; 105}. Syntrophic microbial consortia are widely used in microbial fuel cells, where multiple fermentative bacteria degrade a mixture of complex organic pollutants and exoelectrogenic microorganisms (typically *Geobacter* species) rapidly convert fermentation intermediates into electrical current or chemicals/biofuels thereby eliminating their feedback inhibition on fermentative bacteria¹⁰⁶. Synthetic microbial consortia have been used to design more efficient microbial fuel cells^{107; 108; 109; 110; 111; 112; 113}. A notable example is the study by Venkataraman et al¹¹⁰, which demonstrated that a mutualistic co-culture of *Pseudomonas aeruginosa* and *Enterobacter aerogenes* in a bioelectrochemical system leads to up to 14-fold increase in electric power generation compared to either of the monocultures. The mutualistic interactions are mediated by 2,3-butanediol, which is a by-product of glucose fermentation

by *E. aerogenes* and is subsequently used by *P. aeruginosa*. The increased current production was attributed to the removal of fermentation by-product by *P. aeruginosa* as well as the enhanced production of pyocyanin (electron shuttles) by *P. aeruginosa*¹¹⁰. A more recent study addressed the issue that butyrate, which is an anaerobic fermentation by-product is not directly used by *Geobacter sulfurreducens* in anode. A synthetic consortium composed of two different but complementary mixed cultures was designed for the anode to overcome this issue, where one oxidizes butyrate to acetate and the other (enriched in *Geobacter* species) produces electric current by the consumption of acetate¹¹². This synthetic community led to an enhanced production of electric current that outperformed the previous naturally derived communities.

Mathematical modeling and computational analysis of microbial communities

Despite the growing availability of high-throughput experimental data (especially metagenomic sequences) for a diverse range of complex natural microbial communities, the full characterization and understanding of these communities is still a challenging task. This is partly due to the fact that it is very difficult, if not impossible, to measure the extent and direction of inter-species interactions (a key determinant of community's function and dynamics^{11; 12}) even using the state-of-the-art experimental techniques. Furthermore, engineering synthetic consortia to perform sophisticated tasks for application areas reviewed above requires searching through a complex web of organisms and interactions in time and space, which can no longer be achieved by empirical tinkering. The development of efficient computational techniques and/or mathematical modeling tools can address some of these questions and shed light onto the experimentally inaccessible aspects of microbial communities. These models are critical in addressing a variety of ecologically and evolutionary relevant questions such as quantifying the impact of inter-species interactions and environmental factors on the emergence of cooperation, coexistence of cooperators and cheaters and the evolutionary fate of the communities. More importantly, they can play a critical role in the rational design of synthetic consortia for desired applications. In the following, we review some of the most common techniques for modeling microbial communities.

Ecological based modeling

Ecological theories of inter-species interactions—Two important models from theoretical ecology that have been successfully employed to analyze inter-species microbial interactions are resource ratio theory (RRT) and the maximum power principle (MPP). RRT models the competition between two or more species for a limiting resource based on the assumption that the outcome of competition is determined by the ratio of supply rates of the limiting nutrient(s)¹¹⁴. RRT has been primarily used to model competition^{115; 116; 117; 118}, but has been extended to account for cooperative interactions as well¹¹⁹.

MPP is another interesting model, which relies on the assumption that all biological systems are coordinated to increase power (i.e., metabolic rate) whenever constraints allow^{120; 121}. By comparing model predictions with experimental observations, DeLong¹²² showed that

MPP can successfully predict various outcomes of competition in two-species microcosm communities. In particular, it was concluded that in the case of competitive exclusion the winning species uses more power (i.e., it acquires and uses more energy and thus reproduces faster) than the losing species. In the case of coexistence, either the community-level power exceeds that of individual species in isolation, or the power that a species can attain within the community is higher than what could be reached in isolation ¹²³.

In addition to RRT and MPP, a number of other ecological models and theories have been proposed recently, which have a great potential for modeling microbial interactions ^{124; 125; 126}. An example of such a theory, which offers a new perspective on the emergence and evolution of costly cooperation in microbial communities is the Black Queen Hypothesis (BQH) ¹²⁵. It posits that cooperation among species may emerge due to purely selfish traits. The most important assumption of BQH is that some costly microbial functions are often leaky, such that the resulting public goods can be used by other species. It further hypothesizes that since these functions are costly and thus undesirable, adaptive loss of the corresponding genes may happen in some species. This inevitably turns some community members to “helpers” and the rest to “beneficiaries” and builds an obligatory association between helpers and beneficiaries. The “black queens” here refers to these costly functions that most species strive to avoid, analogous to the queen of spades in the game Hearts ¹²⁵. This theory has the potential to devise a possible evolutionary path for the emergence of cross-feeding, whereby leakiness and gene loss may be followed by the evolution of costly cooperative traits in beneficiaries to maximize the production of the vital by-product by the helpers ¹²⁶. Recently, Oliveira et al ¹²⁷ examined the evolution of cross-feeding based on BQH by developing a ecoevolutionary model that accounts for multiple secretions by each species, which can be exchanged among genotypes. They concluded that the evolution of cooperative exchanges reduces the community productivity relative to an autonomous strain performing all vital functions it needs, and that this type of cooperative behavior evolves only under specific demographic regimes characterized by intermediate genetic mixing ¹²⁷.

Population dynamic models—A traditional way of modeling the dynamics of microbial communities rooted in theoretical ecology is the use of coupled differential equations describing the temporal evolution of microbial species abundances. The most widely-used such model is the Lotka–Volterra (LV) model, originally developed for modeling predator–prey dynamics, and later generalized to model combinations of competitive and cooperative interactions ^{128; 129}. The LV equations can, in general, be written as follows ¹²⁸:

$$\frac{dN_k}{dt} = \left(r_k + \sum_{k'=1}^K b_{kk'} N_{k'} \right) N_k \quad k=1, 2, \dots, K, \quad (1)$$

where N_k is the abundance of species k , K denotes the total number of species, r_k is the intrinsic net growth (i.e., growth minus decay) rate and b_{kk} denotes the interaction coefficients (or strengths) measuring the effect of one individual in population k' on the growth of one individual in population k , which can assume a negative, zero or positive value denoting a negative, neutral, or positive interaction, respectively. In this form, the LV

model takes into account the impact of the presence or absence of other species implicitly through the interaction coefficient, but cannot capture explicitly indirect interactions through e.g., metabolite exchange or quorum sensing¹³⁰. In an attempt towards addressing this limitation, a recent study proposed to include in LV models the explicit dynamics of exchanged metabolites in a one-way mutualistic interaction where one species grows on the waste product of another species¹³¹. Generalized LV models (Equation 1) have been used to model bacteria-bacteriophage interactions^{132; 133; 134} as well as microbial interactions in the gut^{135; 136} and in a cheese microbial community¹³⁷. For example, Fisher and Mehta¹³⁵ used sparse linear regression to infer interaction coefficients in a discrete LV model of microbial dynamics using species abundance data for the gut microbiota of two individuals. In another effort, the generalized LV models were extended further to account for the impact of time-dependent external perturbations¹³⁶. After using linear regression to infer interaction coefficients, this extended model was used to computationally assess the impact of infection by pathogens and antibiotic administration on the dynamics and stability of the mouse gut microbiota. It is worth noting that in addition to LV models, various other ODE-based models have been used to describe the dynamics of interacting microbial populations in different settings^{23; 87; 138; 139; 140; 141}.

Spatial modeling—Except for laboratory setups, most natural microbial communities display highly complex spatial structure. As a result, community interactions and abundances vary not only with time but also with space due to the heterogeneity of their habitat, the existence of natural gradients (e.g. different amounts of oxygen penetrating through a biofilm), and self-organization properties of the microbes themselves. For example, a given resource may be differentially available to a given species in different spatial locations, thus affecting significantly the function, stability, dynamics and evolution of the entire ecosystem. In this case, instead of ODEs, the dynamics of the system is best captured by partial differential equation (PDE) models. The most widely-used PDE model is the reaction-diffusion equation, which determines the density of each species at different time points and different locations in space due to diffusion and population dynamics^{142; 143}:

$$\frac{\partial C_k}{\partial t} = D_k \left(\frac{\partial C_k}{\partial x} + \frac{\partial C_k}{\partial y} + \frac{\partial C_k}{\partial z} \right) + r_k(x, y, z, t, C_1, C_2, \dots, C_K) \quad k=1, 2, \dots, K, \quad (2)$$

where C_k is the concentration (or density) of species k at time t in location (x, y, z) , D_k is the diffusion coefficient of species k in the medium (measuring dispersal rate), and r_k denotes growth or decline in population k due to population dynamics, which can be determined at a given point (x, y, z) in space by using any population dynamic model described in the previous section. Note that the first term in the right-hand side of Equation (2) determines dispersal due to diffusion. This equation is thus reduced to population dynamic models (based on ODEs) for a homogeneous environment. This same equation can be used to model the spatio-temporal variations in the concentration of shared compounds in a microbial community in which case the term r_k stands for the net production rate of a compound k by different members of the community.

This class of PDE-based models and their extensions/simplifications have been used in conjunction with population-based models to study a variety of ecological phenomena related to spatial effects such as range expansion and diffusion-based spatial patterning^{144; 145; 146; 147; 148; 149}. For example, by using a one-dimensional reaction-diffusion equation, Datta et al¹⁴⁶ successfully modeled the wave front profiles observed in a range expansion experiment involving populations of cooperator and cheater yeast strains. They also used a similar reaction-diffusion equation to model the spreading of cooperator and defector alleles and to analytically derive the velocity of defectors invading a spatially extended population of cooperators¹⁴⁶. In a subsequent related study, a spatial model of a mixed population of cooperators and defectors coupling changes in both population density and allele frequencies was developed and it was shown that cooperators are favored at the edge of an expanding population, and under certain conditions, they can spread into new territories faster than they are invaded by defectors¹⁴⁶. More recently, a reaction-diffusion model was used to construct a model of cross-feeding mutualism that explicitly accounts for the production, consumption and diffusion of public goods¹⁴⁹. Interestingly, this study showed that while species migration improves mutualism and stabilizes coexistence, cooperation is lost beyond a critical diffusivity of public goods. Furthermore, for the case of unequal diffusivity of public goods, the species with slower-diffusing public goods will dominate the co-culture and destroy cooperation by driving the other species to extinction.

Game theoretical models

The complex balancing of benefits and costs associated with inter-species interactions in microbial communities can also be effectively addressed by using game theory and evolutionary game theory approaches^{45; 87; 150; 151; 152; 153; 154; 155} (see also¹⁵⁶ and¹⁵⁷ for comprehensive reviews). Game theory is a general mathematical framework to model strategic interactions among a number of agents (players) where the payoff of each agent (i.e., how happy each agent is) is not only a function of its own strategy (action) but also a function of other players' strategies.

The payoffs are mathematically represented as a (multi-dimensional) matrix whose entries represent the payoff of each player for a given strategy profile. This payoff matrix is used to determine the equilibrium of the system, a state where no player has any incentive to deviate from its current strategy given all other players' strategies, because no change in strategy would increase the player's payoff. In evolutionary game theory, the payoff of each player depends not only on the action of other players but also on their relative abundances. This payoff is then used to determine the reproductive fitness of each player. The most popular way of modeling the reproduction dynamics of mixed interacting populations in evolutionary game theory is using the replicator's equation¹⁵⁸:

$$\frac{dx_k}{dt} = (f_k(\mathbf{x}) - \phi(\mathbf{x}))x_k \quad k=1, 2, \dots, K, \quad (3)$$

$$f_k(\mathbf{x}) = \sum_{k'=1}^K a_{kk'} x_{k'} \quad k=1, 2, \dots, K, \quad (4)$$

$$\phi(\mathbf{x}) = \sum_{k'=1}^K f_{k'}(\mathbf{x})x_{k'}. \quad (5)$$

Here, $\mathbf{x} = [x_1, x_2, \dots, x_K]^T$ represents the composition of the community with x_k being the relative abundance (frequency) of species K , $f_k(\mathbf{x})$ is the fitness of species k , $\phi(\mathbf{x})$ denotes the average fitness of the community and $a_{kk'}$ represents the payoff of species k confronting species k' (extracted from the payoff matrix of the game). According to Equation (3), the frequency of species k increases, decreases or remains constant, based on whether its fitness is greater than, less than or equal to the average fitness, respectively. Even though this model does not explicitly capture the emergence of new phenotypes due to mutation, it is usually used to assess whether a pre-specified mutated phenotype can invade an existing phenotype. It has been shown that the replicator equation for a game with K strategies can be transformed into the generalized LV model with $K - 1$ species¹⁵⁸. This alludes to a fundamental link between evolutionary game theory and theoretical ecology.

A prominent example of modeling microbial interactions using game theory is the work by Gore et al⁴⁵. They experimentally assessed and modeled the outcome of interactions between a wild-type cooperator strain of *Saccharomyces cerevisiae*, which produces the invertase enzyme to hydrolyze sucrose and convert it to glucose and fructose, and a cheater mutant strain of *S. cerevisiae*, which benefits from the sugars resulted from sucrose hydrolysis but does not endure the cost of producing invertase. In this work, instead of assuming that fitness is a linear function of the relative abundance of species (see Equation 4), experimental data were used to formulate a fitness function that depends nonlinearly on the relative abundance of both species, and on the production cost of invertase. This nonlinear model could explain the experimentally observed coexistence between cheaters and cooperators, which is the reminiscent of a classical game theory scenario, termed the snowdrift game. In another study, the dynamics of a game between two bacterial species competing for a limiting resource in a fluctuating environment was captured by an extension of the LV model that allows switching from one species (strategy) to another¹⁵¹. Each species was assumed to take either of the two strategies constant (environment-insensitive) growth and susceptible (environment-dependent) growth. This analysis showed that the constant growth strategies always outcompete or evenly match with its competing strategy. Despite the limitations associated with quantifying the payoffs in biological systems, game theory and evolutionary game theory remain an attractive mathematical tool to model microbial interactions. For example, they can be used to assess whether an engineered microbial consortium for a desired biotechnological application can be invaded by cheaters, or to determine the range of environmental conditions where cheaters are dominated by or coexist with cooperators (e.g., see¹⁵⁹).

It is worth noting that game theory/evolutionary game theory models were also extended to capture the impact of spatial structure by assuming that game players are located on the vertices of a non-complete graph and preferentially interact only with their neighbors¹⁵⁸. This approach was recently used to investigate the emergence and fate of cooperation in “diffusible public good dilemmas” in microbial communities¹⁶⁰. In this model, both colony

geometry and public good diffusion are described by graphs and it was found that cooperation is favored when public goods decay and diffuse slowly and when colonies are flatter ¹⁶⁰.

Individual-based modeling

Individual-based (also known as agent-based) models (IBMs) explicitly treat each individual cell as a discrete independent entity that interacts with other individuals and with its continuous environment. These models allow the introduction of individual variability (e.g., in growth rates, substrate uptake and secretions rates, cell mass, cell volume, etc.). This modeling formalism is a bottom-up approach where the dynamics and function of the whole system is governed by that of individual cells in their pursuit of optimal fitness ¹⁶¹. While the biomass spreading is modeled using the discrete individual-based approach (where cells are modeled as spheres spreading only when they get too close to each other), changes in concentration of soluble substrates in the continuous environment can be modeled using reaction-diffusion equations. Therefore, in contrast to previously described PDE models, here reaction-diffusion models are used only to follow the dynamics of the shared compounds rather than microbial biomass. The discrete and continuous models are then integrated numerically using the so-called “hybrid Eulerian–Lagrangian approach” ¹⁶².

These models have been employed extensively to analyze microbial interactions ^{163; 164; 165; 166; 167; 168; 169}. For example, Nadell et al ¹⁶⁵ used a two-dimensional individual-based model to uncover how cooperative and cheater cells can spontaneously segregate from each other in space as the size of a biofilm colony expands. This spatial segregation allows cooperative cells to preferentially interact with other cooperative cells thereby avoiding exploitation by cheaters and favoring the evolution of cooperation. This same modeling framework was used in a later study ¹⁶⁷ to assess how addition of a new species to a biofilm microbial community will affect the evolution of cooperation. Momeni et al ¹⁶⁸ also used an individual-based model to systematically explore how different types of ecological interactions affecting the fitness of species can lead to distinct patterning in three-dimensional communities grown from two fluorescently-marked populations of cells initially distributed randomly on top of a surface. This model predicted that interactions benefiting at least one population could allow initially disparate partner ratios to converge over time. Furthermore, it revealed that strongly cooperative cells are inter-mixed by forming patches successively accumulating on top of each other.

Genome-scale metabolic network modeling

All the modeling approaches described so far aim at predicting the dynamics of microbial communities based on the description of the abundance of different species and on a crude description of how each species affects other species. Interaction terms, described for example as a matrix in Equation 1 or Equation 4, constitute a simplified abstraction of inter-species interactions driven by the complex network of molecular processes that take place within individual cells. Mathematical modeling of intracellular networks is a growing research area, and one of the pillars of systems biology. The question is whether and how one can build a bridge between systems biology and microbial ecology in order to study

ecosystem-level function and dynamics by modeling the detailed wiring present within each cell.

A major progress in this direction has been made possible, in the last decade, by the rapid advances in the construction of genome-scale stoichiometric-based models of metabolism. Each model consists of a compilation of all biochemical reactions occurring in an organism derived from its annotated genome. These models also typically contain a fictitious reaction (referred to as *biomass reaction*) whose reactants are precursors essential for cellular growth and whose stoichiometric coefficients correspond to the relative contributions of these precursors to the cell's dry biomass. The flux of this reaction is considered to be an indicative of cell's growth capacity. Genome-scale stoichiometric models of metabolism are now available for a wide variety of organisms, ranging from bacteria to archaea and plants^{170; 171; 172; 173}.

Flux Balance Analysis (FBA)¹⁷⁴ is a mathematical modeling approach that utilizes these stoichiometric models to analyze how cells allocate environmentally available resources for homeostasis and reproduction. It is capable of making quantitative predictions of intracellular reaction fluxes, the export and secretion rates of metabolites and the cell's growth rate under the pseudo steady-state without requiring any kinetic parameters. Toward this end, a core assumption in FBA is that metabolic fluxes in the cell are close to a predictable optimum (e.g., maximum biomass production) describing a state achieved by the cell through evolutionary adaptation (e.g., adaptation toward maximum growth). This optimality criterion is formulated as a linear programming problem (for a single-species system):

$$\begin{aligned} & \text{maximize} && v_{biomass} && [FBA] \\ & \sum_{j=1}^N S_{ij} v_j = 0 && \forall i \in I, && (6) \end{aligned}$$

$$\begin{aligned} & LB_j \leq v_j \leq UB_j && \forall j \in J, \\ & v_j \in \mathbb{R} && \forall j \in J, && (7) \end{aligned}$$

where I and J denote the set of metabolites and reactions in the network, S_{ij} is the stoichiometric coefficient of metabolite i in reaction j (an entry of the stoichiometric matrix), LB_j and UB_j are lower and upper bounds on the flux of reaction j , respectively, v_j denotes the flux of a reaction j serving as optimization variables and $v_{biomass}$ is biomass production flux. Constraint (6) represents steady-state mass balance for each metabolite in the network and Constraints (7) impose lower and upper bounds for each reaction flux. In addition to the maximization of biomass flux, other studies have explored alternative objective functions¹⁷⁵ or the sampling of all flux values irrespective of any optimality principle¹⁷⁶. FBA has been experimentally tested for several systems, and successfully used for model-driven biological discovery as well as for a variety of biomedical and biotechnological applications (see^{177; 178; 179; 180} for comprehensive reviews).

The ability to model the metabolism of an organism at genome-scale paved the way for an unprecedented opportunity to transition from phenomenological modeling (e.g. LV

equations) to mechanistic modeling of microbial communities at genome-scale resolution. This enabled researchers to ask many questions that could not be directly addressed using other modeling approaches: Can one infer, instead of assuming, inter-species interaction networks from intracellular metabolic networks (see Fig. 3)? Can one predict whether compounds secreted by one organism could be used by a different organism? Can these metabolic interactions lead to an overall efficient resource utilization? How often do cross-feeding or competition arise? These questions spurred the development of metabolic models for simple multi-species microbial systems. These models evolved from steady-state analysis to dynamic and spatiotemporal analysis of microbial communities (see Table 2 and Fig. 4).

Steady-state models—Metabolic modeling of microbial communities was pioneered by Stoliar and colleagues¹⁸¹ who reconstructed a stoichiometric metabolic model of a simple mutualistic microbial community consisting of *Desulfovibrio vulgaris* and *Methanococcus maripaludis*. This analysis treated a multi-species community analogously to multi-compartment metabolic models of eukaryotes, such as *Saccharomyces cerevisiae*^{182; 183}. In these eukaryotes models, multiple organelles are modeled by defining suitably labeled compartment-specific metabolites and reactions, and adding transport reactions across compartments, as dictated by the knowledge of diffusion or transporters. In a community-level model, members can be similarly treated as compartments embedded in a meta-compartment that represents the shared environment. Formally, the stoichiometric matrices of individual species can be combined with each other in a larger block matrix, to construct a community-level stoichiometric matrix. One subtle aspect of implementing FBA simulations for a microbial community based on this compartment-based stoichiometry is the identification of an appropriate objective function. In the *Desulfovibrio-Methanococcus* model, the widely-employed FBA objective of biomass maximization was replaced with the maximization of a weighted sum of the biomass production fluxes for the community members (see Figure 4A). This community-level FBA problem can then be formulated as follows:

$$\begin{aligned} & \text{maximize} \quad \sum_{k=1}^K w^k v_{biomass}^k \\ & \sum_{j=1}^{N^k} S_{ij}^k v_j^k = 0 \quad \forall i \in I^k, k \in \{1, 2, \dots, K\}, \end{aligned} \quad (8)$$

$$LB_j^k \leq v_j^k \leq UB_j^k \quad \forall j \in J^k, k \in \{1, 2, \dots, K\} \quad (9)$$

$$\begin{aligned} -v_{EX_{i(e)}} + \sum_{k \in K^{export,i}} v_i^{k,export} - \sum_{k \in K^{uptake,i}} v_i^{k,uptake} = 0 \quad \forall i \in I^{shared}, \\ v_j^k \in \mathbb{R} \quad \forall j \in J^K, k \in \{1, 2, \dots, K\}, \end{aligned} \quad (10)$$

where all basic parameters and variables are analogous to those defined for the single-species FBA problem (Equations 6–7), except for the additional superscript label k , which denotes the community member k to which they belong. In addition, w^k is a pre-specified weight for the biomass flux of each community member in the objective function, I^{shared} is

the set of shared metabolites, and $K^{export, i}$ and $K^{uptake, i}$ are the sets of community members exporting and uptaking a shared metabolite i , respectively. Moreover, the variable $v_{EX_i(e)}$ represents the community's net exchange rate of shared metabolite i with the surrounding environment (a positive value implies export while a negative value implies uptake). Constraints (8) and (9) are similar to Constraints (6) and (7), respectively, for community member k and Constraint (10) represents a steady-state balance on the shared metabolite i in the extracellular compartment, where it is produced by some community members and is consumed by some others.

This compartment-based approach and its variants were used in many subsequent community metabolic modeling studies, ranging from the study of the gut microbiota^{184; 185; 186} and interactions between multiple tissues in human and plants^{187; 188; 189} to the overproduction of chemicals¹⁹⁰ and the study of a variety of other synthetic and natural multi-species systems^{22; 56; 191192}. For example, Heinken and Thiele¹⁸⁶ constructed a metabolic model of pairwise interactions between 11 representative microorganisms in the gut, in conjunction with a metabolic model of human small intestinal enterocytes subject to three different diets. Here, the host and microbes interact through a compartment simulating the intestinal lumen serving as a pool for nutrients derived from the diet and enterocytes. This study suggests the presence of species-specific commensal, parasitic, mutualistic, or competitive interactions among these microbes. In another effort, as a step toward modeling a whole plant system a multi-tissue model consisting of six different tissues related to root, stem and leaf were reconstructed¹⁸⁹. This model was utilized to probe the division of labor between the source and sink tissues assuming that all tissues work in concert for plant growth by minimizing energy usage (photon capture) as the objective function. Nonlinear objective functions to model microbial communities have been also explored by extension of the minimization of metabolic adjustment (MOMA) framework¹⁹³ to model synthetic crosstalk between pairs of auxotrophic *E. coli* mutants²². On another front, this multi-compartment approach was used to show computationally that it is in principle possible to induce a cross-feeding interaction between two microbial species by cultivating them in an appropriately designed medium⁵⁶. The search for such syntrophy-inducing media used a mixed-integer linear optimization approach to minimize the number of exchanged metabolites under the constraint that the biomass production by each species must be greater than a pre-specified threshold. Conceptually, this study illustrated the possibility of engineering interactions and communities by tweaking the environment rather than manipulating the genomes of the organisms. Other studies pursued an extension of FBA that directly accounts for species abundances based on the concept of balanced growth of microorganisms (community FBA)¹⁹⁴, or stoichiometric-based metabolic modeling of microbial communities independent of an optimality assumption, such as elementary mode analysis¹⁹⁵ or the analysis of (topological-based) metrics quantifying the degree of inter-species competitive/cooperative metabolic interactions^{196; 197; 198; 199; 200}.

One of the delicate issues, both conceptually and technically, in handling stoichiometric FBA-based models of communities is the interplay and trade-offs between individual organisms' objectives and potential ecosystem-level ones. An approach explicitly designed to address this interplay, and to aid in the development of engineered communities capable

of defined tasks is the multi-level multi-objective optimization method, OptCom²⁰¹. This is a nested optimization problem where some of the constraints are another optimization problem (referred to as inner-level problems)¹⁸⁰. OptCom couples distinct FBA problems for each community member (as inner problems with potentially conflicting objective functions), with a community-level objective function (as the outer-level objective). Species-specific inner problems are linked together through the inter-species interaction constraints in the outer problem that express the metabolite exchange among community members (i.e., Equation 10). The objective function of the outer-level problem is to optimize a community-level fitness function (see Figure 4B). Even though a universal community-level objective function is hard to identify, maximization of the entire community biomass has been used as a first approximation, assuming that the whole community works as a “super-organism” striving to maximize its growth²⁰¹. Alternatively, one can impose a desired bioengineering objective for the outer-level problem (such as the overall production of a given compound) to provide guidance on what type of interactions are needed to achieve this goal. OptCom was used to examine the addition of a new member to an existing community representative of those in subsurface anaerobic environments²⁰¹ and to model interactions between *Bifidobacterium adolescentis* and *Faecalibacterium prausnitzii* in the gut microbiota²⁰². It is worth noting that due to the fact that it relies on a multi-level optimization framework, OptCom is computationally more costly than the conventional single-level FBA formulations. Therefore, it is most appropriate for cases where different species- and community-level objective functions are sought. Furthermore, OptCom is not a suitable modeling framework for purely competitive microbial communities as a community-level objective function is biologically irrelevant for such systems.

Dynamic models—The FBA-based community-level approaches described above, while useful for several applications, have some inherent limitations, due to the nature of FBA. These limitations include the lack of explicit temporal scales, the incapacity to predict microbial species abundances as well as the need to define *a priori* a community-level objective function. Interestingly, a lot of these issues are naturally resolved by extending steady-state genome-scale methods to approaches that explicitly model the dynamics of microbial growth and of environmental metabolites^{203; 204; 205; 206; 207; 208; 209}. These methods (see²¹⁰ for a review) are mostly based on the extension of dynamic FBA (dFBA)²¹¹ for single-species systems (see Figure 4C). As a first attempt in this direction Zhuang et al²⁰³ proposed Dynamic Multi-species Metabolic Modeling (DMMM) framework, where cell densities are updated similarly to dFBA, while shared metabolite concentrations evolve by taking into account all species producing or consuming each shared metabolite:

$$\frac{dC_k}{dt} = \mu_k C_k \quad k=1, 2, \dots, K, \quad (11)$$

$$\frac{dC_i}{dt} = \sum_{k \in K^{export,i}} v_i^{k,export} C_k - \sum_{k \in K^{uptake,i}} v_i^{k,uptake} C_k \quad \forall i \in I^{shared} \quad (12)$$

Here, C_k and C_i denote the density of species k (g/l) and the concentration of shared metabolite i ($mmol.gDW^{-1}.h^{-1}$), respectively and μ_k (h^{-1}) is the specific growth rate of species k . Constraint (11) represents the exponential growth phase and Constraint (12) expresses the conservation of mass for the shared metabolite i in the extracellular space. These differential equations are discretized using a finite differences method, in which the specific growth rate μ_k for species k at each time point is determined by solving the corresponding FBA problem. Notably, in the solution of each FBA problem, uptake limits for the shared metabolites are determined by using kinetic expressions (such as Monod kinetics), which estimate the uptake flux upper bound as a function of the extracellular concentration of that metabolite.

DMMM was used to model competition between *Geobacter sulfurreducens* and *Rhodospirillum rubrum* in natural and manually perturbed anoxic subsurface environments²⁰³ and a co-culture of *Clostridium acetobutylicum* and *Clostridium cellulolyticum* for consolidated bioprocessing of lignocellulosic material²⁰⁴. An extension of the DMMM framework²⁰³ was also developed to identify the optimal acetate and Fe(III) addition rates for the effective uranium reduction by controlling the relative abundance of *Geobacter sulfurreducens* and sulfate-reducing bacteria²⁰⁵. In addition to addressing environmental issues, these approaches have been used for exploring possible ways to increase the production of chemicals and biofuels^{206; 207; 209; 212}. For example, a multi-species dFBA framework was combined with flux variability analysis (FVA)²¹³ to identify the biosynthetic capacity of a large number of synthetic two-species communities consisting of over 100 microbial species, where the co-culture enables the production of metabolites that cannot be produced by mono-cultures in the same growth medium²¹². The dynamic nature of this analysis revealed two phases where new biosynthetic capabilities emerge: One as soon as the organisms are introduced into the same medium and the other toward the end of the growth period in a nutrient-depleted growth medium.

In line with these advances in dynamic analysis tools, an extension of the OptCom procedure²⁰¹, termed d-OptCom (dynamic OptCom), was proposed for the dynamic multi-objective metabolic modeling of microbial communities²¹⁴ (see Figure 4D). Here, time-dependent conservation of mass for the biomass of community members and shared metabolites (Equations 11 and 12) as well as kinetic expressions determining the uptake rate of shared metabolites are included as new constraints in the outer-level problem. While, similarly to OptCom, the inner-level optimization problems are species-specific FBA problems subject to uptake rates determined by the outer problem, maximization of the total community biomass concentration was used as the outer-level objective function (instead of maximizing the sum of biomass fluxes in OptCom). d-OptCom was used to model the dynamics and biomass composition of synthetic two-species consortia composed of two cross-feeding *E. coli* mutants and that of a uranium-reducing community comprised of *Geobacter sulfurreducens*, *Rhodospirillum rubrum*, and *Shewanella oneidensis*²¹⁴. Notably, the use of community-level objective function allowed for the emergence of costly cooperation in the former case study, which cannot be captured by DMMM-like approaches. Nevertheless, in cases where a community-level objective is biologically irrelevant (e.g., for a pure competitive ecosystems) or uncertain, DMMM-like approaches are preferred since

they have lower computational cost and they allow the whole community's function and dynamics to emerge solely from the selfish behavior (i.e., growth maximization) of its constituent species.

Spatio-temporal models—As noted earlier, in addition to temporal variability, microbial communities generally display high spatial heterogeneity, due both the inhomogenous nature of the surrounding environment, and the locality of interactions between different microbes in the absence of vigorous mixing. By adding a spatial component to the community-level dynamic FBA concept described above, it is possible to explicitly model inter-species interactions in structured environments, such as layered biofilms, or colonies on a Petri dish. The development of such an integrated platform was the goal of a recent modeling framework termed COMETS (Computation Of Microbial Ecosystems in Time and Space)²¹⁵. The COMETS framework directly couples community-level dFBA with diffusion models to enable the spatio-temporal analysis of microbial communities using genome-scale metabolic models²¹⁵ (see Figure 4E). A heterogeneous environment is approximated by a spatially structured lattice. Each point in this lattice may contain an arbitrary number of species and different concentrations of shared metabolites. Simulations consist of two fundamental steps including (i) cellular growth and metabolite secretion/production at each lattice point modeled by a multi-species dFBA framework similar to DMMM²⁰³ and (ii) a finite difference approximation of the shared metabolites and biomass diffusion in the lattice. COMETS predictions were tested experimentally for two-species and three-species synthetic consortia involving *E. coli*, *Salmonella enterica* and *Methylobacterium extorquens*. COMETS enabled the study of how pairs of cross-feeding colonies may be affected by the interposition of a third colony in between them, highlighting the complex dependency of inter-species interactions on spatial organization. A modeling strategy similar to COMETS was used to model the emergence of acetate cross-feeding sub-populations in colonies of *E. coli* growing on an agar plate²¹⁶.

Current challenges in modeling microbial communities

A long-standing challenge in the model-driven analysis of microbial communities is the increase in demand for computational resources for complex and/or large-scale microbial communities. For example, such problems arise for individual-based models, where one typically deals with a considerably large number of individual cells, as well as for FBA-based methods, in which it may need to handle a large number of community members whose genome-scale metabolic models could contain of the order of a thousand reactions and metabolites. These issues become even more prohibitive if spatial heterogeneity is taken into account, especially in two- or three-dimensional discretized space. Due to these limitations both IbM and FBA studies that include spatial heterogeneity have been so far applied to small-scale (from micrometers to centimeters) environments^{163; 215; 217}. In addition to more powerful computers and more efficient core algorithms, these issues can be addressed by reducing the complexity of the problem in a number of ways. For IbMs, for example, a proposed solution has been to work with “super-individuals”, which are representatives of a large number of individuals with similar traits²¹⁸. This approach, however, weakens the intrinsic advantage of IbMs, which is to capture variability at the individual cell level¹⁶².

Similarly to super-individuals, one can reduce the number of necessary genome-scale metabolic models in a complex microbial community by using the concept of “a functional guild”. A guild is a group of organisms that use the same class of environmental resources in the same manner²¹⁹. Instead of considering all species in a community one could work with a few representative guilds without the significant loss of accuracy. This strategy has been used before in metabolic modeling of microbial communities from Octopus and Mushroom Springs in Yellowstone National Park^{195; 201}. Alternatively, it may prove useful to build and employ, for example, genus-level models containing all or most abundant metabolic reactions from their member species.

A possible strategy to address high computational demands for modeling large-scale heterogeneous environments is creating a “look-up” table. This table contains precomputed solutions for a computationally manageable list of combinations of environmentally-relevant cell and nutrients distributions in space (e.g. a matrix of possible values for nitrogen and carbon source concentrations in the medium). This approach has been pursued for single-species FBA simulations in a large heterogeneous environments²²⁰ but can be easily extended to both multi-species FBA simulations or IbMs. In this way, instead of directly solving a FBA problem for each species at each time and grid point, the required reaction fluxes are updated simply by interpolating within this loop-up table. Future developments could use combinations of pre-computed solution tables and new calculations, as dictated by environmental conditions.

As a complementary strategy to reduce the computing time, one can invest in the development of efficient high-performance numerical and computational methods that can alleviate the CPU demand of synthetic ecology algorithms. An example of progress in this direction has been recently reported through the development of a software tool termed Biocellion²²¹ for the individual-based modeling of large communities containing millions to billions of cells. By utilizing efficient numerical and parallelization techniques, it was possible to reduce the required CPU time for a case study on pattern formation in microbial communities¹⁶⁸ from a week to a few hours²²¹.

In addition to computational complexity, the development of stoichiometric-based algorithms for synthetic ecology needs to address a number of other challenges. Some of these challenges are rooted in our limited capacity to translate genomes and metagenomes into complete and accurate lists of functions for individual species in a community. Despite the availability of automated metabolic model reconstruction pipelines such as MetaFlux²²², ModelSeed¹⁷¹ and the RAVEN toolbox²²³, all these algorithms can only partially compensate for the lack of knowledge of gene function. These algorithms provide only first-draft reconstructions, whose conversion to a computationally reliable model still requires extensive and time-consuming manual curation. New algorithms, e.g. using likelihood-based gene annotation and gap filling²²⁴ can alleviate to some extent the need for manual curation, but they do not fully resolve the need for manual curation. A major leap towards resolving this problem at its root may result from coordinating big community endeavors for the prediction and experimental validation of individual gene functions, as pioneered for example by the COMBREX consortium²²⁵.

More broadly, the granularity at which taxonomy should be represented in stoichiometric metabolic models constitutes a technically and conceptually fascinating question. Brought to the extreme, one could ask whether it is possible to build community-level stoichiometric models as “soups of enzymes” irrespective of the knowledge of what reaction is performed by what species, similar to previously proposed models of biosphere-level metabolism^{226; 227} (also see¹⁹⁵). One of the advantages of this approach would be the possibility of building community-level models straight from metagenomic sequencing data. Such metagenomic-based metabolic models could indeed conveniently capture the entire repertoire of functions of the community as a whole. However, the lack of metabolite and reaction compartmentalization in these models could lead to significant complications or predictions errors, for example due to the disruption of membrane gradient-mediated processes²²⁸.

Conclusions and future perspectives

The emergence of synthetic ecology has provided an attractive alternative to engineering single-species systems for a wide variety of tasks ranging from the discovery of key ecological features of natural microbial communities to the targeted design of synthetic consortia for biotechnological and biomedical applications. While several examples of promising small synthetic ecosystems have demonstrated the feasibility of this approach, the most exciting opportunities of synthetic ecology require coping, in a more systematic way, with the complexity of microbial systems, and in particular with a hierarchy of nested networks, from those within microbes to those between them. Predictive mathematical modeling approaches can play a key role in addressing these challenges: they can help decipher how inter-species interactions in natural microbial communities govern community dynamics and evolution, and they can translate harvested knowledge into methods for the design of new communities with desired properties. The successful development of these predictive tools will require both revisiting existing modeling approaches to cope with the inherent complexity of microbial ecosystems, and inventing new ones.

When combined with engineering principles, these modeling approaches have a great potential in complementing experimental efforts (by reducing or prioritizing costly experiments) to address a number of current challenges in biotechnology, and biomedicine. The most immediate application is the rational design of microbial communities capable of performing a desired task. This brings to the forefront the need to develop efficient “computational consortia design” algorithms similar to strain design algorithms for single-species systems, which have been successfully applied to bioenergy and metabolic engineering applications (see¹⁸⁰ for a review of these tools). For example, as mentioned before, mathematical modeling tools such as OptCom²⁰¹ and d-OptCom²¹⁴ can be easily re-purposed/adjusted for engineering applications by mathematically describing the desired engineering objective as the outer-level objective function, while the inner-level problems simulate species-specific fitness criteria as before. There are also several ways of incorporating engineering interventions in these and other similar frameworks. For example, one can use binary variables to determine whether a gene in a specific community member must be knocked out or whether a community member or a nutrient in the growth medium should be removed or if a new one should be added, to optimize the desired engineering

objective^{56; 201; 214}. Moreover, new computational approaches, combining ideas from synthetic biology, metabolic engineering and ecosystem-level modeling could enable the concurrent design of environments and strains to achieve a desired behavior. At a finer scale, taking into account the interplay between multiple biological processes contributing to the ecological behavior of a community member can enhance the predictive power and the scope of modeling frameworks. For instance, recent advances in whole-cell modeling²²⁹ and integrated metabolic-expression (ME) models²³⁰ have enabled capturing several ecologically relevant biological processes that are not addressed by stoichiometric-based metabolic models such as gene expression, regulation and signal transduction. Although the current studies using these models have focused on individual species so far^{231; 232}, it is likely that these models will play a crucial role in constructing more predictive models of microbial communities.

Another important aspect for which modeling can play a key role in guiding the design of synthetic communities is the assessment of synthetic consortia stability. Similarly to engineered single species, instability is a major problem associated with engineered consortia, either based on wild-type or on genetically modified strains. Many existing consortia design strategies rely on the assumption that interactions among community members are fixed. However, patterns of inter-species interactions can be significantly modulated by environmental changes, and can change during the course of evolutionary adaptation²³³. Thus, understanding processes that may guarantee stability and resilience in presence of these changes would require taking explicitly into account the volatility and context-dependence of interactions. Knowledge from the large body of evolutionary and ecological theory literature dedicated to addressing the problem of stability and the evolutionary fate of inter-species interactions in communities (e.g.,^{234; 235; 236; 237}) could be used to inform computational consortia design tools to arrive at evolutionary stable communities. The importance of this integration has been recognized before²³⁸ but has not been realized so far. Given that natural microbial communities are generally stable and robust to perturbations, some studies suggest the use of a top-down approach to address the stability issue. This was achieved by using artificial selection to sequentially screen a natural microbial community and arrive at a refined community capable of efficiently and stably performing the desired function^{239; 240}. For example, this approach was used to select for an efficient 3-chloroaniline degrading community²⁴⁰. The promise of artificial selection was also shown by using individual-based modeling in a later study²⁴¹.

Even though both bottom-up and top-down approaches use different formalisms and assumptions, a synergy between these two could have great potential and applications. For example, a community obtained through sequential screening or laboratory evolution experiments could be later scrutinized to reveal its key species and interactions. Moreover, in analogy to a process referred to as “refactoring” in synthetic biology²⁴², one could build a more tractable, standardized and simplified version of such a community. Refactoring a microbial community would entail redesigning a screened natural community from the bottom up by systematically eliminating undesired and poorly understood organisms and interactions, and replacing them with well-characterized ones. A refactored community could achieve the same functionality of the original one, but with enhanced stability and tractability. Thus, similar to the standardization of biological parts (biobricks) for synthetic

biology²⁴³, we envision that the field of synthetic ecology may similarly benefit from a registry of standard “ecological parts”. This registry would catalogue well-characterized ecological parts such as microorganisms and small ecological modules (motifs) with defined functions, inputs/outputs, interaction properties and, ideally, evolutionary characteristics. Such a registry would facilitate the experimental construction of increasingly complex consortia and could be complemented with new modeling tools to identify the required building blocks and the ecological circuit connectivity that bring about a desired function.

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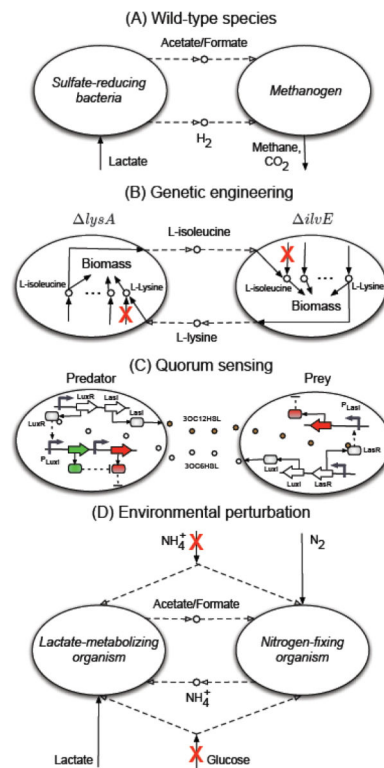
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Highlights

- Synthetic microbial consortia are implicated in environmental sustainability and biomedicine
- Mathematical models are an essential tool for the rational design of synthetic ecosystems
- A main goal is to infer community outputs and interactions based on genomes
- Faster computation, data/model integration and standardization needed for progress

**Fig. 1.**

Examples of various mechanisms for establishing a synthetic microbial community. Dashed and solid arrows in (A), (B) and (D) denote interspecies metabolite exchanges and the measurable uptake/export of metabolites by community, respectively. (A) Co-culturing wild-type species under conditions resembling naturally occurring environments. An example is the association between sulfate-reducing bacteria and methanogens. Here a sulfate-reducing bacterium utilizes lactate as the sole carbon source and produces acetate/formate, which is used by a methanogen that is incapable of using lactate. The methanogen bacteria scavenge hydrogen in return thereby providing a thermodynamically favorable condition for the growth of sulfate-reducing bacteria^{25; 28}. (B) Using genetically engineered species to induce metabolite exchanges. An example is the association between two (or more) mutant strains where each strain relies on its partner for the essential amino acid it cannot produce on its own^{22; 23}. (C) Using synthetic genetic circuits to induce interactions through quorum sensing. Generally, each species may produce a signaling molecule that activates or represses the transcription of one (or more) gene(s) in another species. The example shown here is a predator-prey system⁴⁸, where empty and filled circles represent signaling molecules 3OC6HSL and 3OC12HSL (synthesized by LuxI and LasI), respectively, solid arrow denote protein production, dashed arrows represent activation and inhibition/killing. At low density of the prey, the predators die due to the constitutive expression of a killer protein (shown by red). At high concentration of the prey, 3OC6HSL activates LuxR in predators, which in turn induces the expression of antidote gene (shown by green) thereby rescuing the predators. At the same time, 3OC12HSL (produced by predators) activates LasR in preys, which induces the expression of a killer protein. (D) Using environmental perturbations. Here, we show a hypothetical example, where each

microorganism can grow on its own in the presence of glucose and ammonium. However, in the absence of these two compounds they rely on each other for the carbon and nitrogen source as only one can fix nitrogen and only one can metabolize lactate. The potential for such interactions between *Desulfovibrio vulgaris* and *Methanococcus maripaludis* has been reported using computational modeling ⁵⁶.

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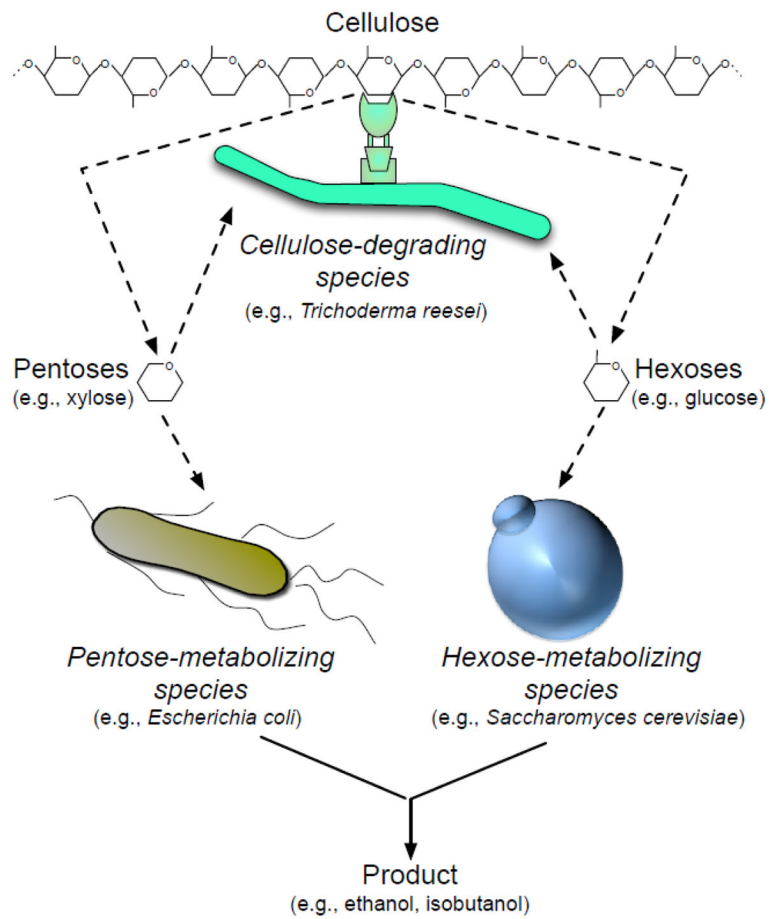


Fig. 2. Consortia-based conversion of lignocellulosic feedstock to biofuels by division of labor. One organism is proficient in the degradation of lignocellulosic feedstock into soluble sugars (e.g., *Trichoderma reesei* and *Clostridium cellulolyticum*), one in metabolizing pentoses (e.g., *Escherichia coli*) and the other in metabolizing hexoses (e.g., *Saccharomyces cerevisiae*). See also Table 1.

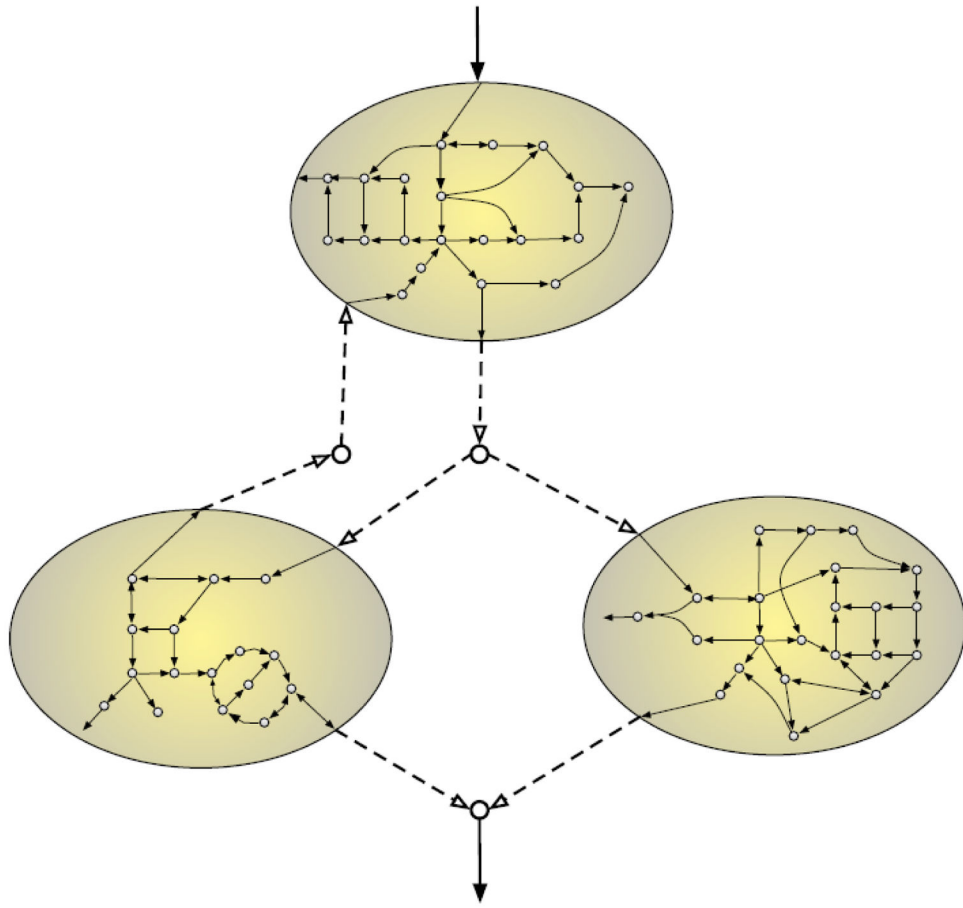


Fig. 3. Metabolic modeling of microbial communities provides an opportunity to infer, rather than assuming, inter-species interactions network from the intracellular metabolic networks of community members.

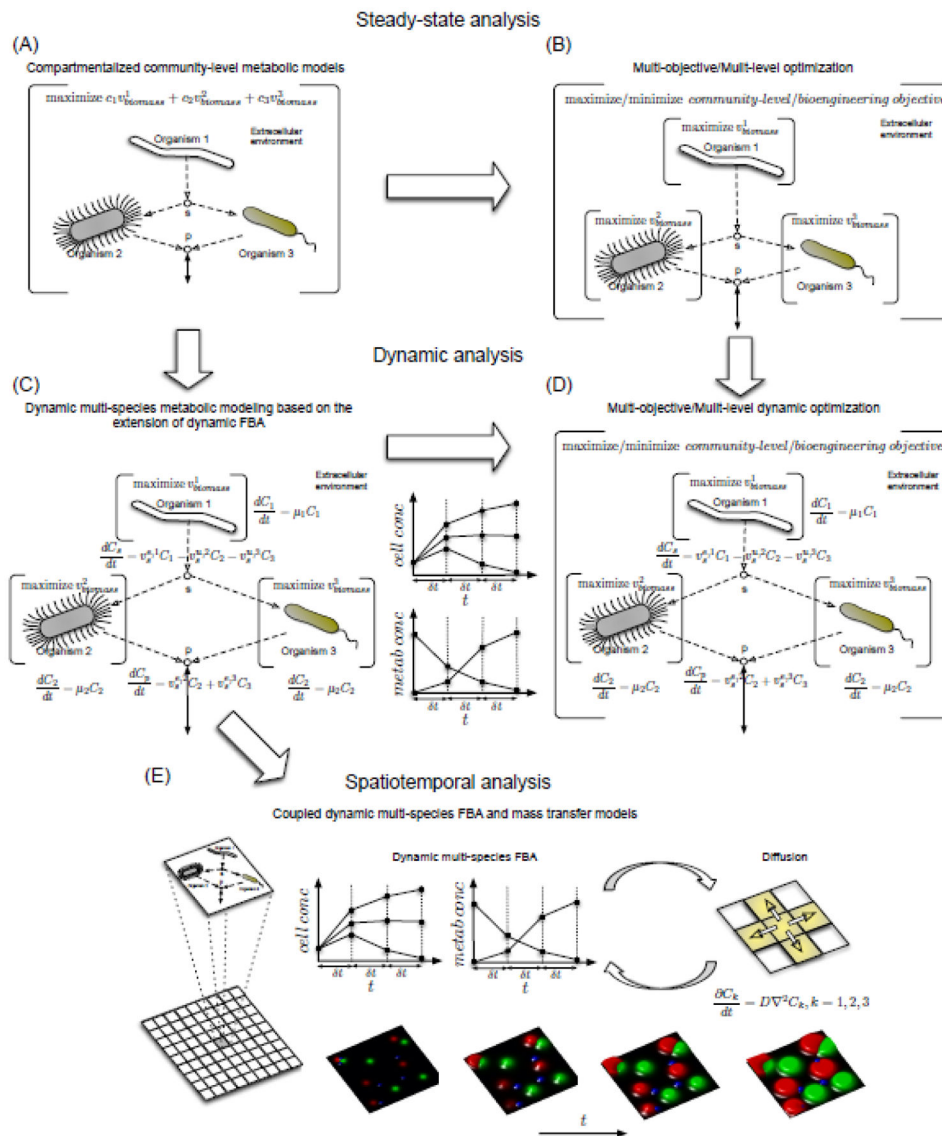


Fig. 4. Evolution of the metabolic modeling of microbial communities from steady-state to dynamic to spatio-temporal analysis. Brackets represent an optimization problem, empty circles represent shared metabolites (labeled as s and p), dashed arrows represent inter-species metabolic extracellular interactions (metabolite exchanges) and solid arrows denote the uptake and export of metabolites. (A) Steady-state analysis using compartmentalized community-level metabolic models with the objective function being a weighted sum of biomass fluxes of community members ¹⁸¹. (B) Steady-state analysis using a multi-objective and multi-level optimization procedure (a nested optimization problem, where some of the constraints are another optimization problem referred to as inner-level problems ¹⁸⁰). Here, a separate FBA problem is defined for each community member serving as inner-level optimization problems and capturing species-level fitness criteria. These FBA problems are integrated by using constraints in the outer-level problem representing metabolite exchanges among community members. The objective function of the outer-level problem captures a

community-level fitness criterion (e.g., maximization of the total community biomass) or a desired community-level bioengineering objective (e.g., the overproduction of a desired compound) ²⁰¹. (C) Dynamic analysis by using the dynamic multi-species metabolic modeling ²⁰³ based on an extension of dynamic FBA for single species ²¹¹. Here, biomass and shared metabolite concentrations are dynamically updated using a finite difference approximation of the conservation of mass equations. A separate FBA problem is solved for each species at each time point to determine their growth rate. (D) Dynamic analysis using a dynamic multi-level and multi-objective optimization procedure that combines procedures in (B) and (C) ²¹⁴. Here, species-level FBA problems are coupled with a community-level or a desired bioengineering objective function using the conservation of mass equations. (E) Spatio-temporal analysis by the direct coupling of dynamic metabolic analysis in (C) and diffusion models ²¹⁵.

Table 1

Examples of consortia-based cell factories to produce biofuel from lignocellulosic material.

(Ligno)cellulose- degrading species	Pentose fermenting species	Hexose fermenting species	Product(s)	Reference
<i>Trichoderma reesei</i>	<i>Escherichia coli</i>	<i>Escherichia coli</i>	Isobutanol.	Minty et al ⁸⁷
<i>Trichoderma reesei</i>	<i>Scheffersomyces stipitis</i>	<i>Saccharomyces cerevisiae</i> ,	Ethanol	Brethauer et al ⁸³
<i>Clostridium thermocellum</i>	<i>Clostridium beijerinckii</i>	<i>Clostridium beijerinckii</i>	Acetone, butanol and ethanol	Wen et al ²⁴⁴
<i>Clostridium thermocellum</i> and <i>Clostridium thermolacticum</i>	<i>Clostridium thermolacticum</i>	<i>Clostridium thermocellum</i>	Ethanol	Xu and Tschirner ⁸⁵
<i>Clostridium thermocellum</i>	<i>Thermoanaerobacter</i> strains (X514 and 39E)	<i>Thermoanaerobacter</i> strains (X514 and 39E)	Ethanol	He et al ⁸⁶
<i>Clostridium phytofermentans</i>	-	<i>Candida molischiana</i> or <i>Saccharomyces cerevisiae</i> cdt-1	Ethanol	Zuroff et al ⁵⁷

Table 2

A summary of various categories of community modeling approaches using genome-scale metabolic models.

Modeling formalism	Modeling condition	Type of optimization problem	Reference
Compartmentalized community- level metabolic models based on FBA	Steady-state	Linear programming	Stolyar et al ¹⁸¹ , Shoaie et al ¹⁸⁴ , Heinken and Thiele ¹⁸⁶ , Bordbar et al ¹⁸⁷ , Klitgord and Segre ⁵⁶ , Gomes de Oliveira Da' Molin et al ¹⁸⁹ , Bizukojc et al ¹⁹⁰ , Merino et al ¹⁹¹ , Nagarajan et al ¹⁹²
Compartmentalized community- level metabolic models based on MOMA	Steady-state	Quadratic programming	Wintermute and Silver ²²
(De-)Compartmentalized community-level metabolic models based on elementary mode analysis	Steady-state	NA	Taffs et al ¹⁹⁵ ,
Analysis of metabolic model- derived metrics quantifying the degree of cooperation and/or competition	Steady-state	NA	Zelezniak et al ¹⁹⁶ , Kreimer et al ¹⁹⁷ , Levy et al ^{198;200} , Borenstein and Feldman ¹⁹⁹
Community FBA based on the balanced growth of microorganisms	Steady-state	Linear/Nonlinear programming	Khandelwal et al ¹⁹⁴
Multi-level and multi-objective modeling	Steady-state	Nonlinear programming	Zomorodi and Maranas ²⁰¹ , El-Semman et al ²⁰²
Dynamic multi-species metabolic modeling based on the extension of dynamic FBA ²¹¹ for single species	Dynamic	Linear programming	Zhuang et al ²⁰³ , Salimi et al ²⁰⁴ , Hanly and Henson ^{206;207209} , Tzamali et al ²⁰⁸ , Chiu et al ²¹²
Multi-level and multi-objective dynamic metabolic modeling	Dynamic	Nonlinear programming	Zomorodi et al ²¹⁴
Direct integration of community-level dynamic FBA and diffusion models	Spatiotemporal	Linear programming	Harcombe et al ²¹⁵ , Cole et al ²¹⁶