



Towards Predictive Models of the Human Gut Microbiome

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Abstract

The intestinal microbiota is an ecosystem susceptible to external perturbations such as dietary changes and antibiotic therapies. Mathematical models of microbial communities could be of great value in the rational design of microbiota-tailoring diets and therapies. Here, we discuss how advances in another field, engineering of microbial communities for wastewater treatment bioreactors, could inspire development of mechanistic mathematical models of the gut microbiota. We review the state of the art in bioreactor modeling and current efforts in modeling the intestinal microbiota. Mathematical modeling could benefit greatly from the deluge of data emerging from metagenomic studies, but data-driven approaches such as network inference that aim to predict microbiome dynamics without explicit mechanistic knowledge seem better suited to model these data. Finally, we discuss how the integration of microbiome shotgun sequencing and metabolic modeling approaches such as flux balance analysis may fulfill the promise of a mechanistic model.

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Introduction

Mathematical models of multispecies microbial communities have a long tradition in design and control of environmental biotechnology processes. For more than 20 years, sophisticated mechanistic models have assisted engineers in understanding the relationship between operational parameters (e.g., flow rate, temperature and oxygenation) and microbial composition in wastewater treatment bioreactors [1]. Recent advances in metagenomics have reinforced the notion that the intestinal microbiome is composed of multiple microbial species in competition for limited nutrients and attachment sites and differentially susceptible to external perturbations, which is similar to bioreactors. Both the gut microbiota and wastewater have very high microbial biodiversity [2]. However, whereas the perturbations in bioreactors usually consist of changes in the operational variables such as flow rate or composition of the influent, in the microbiota, the perturbations are such as antibiotic treatment, changes in diet and exposure to external microbes (Fig. 1). There is a significant interest in

optimal microbiome management due to its relevance to human health [3]. While most of our current insights come from experimental studies [4], it should be possible to develop mechanistically based mathematical models to assist in the design of intervention strategies, similarly to how engineers apply mathematical models in the design and management of bioreactors.

Mathematical Models in Environmental Biotechnology

Biological wastewater treatment is the process of clearing sewage water by converting the dissolved nutrients, which would otherwise cause eutrophication and bad water quality in the receiving water bodies, into microbial biomass, which can then be disposed or recycled. In these bioreactors, multispecies microbial communities grow by consuming carbon-, nitrogen- and phosphorus-rich organic compounds in the wastewater, effectively cleaning the water of these compounds. The microbial community can be very

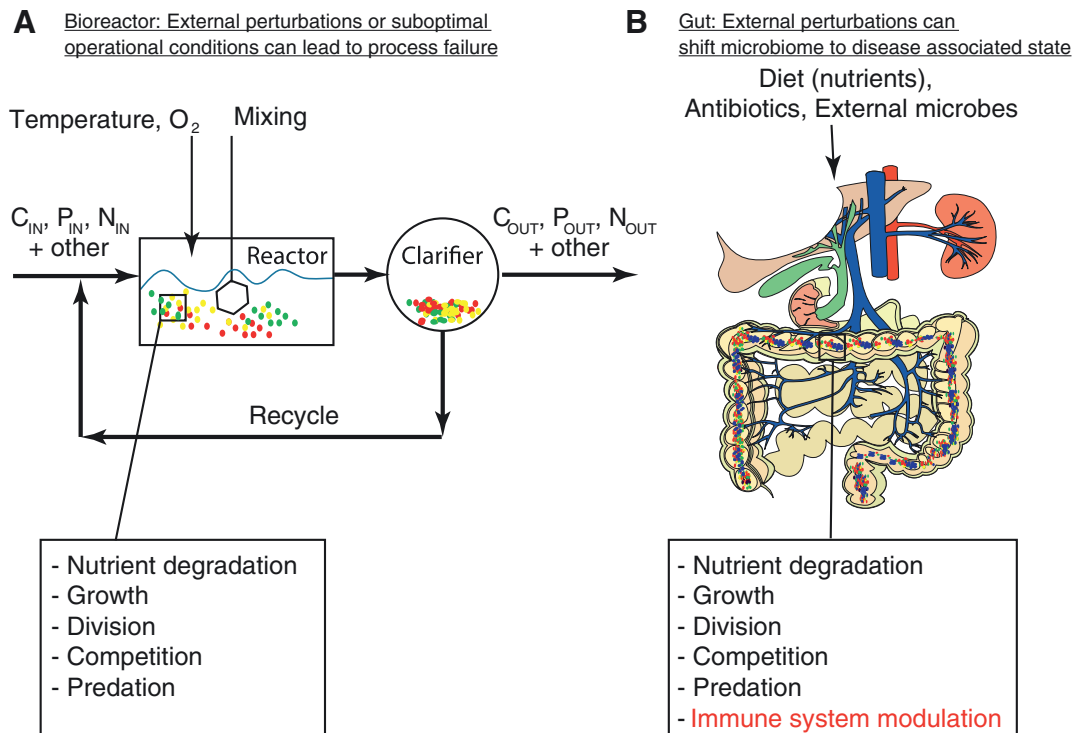


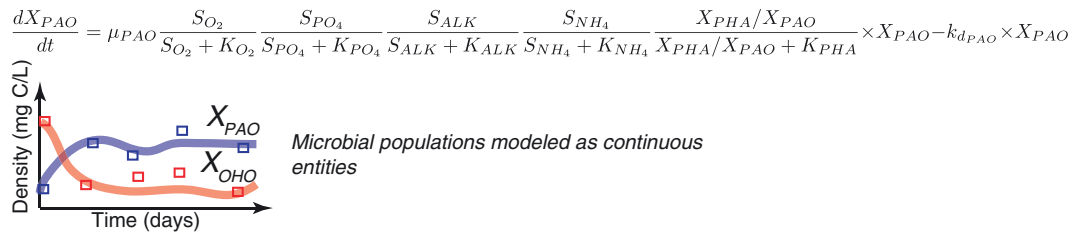
Fig. 1. Analogy between environmental engineering bioreactors and the human intestinal microbiome. Bioreactors, such as the activated sludge (A), use multispecies microbial communities to degrade waste (e.g., C = organic carbon) and other nutrients (e.g., N = nitrogen as ammonia, P = phosphorous) that if untreated can lead to environmental pollution problems. The optimal microbial community is selected by the bioengineer by tuning external parameters such as mixing, aeration and temperature. Composition shifts due to improper operational conditions can lead to system failure. (B) The intestinal microbiome is the multispecies microbial communities harbored in the human intestine. Similar to the environmental bioreactor communities, the microbiome degrades nutrients and is susceptible to external perturbations such as diet change and antibiotic application. Its composition shifts due to changes in diet or antibiotic use, and some community alterations have been associated with disease.

diverse both at the phylogenetic level and at the functional level [5]. While it is essential to maintain a proper microbial composition to assure efficient wastewater treatment [6,7], the bioengineer has only a limited number of operational handles on the system. The operational variables include aeration, mixing and flow rate, which if not optimally chosen can lead to bioreactor failure [8]. Mathematical models can be valuable tools to assist in the operation and control of these bioreactors, in order to enrich microbial composition in the right type of microbes [9].

Traditional mathematical models of wastewater treatment are based on differential equations that describe microbial population dynamics and the dynamics of chemical compounds in solution or in suspension. Most models adopt variations of the Activated Sludge Model (ASM) [1]. There are presently five versions of the ASM [10] that differ in their level of detail. In general, these models include bioreactions such as degradation of soluble, particulate and colloidal organic carbon sources and of nitrogen (as ammonia, nitrate and nitrite) and phosphorus sources. Microbial processes from mainly three

functionally relevant microbial groups mediate these reactions. These microbial groups are ordinary heterotrophic organisms (i.e., organisms that use organic carbon for growth), autotrophic nitrifying organisms (organisms that use inorganic nitrogen sources such as ammonia) and phosphorus accumulating organisms (PAOs; which store phosphorous internally in the form of polyphosphates). This functional grouping was chosen because the goal of wastewater treatment is to remove nutrients before municipal or industrial effluents are released to the environment. In this functional grouping, the microbes do not necessarily belong to the same phylogenetic taxa. An example equation corresponding to PAOs net growth from the ASM2 model is displayed in Fig. 2A.

Even though many engineers rely on ASM to characterize bioreactor properties and designs [11], there is active research to introduce additional levels of detail [12]. Some of the limitations of ASM are due to oversimplified model representation (functional groups) and parameterization [13]. For example, ASM2 was modified to simulate phosphorous removal by PAOs and to include the presence of

A Population Level Modeling (e.g. ASM)**B Individual Based Modeling (e.g. Framework, iDynoMiCS, iAlgae)**

$$\frac{dX_{PAO}^i}{dt} = \mu_{PAO}^i \frac{S_{O_2}}{S_{O_2} + K_{O_2}^i} \frac{S_{PO_4}}{S_{PO_4} + K_{PO_4}^i} \frac{S_{ALK}}{S_{ALK} + K_{ALK}^i} \frac{S_{NH_4}}{S_{NH_4} + K_{NH_4}^i} \frac{X_{PHA}/X_{PAO}^i}{X_{PHA}/X_{PAO}^i + K_{PHA}^i} \times X_{PAO}^i - k_{dPAO}^i \times X_{PAO}^i$$

$$X_{PAO}^{Total} = \sum_{i=1}^L X_{PAO}^i \quad \text{Microbial populations modeled as sum of individual cells}$$

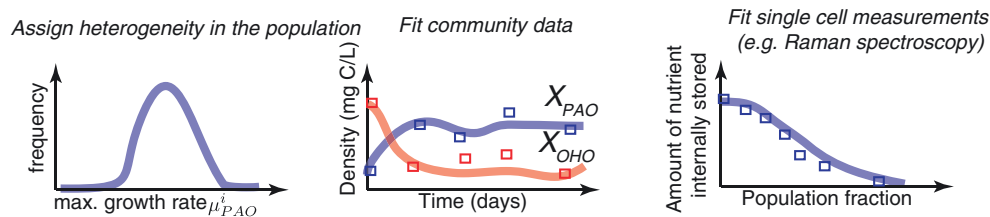


Fig. 2. Population- and individual-level approaches used to model environmental bioreactor microbial communities. (A) Population-level modeling, such as the ASM, considers microbial population as continuous entities. These models do not account for population heterogeneity in parameters and internally stored nutrients and are used to reproduce bulk community measurements. μ_{PAO} is the maximum growth rate of phosphate accumulating organisms (PAOs), S_{O_2} represents the oxygen bulk concentration, S_{PO_4} represents the bulk phosphate concentrations, S_{ALK} is the alkalinity of the wastewater, S_{NH_4} represents the bulk ammonia concentrations, X_{PHA} is the poly-hydroxy-alkanoates internally stored concentration for PAOs and X_{PAO} is the biomass density of PAOs in the wastewater. K_{O_2} , K_{PS} , K_{ALK} , K_{NH_4} and K_{PHA} are the half-saturation constants for the respective saturation kinetics. (B) IbM has been used to respond to the increasing number of single-cell measurements (e.g., Raman spectroscopy [14,15]). These measurements show that microbial functionally relevant groups in bioreactors are heterogeneous, such that they harbor different level of internally stored nutrients. Fitting single-cell observations and bulk community data with IbM lead to a deeper understanding of the system under investigation. In the IbM approach, total PAOs growth is an emergent property of the summation over each individual cell contribution to growth which is dependent on the current state of the individual cell, its parameters and the local concentrations of solutes. With superscript i , we indicate the parameters corresponding to individual cell $i = 1 \dots L$ and L is the total number of individuals. Examples of IbMs are the Framework [16], iDynoMiCS [17] and iAlgae [15].

another microbial subgroup, the glycogen accumulating organisms, which compete with the PAOs for carbon uptake therefore limiting the bioreactor performance in phosphorous [18]. Nevertheless, a recent review still argues that the metabolic differences among microbial subgroups within the PAOs (or glycogen accumulating organisms), which results from lumping phylogenetically different species into the same category, are responsible for some of the inconsistencies of the application of these models [19].

Individual-Based Models

Most ASMs ignore the fact that microbial populations, even isogenic ones, can be highly heterogeneous phenotypically [20]. Individual bacterial cells in

the same population can differ, for example, in their growth rate or the level of internally stored nutrients. The latter has been recently shown experimentally using single-cell Raman spectroscopy to measure intracellular metabolites coupled with fluorescence *in situ* hybridization microscopy and may have strong implications in the biology of environmental bioreactor microbial communities [14]. Population-level models such as the ASM typically neglect this information. However, parameters obtained by bulk population measurements can be poor estimations of the actual mechanistic parameters and therefore limit the applicability of a calibrated model to different scenarios, which is a consequence of the mathematical concept known as Jensen's inequality explaining that the weighted average of a given property of the individuals in the population can be greater than the simple average value in the population [21]. This

shortcoming can be to some extent resolved using individual-based modeling (IbM) that accounts for cell heterogeneity by explicitly simulating individual bacterial cells within the population [21]. The global population-level behavior results from the cumulative behavior of the individuals and their interactions (Fig. 2B). A recent study showed the importance of accounting for heterogeneity in enhanced biological phosphorus removal systems by showing that IbMs are able to predict the distribution of internal stored nutrients obtained by single-cell Raman spectroscopy methods [15].

Another important source of individual variability comes from environmental heterogeneities. In microbial communities such as thick biofilms, bacterial growth will be limited by diffusion of nutrients [22]. Because diffusion of nutrients is a slow process compared to their consumption by bacteria, spatial gradients will form in a biofilm. Gradients basically mean that bacteria in different locations of the biofilm will experience different local concentrations. The spatial heterogeneity has consequences to the way of bacteria interaction within a biofilm. IbMs that take diffusion into account have been of great importance to unveil the role of gradients in biofilm processes [17,22–28].

Although IbMs are more computationally demanding [18] than equivalent population models, these models are increasingly being adopted to study microbial processes, particularly in surface-attached (or biofilm) communities [29,30]. IbMs allow a greater level of detail at microscopic scales with implications for the overall bioreactor functioning [21]. One notable example of a successful IbM is the multiyear, multigroup project to develop a general IbM for microbial dynamics in surface-attached reactors called iDynoMiCS [17]. iDynoMiCS was already applied to evaluate reactor performance as a function of the relative ratio between biomass limitation and diffusion limitation in a membrane biofilm reactor [31]. Similar IbMs were used to design optimal strategies for nitrogen removal in granular bed sludge reactors [16] and in other areas of biofilm biology [27,32,33].

Metagenomics: Unveiling the Intestinal Microbiome

Like wastewater treatment communities, the intestinal microbiota is a highly diverse ecosystem that performs important nutrient degradation functions [34]. The gut microbiota has the additional roles of providing resistance against colonization by enteropathogens [35–38] and modulating immunity [38–40]. Recent studies, supported by next-generation DNA sequencing, have established novel connections between the intestinal microbiota composition and disease [41,42]. Compositional shifts in the intestinal microbiota have been linked to chronic diseases such

as obesity [43], diabetes [44] and Crohn's disease [45]. Interestingly, even drug-induced transient changes increase the risk of developing acute intestinal infections [46] or pulmonary viral infections in mammalian hosts [47].

Two main methods are currently used to investigate this community with high-throughput DNA sequencing [48]. The first one, amplicon or targeted sequencing, uses pooled sequencing of the PCR product of 16S ribosomal RNA (16S rRNA) genes followed by mapping the resulting uniquely identified sequences to a taxonomic database (see Ref. [49] as example). The second method, shotgun sequencing, aims to characterize the whole metagenomic content in a sample (the microbiome) and identifying function by mapping sequencing reads to a database of genes with presumably known function (KEGG, COG) [50]. While 16S analysis provides mostly demographic information (which microbes are present and how abundant they are), shotgun sequencing can provide information on the processes available in this ecosystem (e.g., pathways) [51].

Current microbiome analyses provide a unique view into this, still little known, microbial ecosystem. Nonetheless, the quantitative analyses presented so far are mostly descriptive, such as comparisons of sample composition using quantitative indices and correspondence analysis [52] or cross-sectional statistical tests [53,54]. Top-down modeling frameworks such as Singular Value Decomposition [55] or Mixture Models Engines [56] that aim to identify patterns in the data have been employed to individuate stereotypical microbial responses to external perturbations but provide little information on community interactions. Correlation analyses have been used to evaluate interactions among species and the effect of external perturbations [57,58], but these methods lack in mechanistic insight. The temporal predictability of a microbial ecosystem was recently quantified using time-decay similarity indices [59] but this method cannot be used to predict the intestinal microbiota dynamics [60] (Fig. 3A).

Environmental engineering provides mathematical frameworks to predict temporal dynamics of microbial communities as a function of multiple external stimuli and perturbations. The same approach in principle can be applied to the intestinal microbiome. However, there is a large gap between sequencing data and the level of mechanistic detail used in biochemically realistic models. Can we bridge this gap in order to develop models of intestinal microbiota dynamics and its relation to health and disease?

Mechanistic Models of the Intestinal Microbiota

A recent model proposed for the human intestinal flora [61,62] aimed to simulate the microbial digestion

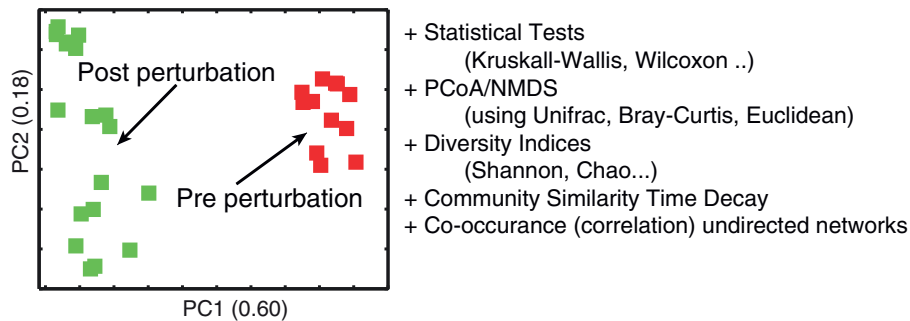
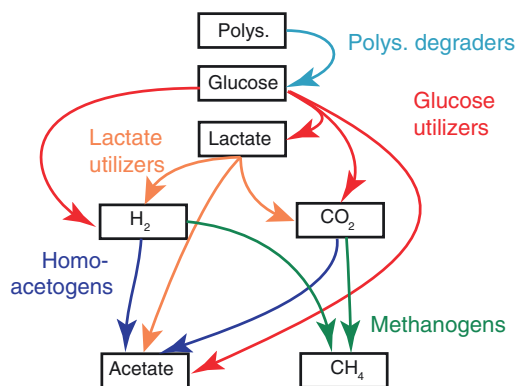
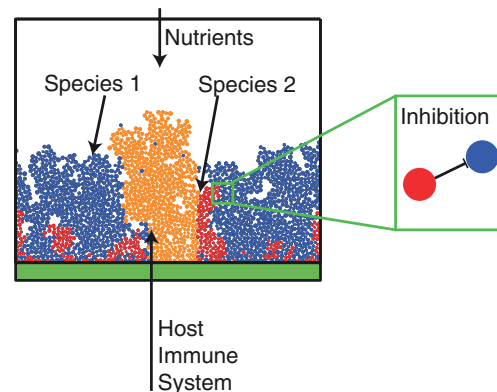
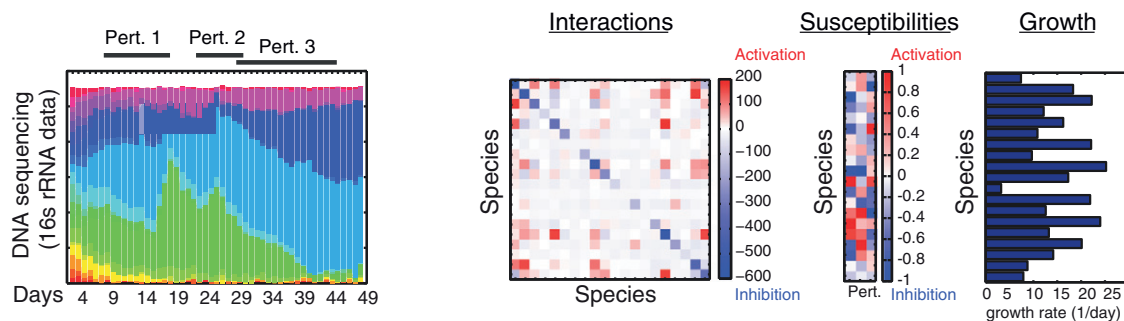
A Current computational analysis of microbiome**B** Bottom-Up model from Munoz-Tamayo et al.**C** eGUT model for mucosal biofilm**D** Generalized Lotka-Volterra model to infer community structure and response to external stimuli from metagenomics

Fig. 3. Computational analysis and mathematical models of the intestinal microbiome. (A) Example and list of current computational approaches used to analyze community data for microbiome (16S rRNA) studies. (B) Schematic of a model to simulate polysaccharide digestion in the intestine [61]. This model is built in a bottom-up approach, which aims to determine population-level behaviors from detailed biochemical reactions. Parameters are often taken from literature. No comparison with data is provided in the original paper. (C) Conceptual scheme of the approach used in the eGUT simulator under development by Jan Kreft. (D) Generalized Lotka–Volterra system of equation with time-variable perturbations approach. The method uses ecological modeling and machine learning to infer network of microbial interactions, susceptibilities to external perturbations and growth rates. The parameters inferred are used in an ecological community model that can then be used to predict ecosystem dynamics by numerical simulations or to identify steady states. The data shown is an example obtained by simulation to validate the inference method and shows microbiota dynamics in the presence of three distinct perturbations representing antibiotic administration [63].

of complex polysaccharides into sugars and end products such as short chain fatty acids, methane and carbon dioxide. The model uses an engineering modeling framework similar to the ASM and models the intestinal tract as multiple interconnected completely mixed reactors, each representing a section of the human intestine. Each compartment is subdivided in two additional subcompartments corresponding to the lumen and the mucosa. Nine chemical compounds and four functional microbial groups are modeled using mass balance equations. As is common in environmental engineering, the bioconversions of microbial growth and chemical substrate transformations are modeled with saturation kinetics using parameters obtained from the literature or previous experiments. The model was applied to investigate effect of diets and host secretion of mucins on microbial composition and function (Fig. 3B).

There have been efforts to apply lbM as well. A recent example investigated how the social interaction between microbial species can be modulated by a third species [26]. Such a systematic analysis of multispecies interactions may eventually be extended to large numbers of species to model biodiverse microbiomes. Another study used a two-species model but included interaction with the host [64]. This individual-based model, perhaps the first to describe host–microbiome interactions, revealed that host secretions could be important in maintaining microbiome diversity. Members of the group that created iDynoMiCS [17] are developing an individual-based model of the mammalian gut microbiota. The project, called eGUT, is still in early stages of development and aims to produce a software tool for the broad scientific community, with the hope that *in silico* investigations may reduce the need for *in vivo* experimentation with animals in the future. Compared to the iDynoMiCS framework, which focuses in inter-species interactions and nutrient consumption, eGUT will also include a description of the intestinal wall, processes simulating immunity and inflammation and a sequence of larger compartments with three regions per compartment corresponding to mucosa, lumen and particles in the lumen (Fig. 3C).

Inferring Community Structure from Metagenomic Data

Mechanistic models such as the ones described above typically rely on a coarse-grained functional definition of microbial groups (e.g., glucose utilizers, methanogens) that is difficult to conciliate with most microbiota sequencing data. 16S amplicon sequencing metagenomics primarily provide information on the phylogeny of the bacteria compositing the consortium, rather than providing information on their function. Nonetheless, methods to integrate these two levels of

information are now under development [51,65]. Such methods can be highly valuable to assist in model development using data from high-throughput sequencing, which have already demonstrated that the presence or absence of a specific bacterium may change ecosystem health and development significantly [66]. Similarly, spatially structured lbMs describe processes at a much finer scale compared with metagenomics and may not benefit from the metagenomics revolution directly.

Modeling population dynamics using the generalized Lotka–Volterra framework is a possible solution for predictive modeling of the intestinal microbiota that can leverage current metagenomic data. This concept has been first suggested by White [67] and very recently led to independent implementations by three distinct research groups [63,68,69]. This type of modeling derives from the classical Lotka–Volterra dynamics used in mathematical ecology to model simple systems such as two-species predator–prey dynamics. The generalized Lotka–Volterra extends the concept to an arbitrary number of species (see the box). The recent papers [63,68,69] propose methods to infer the species–species interaction parameters directly from time series of absolute abundances of microbial groups, which may be obtained from longitudinal metagenomics studies. This approach is similar in concept to methods of inference of molecular networks such as metabolic modeling using S systems [70].

Importantly, like in other network inference methods, the data-driven inference of Lotka–Volterra dynamics from metagenomics time series allows measuring parameters in the natural setting where the microbes reside. This is important for two key reasons. First, it enables measuring growth parameters of unculturable microbes, which would be impossible to measure by traditional methods requiring microbial isolation. Second, even when microbes are culturable, growth rates measured *in vitro* are unlikely to match *in vivo* growth rates because it is difficult to recreate the exact conditions experienced by microbes in the gut. Perhaps another key feature is that the generalized Lotka–Volterra can be extended to model time-dependent external perturbations [61]. Under this extension, the temporal variation in total abundance of species can be computed as the balance between growth, interaction with itself and all the other present in the ecosystem but also a response to multiple time-varying external perturbations (see Box 1). In the case of an antibiotic perturbation, the method provides a quantitative estimation of the sensitivity to antibiotic of every microbial group [61]. The method can also be used to assess the effect of other perturbations such as changes in diet.

The novelty of generalized Lotka–Volterra approaches lies in the fact that the inferred ecological structure of the intestinal microbiota and its response

Box 1

Intestinal microbiome community structure inference and dynamic predictions using generalized Lotka–Volterra with time-dependent perturbations.

The time derivative of the microbial species x_i is computed as a balance between growth, interactions and response to time-varying external stimuli (e.g., antibiotic, diet) and is computed as

$$\frac{dx_i(t)}{dt} = x_i(t) \left(\mu_i + \sum_{j=1}^L M_{ij} x_j(t) + \sum_{k=1}^P \int_{ik} u_k(t) \right).$$

Here μ_i denotes the unlimited growth rate of unit i in the absence of any competition, the interaction term M_{ij} characterizes the effect of unit j on i . In particular, $M_{ij} > 0$ stands for activation and $M_{ij} < 0$ stands for repression. (No interaction is accordingly indicated by $M_{ij} = 0$). Effect of external perturbations is described by $\sum_{k=1}^P \int_{ik} u_k(t)$ where u_k represents an external, time-variable stimulus of a perturbation $k = 1 \dots P$ whose relative susceptibility for each unit i is represented by ϵ_{ik} . This model can be discretized and rewritten in matrix formulation such that the parameters corresponding to growth, interaction and susceptibilities can be inferred from temporal high-throughput microbiota observations using cross-validation techniques and Tikhonov regularization-based optimization approaches [61].

to external perturbations can be used for temporal predictions across short and long time scales (Fig. 3D). These models can generate hypotheses that, once properly validated, can lead to mechanistic insight. Possible applications of these models for the rational development of microbiota-tailoring therapies include the optimization of antibiotic dosage and timing to reduce impact on beneficial gut microorganisms.

Another important application of network inference methods is to identify keystone members of the microbial ecosystem [69] and key interactions within the microbiota [63]. Candidate species and interactions could be properly validated with scalable *in vivo* mouse models with well-defined communities [71–73] as another step towards clinical application.

Moving towards Whole-Cell Community Models

Because they rely strictly on 16S rRNA amplicon DNA sequencing, the present generalized Lotka–Volterra models do not consider the whole genomic content and therefore neglect important aspects of selection and evolution such as antibiotic resistance. A species from a specific phylogenetic group that mutates by acquiring antibiotic resistance will still be placed in that same phylogenetic group. This creates inconsistencies in the model, since the model presently assumes that the properties of the group are static. In principle, one can extend the

framework to allow for time-dependent parameters and time-dependent number of interacting entities such as *Enterococcus* and vancomycin-resistant *Enterococcus*, which are relevant in clinical applications [4,46,74]. However, this requires knowledge on the community beyond that obtained by amplicon sequencing. Also, very importantly, composition shifts observed from community profiling may not necessarily correlate with shifts in ecosystem functionality.

The increasing affordability of nucleic acid sequencing is enabling the acquisition of shotgun metagenomics and even metatranscriptomics data [75,76]. Parallel advances in mass spectrometry technologies allow quantifying abundance of metabolic products such as proteins and metabolites [77]. Bioinformatics analysis on these data enables investigating the microbiome at the level of specific pathways [51] and reconstructing *de novo* the genome of (at least) the most abundant bacteria [78–80]. Combined metatranscriptome and metabolome analysis of microbial communities [81] can help identify which genes are actively transcribed [82] and what proteins and metabolites are produced and degraded. This information will be very valuable in next-generation modeling efforts [83].

Recent studies have focused in extending the widely used flux balance analysis (FBA) [84], a mathematical approach for analyzing metabolite fluxes through metabolic networks, to community flux balance analysis (cFBA) [85,86] and dynamic flux balance analysis (dFBA) [87]. As deducible from its name,

cFBA is focused on determining the metabolic fluxes across multiple interacting microbial strains. Compared to traditional FBA, which constrains the single strain metabolic models under the assumption of growth rate maximization, cFBA relies on a multilevel optimization description. For example, OptCom [86], a recently developed computational platform for cFBA, constrains the community metabolic model by considering potential fitness tradeoffs between the individual and the whole community. dFBA instead assumes that the intracellular solute concentrations are in equilibrium with the extracellular environment and, similarly to FBA and cFBA, the resulting underdetermined stoichiometric model is solved under the assumption of a biochemical objective such as growth rate maximization. In dFBA, the metabolic model is instead coupled with metabolite mass balance equations in the extracellular environment. It assumes a pseudo-steady state where the reactions of the internal metabolites are on a shorter time scale compared to the extracellular nutrients and metabolites. The coupling between cellular metabolism and extracellular metabolites is achieved through the constraint of rates of extracellular metabolite uptake and excretion from the cell. Similarly to traditional FBA, due to the underdetermined nature of the systems of equations, optimization algorithms are essential to compute a solution.

The microbial ecology field is rapidly moving towards integrating multiple level of information to evaluate microbial ecosystem functionality. Nevertheless, metabolic modeling at the current state still treats the community as a single entity interacting with the surrounding environment. Agent-based models capable of going beyond the present oversimplified scenarios by including cellular processes in each microbial cell such as DNA replication, transcription and translation of a few key genes and linking the single-cell behavior to that of the whole population could move the field forward [88]. These types of models can provide a scaffold for model calibration and validation using multi-omics data towards a predictive model of the intestinal microbiome.

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Abbreviations used:

ASM, Activated Sludge Model; PAO, phosphorus accumulating organism; lbM, individual-based modeling; FBA, flux balance analysis; cFBA, community flux balance analysis; dFBA, dynamic flux balance analysis.

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