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A guide to deciphering microbial interactions and metabolic fluxes in microbiome communities

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Microbiomes occupy nearly all environments on Earth. These communities of interacting microorganisms are highly complex, dynamic biological systems that impact and reshape the molecular composition of their habitats by performing complex biochemical transformations. The structure and function of microbiomes are influenced by local environmental stimuli and spatiotemporal changes. In order to control the dynamics and ultimately the function of microbiomes, we need to develop a mechanistic and quantitative understanding of the ecological, molecular, and evolutionary driving forces that govern these systems. Here, we describe recent advances in developing computational and experimental approaches that can promote a more fundamental understanding of microbial communities through comprehensive model-based analysis of heterogeneous data types across multiple scales, from intracellular metabolism, to metabolite cross-feeding interactions, to the emergent macroscopic behaviors. Ultimately, harnessing the full potential of microbiomes for practical applications will require developing new predictive modeling approaches and better tools to manipulate microbiome interactions.

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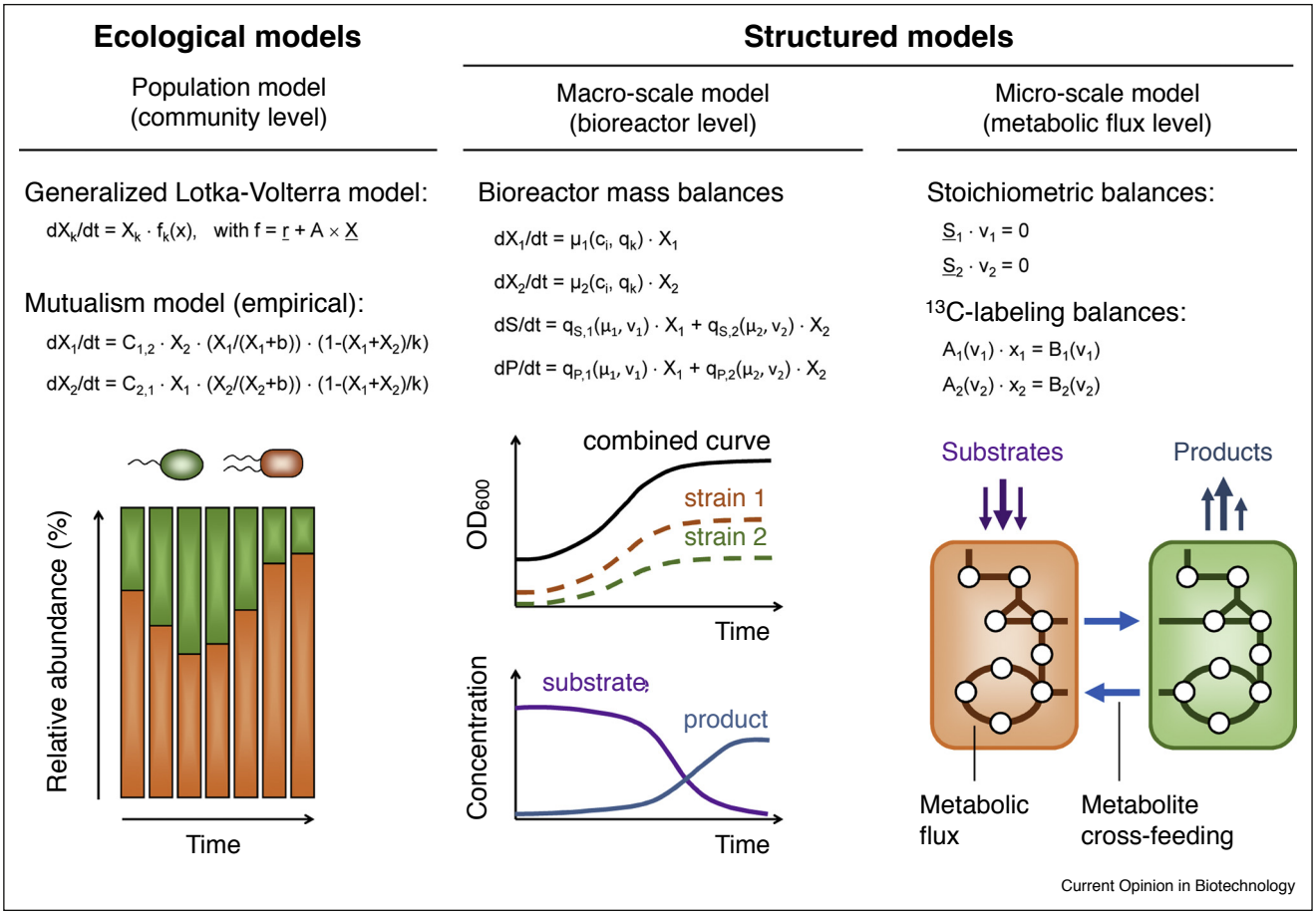
Introduction

Microbial communities are ubiquitous in nature and their impact on Earth's ecosystems is pervasive, from biogeochemical cycles to agriculture and human health [1–3]. Studying microbial communities and their impact on natural environments is therefore essential to understand living systems [4,5]. Moreover, microbial communities have a wide range of practical applications, including in biodegradation of organic chemicals [6], conversion of toxic compounds [7,8], and production of biofuels and other products from single or multiple substrates [9•]. Microbial consortia carry out efficient bio-transformations that result from multiple complementary metabolic systems working together [10,11]. The capabilities of multi-microorganism systems often cannot be predicted by the sum of their parts [12]. Rather, complex interactions at multiple levels result in improved overall performance of these systems [7]. The emerging field of microbiome synthetic and systems biology promises the assembly of complementary metabolic pathways into functional systems [13–17,18•], where the diversity of metabolic capabilities and the ability to exchange metabolites dramatically increases the possible metabolic space [17,19]. This alleviates limitations encountered in single-organism systems [14,20••]. For example, integration of complementary metabolic systems can result in more efficient substrate conversions [7], make communities more robust to fluctuations [16], and accomplish transformations that would be thermodynamically infeasible with single-organism systems [21,22].

Given the prevalence and importance of microbiomes in nature, much effort has been invested in the past decade to unravel the members, structures, functions, interactions, and governing principles of microbial communities [23] (Figure 1). With the advent of next-generation sequencing, metagenomics first emerged as an important tool in the study of microbial communities. It allowed quantitative analysis of the diversity, composition, and dynamics of these systems [24]. Statistical modeling approaches, such as multiple linear regression and multi-dimensional cluster analysis, were then used to interpret the wealth of metagenomics data to identify microbial community trends and correlations between metagenomics data and other observable system variables [25–29]. These statistical modeling approaches provided new hypotheses regarding potential interactions and functions of communities. However, metagenomics data alone could not predict causal relationships in microbial communities. For this, new computational models were needed that allowed a more systematic and rigorous interpretation and interrogation of the huge amount of heterogeneous data that was generated [30,31]. Once a model was constructed, systems biological properties could be analyzed by comparing model simulations with experimentally observed data. Mathematical models of microbial communities thus provided critical tools for generating and testing biological hypotheses to better

Figure 1



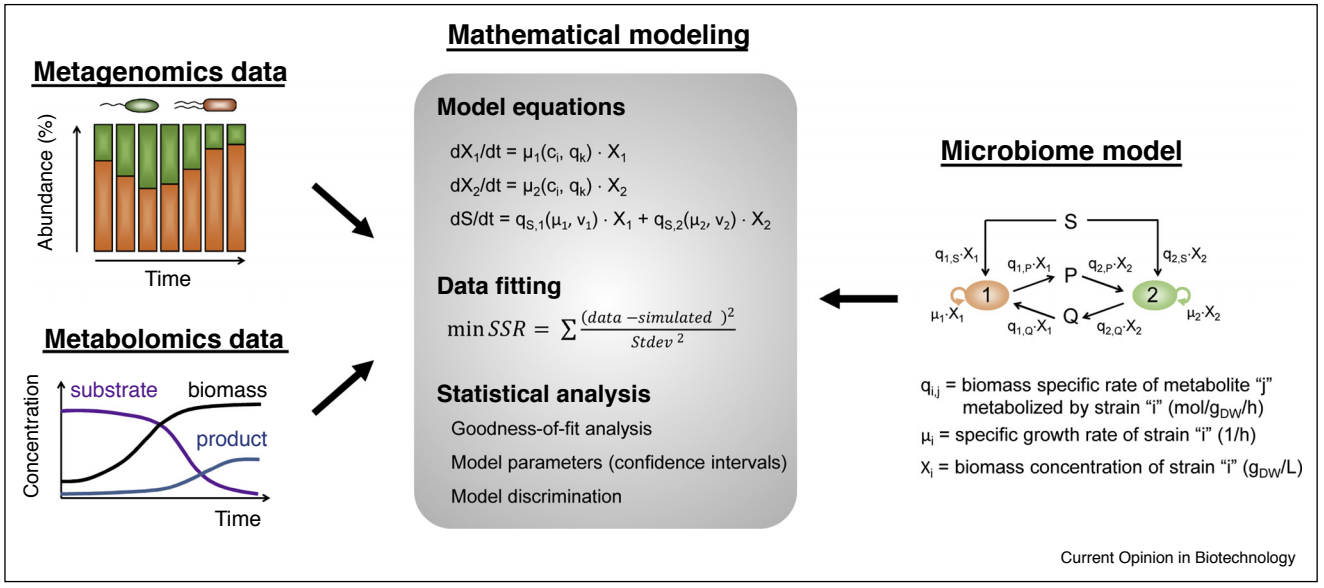
The two primary approaches used to model microbial communities are ecological models and metabolic models. Ecological models focus on describing population dynamics and evaluating the stability of communities under various conditions or perturbations. Metabolic models focus on cellular metabolism and explicit cross-feeding interactions to predict population properties of microbiomes. Recent advances in ^{13}C-metabolic flux analysis also allow intracellular metabolic fluxes and metabolite cross-feeding rates to be measured experimentally in co-cultures.

understand and predict the dynamics and interactions among community members [32] (Figure 2). Looking forward, mathematical models will play an increasingly important role in identifying new targets for the design and optimization of synthetic microbial communities for practical applications in biotechnology, agriculture and medicine [33]. In this review, we provide an overview of the wide range of computational and experimental techniques that have been developed to analyze microbial communities and highlight recent advances that can further enhance our understanding of these complex biological systems.

Ecological models describe population dynamics in communities

The two primary approaches that have been applied to model microbial communities are ecological models and metabolic (e.g. genome-scale) models. Ecological models focus on species abundances and how they change over time given certain relationships between the species. In these models, species abundances are the only system variables, and thus all interactions between community members must be described by suitable mathematical functions to capture the wide range of possible interactions, including mutualistic, neutral, and competitive interactions. In practice, ecological models are often set up as a set of differential equations that can be studied as a dynamic system. The advantage of ecological models is that they can directly use available time-series species abundance data to quantify various properties of communities, such as the stability of biological systems under various conditions and perturbations. The best known ecological model is the generalized Lotka-Volterra model (gLV, also known as predator-prey model), first proposed

Figure 2



Mathematical models of microbial communities provide critical tools for generating and testing biological hypotheses. New computational approaches and software tools are needed that can promote fundamental understanding of microbial communities through comprehensive model-based analysis of omics data sets across multiple scales, from intracellular metabolism, to metabolite cross-feeding interactions between cells, to the emergent behaviors, structures and functions of microbial communities.

in 1920s. In this model, the dynamics of populations of any number of species N (X_1, X_2, . . . , X_N) is described by the following set of ordinary differential equations:

$$\frac{dX_i}{dt} = X_i \cdot \left(\mu_i + \sum A_{i,j} \cdot X_j\right)$$

Here, μ_i is the growth rate of species i, and $\mathbf{A} = [A_{i,j}]$ is the 'community interactions matrix', where the fields represent interactions between species, such that positive $A_{i,j}$ values capture positive interactions (e.g. mutualism), while negative values represent negative interactions (e.g. competition). By integrating the gLV equations, temporal dynamics of populations can be simulated under various interaction assumptions, or perturbations. Inversely, by fitting time-series data of species abundances (e.g. time-resolved metagenomics data) to the gLV model, interaction parameters can be estimated [34]. In the past decade, important systems level properties of microbial communities have been investigated using gLV models such as microbiome stability, resistance to perturbations, and the speed at which systems can reach steady states [30,32,35]. While gLV models have proven their value in explaining global systems properties of communities, they also have important limitations [36]. A notable disadvantage of ecological models is that only certain simplified aggregate interactions between organisms are possible. These interactions are often static and do not relate directly to cellular characteristics such as the metabolic state of cells. Since population abundances are the only system variables in these models, all interactions must be described as a function of these variables. As a result, ecological models typically cannot capture cross-feeding interactions of nutrients between species and provide no information regarding metabolism within each species. Moreover, biochemical production of products and community responses to various substrates are not easily captured by ecological models.

Ecological models of syntrophic co-cultures

Cross-feeding of nutrients is a key characteristic of microbial communities, whereby certain members of the community feed off the products of others, and vice versa [10,11,37]. To better understand the interactions in microbial communities, and in the future to allow rational engineering of microbiomes, it is critical to elucidate syntrophic phonotypes [12,38•,39,40••,41]. For this purpose, auxotrophic *Escherichia coli* strains provide useful model systems to study syntrophic interactions. In a pioneering study, Wintermute *et al.* [12] evaluated 1035 co-cultures of 46 conditionally lethal auxotrophic *E. coli* strains. While none of the 46 strains could grow in minimal medium with glucose as the sole substrate, a surprisingly large fraction (17%) of the paired co-cultures were able to grow on glucose. It was hypothesized that the *E. coli* mutants complemented one another's growth by

cross-feeding essential metabolites, possibly amino acids, although this was not experimentally verified. To describe the observed dynamics a simple empirical ecological model was used to capture the essential features of these co-cultures:

$$\frac{dX_1}{dt} = C_{1,2}\cdot\left(\frac{X_2}{X_1+X_2}\right)\cdot\left(1-\frac{X_1+X_2}{k}\right)$$

$$\frac{dX_2}{dt} = C_{2,1}\cdot\left(\frac{X_1}{X_1+X_2}\right)\cdot\left(1-\frac{X_1+X_2}{k}\right)$$

Here, X_1 and X_2 represent the respective strain abundances, $C_{1,2}$ and $C_{2,1}$ quantify the synergistic cooperation on the part of the two strains, and k is a logistic carrying capacity of the batch culture related to the initial glucose concentration and overall biomass yield. With this model, the population dynamics and final co-culture compositions could be predicted. Later, Mee *et al.* [42] investigated a smaller set of 14 amino acid auxotrophic *E. coli* strains in dual and triple co-cultures. To quantitatively model the co-cultures, slightly revised model equations were used that better captured the observed dynamics according to the authors:

$$\frac{dX_1}{dt} = C_{1,2}\cdot X_2\cdot\left(\frac{X_1}{X_1+b}\right)\cdot\left(1-\frac{X_1+X_2}{k}\right)$$

$$\frac{dX_2}{dt} = C_{2,1}\cdot X_1\cdot\left(\frac{X_2}{X_2+b}\right)\cdot\left(1-\frac{X_1+X_2}{k}\right)$$

These equations were then generalized to describe triple co-cultures, and eventually to model a complex 14-member consortium. Through these investigations, the authors identified that biosynthetically costly amino acids tended to promote stronger cooperative interactions, and more generally, that amino acid auxotrophies could be an evolutionarily optimizing strategy that reduces biosynthetic burden while promoting cooperative interactions between bacteria in microbiomes [42,43].

Genome-scale metabolic models of microbial communities

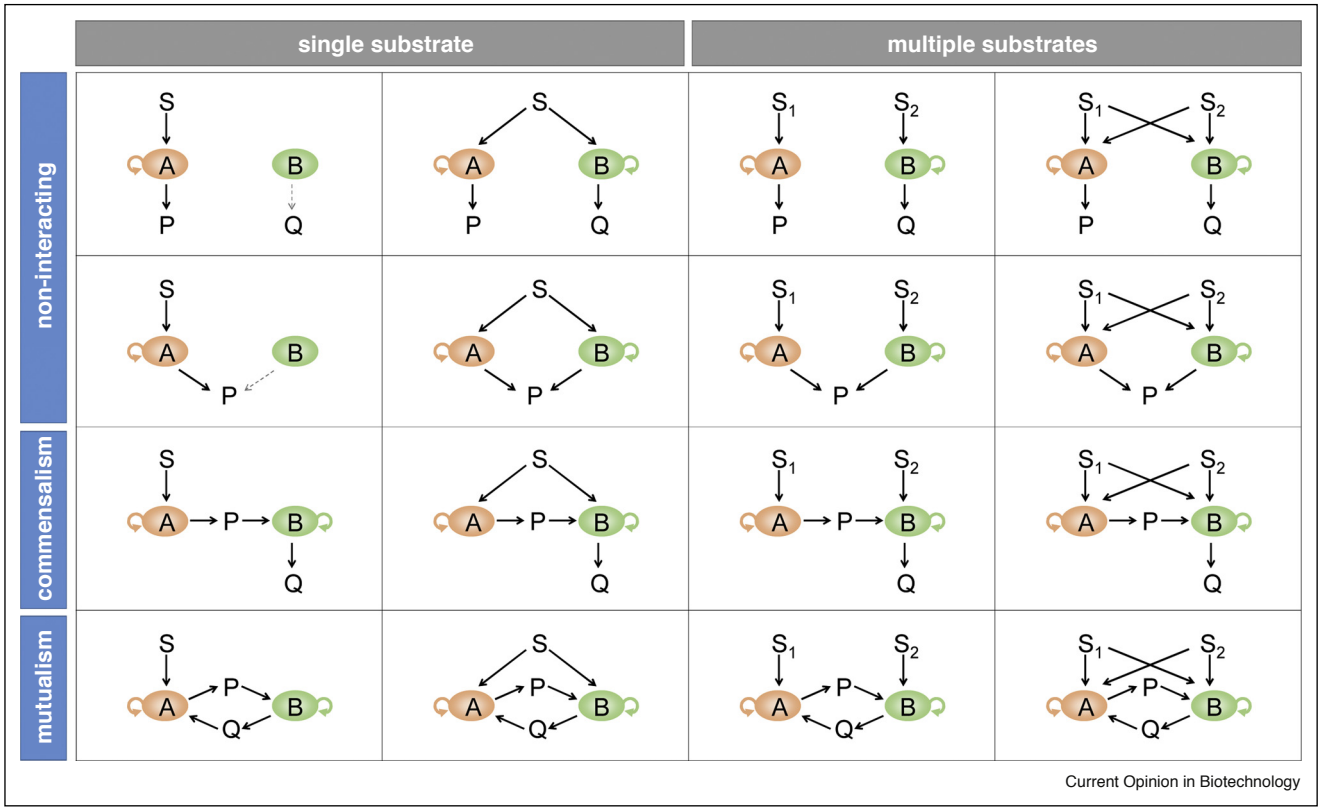
In contrast to ecological models, genome-scale metabolic models focus on cellular metabolism and explicit cross-feeding interactions to predict population properties of microbiomes [44,45]. These models take advantage of the extensive knowledge accumulated over the past two decades regarding metabolic reactions from whole-genome sequencing. In these models, interactions between species are specified at the molecular level by allowing cross-feeding of specific metabolites between community members [46••]. The central mathematical framework for analyzing microbial communities are constraint-based modeling and optimization techniques such as flux balance analysis (FBA) [47], in which a given cellular or community objective is optimized to estimate intracellular metabolic fluxes and cross-feeding rates. Various objective functions have been proposed to model microbial communities [48]. For example, microbial communities have been modeled as a single superorganism with one overall objective function [49,50], or as a set of interacting species each with their own objective functions [51–53].

Based on this framework, various extended modeling approaches have been developed for modeling microbial communities, including the dynamic multispecies metabolic modeling framework (DMMM) [54], the community and systems-level interactive optimization (CASINO) approach [55], the computation of microbial ecosystems in time and space (COMETS) framework [56], OptCom [57], and d-OptCom [58]. While these models can predict certain aspects of microbial communities, important challenges remain that must be addressed in the future. Perhaps the most significant concern with these approaches is their inherent reliance on a specific optimality assumption to predict global system behavior and specific metabolic fluxes and cross-feeding rates. Since different modeling frameworks use different optimality criteria, model predictions become highly dependent on the specific set of assumptions used. Optimality assumptions are notoriously difficult to verify experimentally, even for single-organism systems [59,60], let alone for complex microbial communities. Moreover, metabolic flux predictions based on constraint based approaches can be inaccurate, as they often disagree with experimentally measured fluxes using techniques such as ^{13}C metabolic flux analysis (^{13}C-MFA), as was shown recently for *E. coli* [61]. To improve quantitative accuracy of these models it would be valuable to incorporate kinetic information, which can significantly improve metabolic flux predictions [62].

^{13}C-Metabolic flux analysis for analysis of microbial communities

^{13}C-MFA is the gold standard technique for quantifying metabolic fluxes in living cells. In the past decade, ^{13}C-MFA has become an indispensable tool in metabolic engineering and synthetic biology [63,64]. In contrast to FBA, ^{13}C-MFA does not rely on optimality assumptions to elucidate metabolism. Instead, precise metabolic fluxes are determined through model-based analysis of stable-isotope labeling measurements using well established protocols and modeling approaches [65•,66,67]. Thus far, only a few studies have focused on measuring metabolism in microbial communities using ^{13}C-MFA. In these studies, physical separation of either proteins or cells was required to measure species-specific isotopic-labeling, from which then species-specific fluxes were

Figure 3



Microorganisms can engage in a wide range of possible interactions, including parasitism, commensalism, neutralism, amensalism, competition and mutualism. Even for a simple two-member system there is a large number of positive, neutral and negative interactions possible based on metabolite exchanges and substrate competition.

calculated [68,69]. Separation was accomplished either by direct separation of cells via centrifugation or fluorescence-assisted sorting, or indirect separation through purification of an overexpressed 'reporter protein', where the labeling of the overexpressed reporter protein was used as a proxy for whole-cell protein labeling. However, these approaches for ^{13}C-MFA have a few important limitations. Notably, incomplete separation of cells or proteins was shown to produce inaccurate results [68]. Additionally, fluorescence-assisted cell sorting is a rather slow separation technique, thus making it impractical for routine use in ^{13}C-MFA, while methods based on an overexpressed 'reporter protein' are limited to organisms with well-developed genetic tools. Moreover, these approaches require large sample sizes. Lastly, none of these approaches was able to quantify metabolite cross-feeding fluxes that are of significant interest.

Recently, a new ^{13}C-MFA framework for microbial communities was developed that overcomes previous limitations [70]. This approach doesn't require any physical separation of cells or proteins. Instead, isotopic labeling of the entire sample can be used to resolve species-specific fluxes with high precision and determine inter-species metabolite exchange. This approach for conducting ^{13}C-MFA is based on the idea that different populations in a community will have distinct isotopic labeling patterns and that the measured labeling can be represented as an average population-weighted labeling state. The advantage of this approach is that isotopic labeling of different populations can be computationally deconvoluted from total labeling and species-specific metabolic fluxes and cross-feeding rates between the populations can be quantified with high precision [70]. This approach was recently applied to quantify intracellular fluxes and metabolite cross-feeding interactions between distinct microbial subpopulations in a biofilm [71••]. In the future, by building upon this framework and developing additional tools, for example, for dynamic ^{13}C-MFA and incorporating additional multi-omics data, we envision that understanding of the general principles that govern the structure and function of microbial communities can be further enhanced.

Conclusions and future outlook

One of the key challenges in microbiome research is identifying and manipulating cross-feeding interactions

between community members that drive system dynamics and functions [72,73,74•]. Even for a simple two-member system there are a large number of possible interactions [75••] (Figure 3). To better understand microbiomes and make quantitative predictions, more advanced methods are needed for identifying and experimentally validating these cross-feeding interactions [39,76]. Moreover, to further advance our understanding of the role of metabolism in microbial communities, new mathematical approaches are needed that can span multiple length scales and time scales to enable integrated multiscale analysis of metabolite cross-feeding interactions and community metabolic fluxes [77,78,79•,80•]. With respect to population models, the scope of these models needs to be expanded to allow multiple heterogeneous data sets to be integrated for systems biology analysis of microbial communities. Cross–feeding interactions and other substrate-level effects should be directly incorporated into these new models. The basis for these models could kinetic growth models that have been used extensively in the past to simulate and optimize large-scale industrial fermentations. These predictive models are based on mass balances for all relevant system components (i.e. biomass, substrate, product, oxygen), combined with kinetic expressions for cell growth, product formation and gas-liquid mass transfer. For modeling microbial communities, the kinetic growth rate expressions of individual species must be carefully chosen, since in microbial communities cell growth is often directly linked to cross-feeding rates, rather than to substrate concentrations as in traditional Monod kinetic growth models. To further advance the use of quantitative modeling and analysis techniques for interrogating microbial communities [81,82], new software tools are also needed that can seamlessly integrate the various modeling strategies and analysis functions, so that these approaches are available to a broader cross-section of the scientific community. Ultimately, we envision that integrated models of microbiomes will enhance our fundamental understanding of microbial communities and establish new theories and mechanisms that govern the structure and function of these complex biological systems.

Conflict of interest statement

Nothing declared.

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- •• of outstanding interest

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