Modeling Microbial Communities: A Call for Collaboration between Experimentalists and Theorists

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With our growing understanding of the impact of microbial communities, understanding how such communities function has become a priority. The influence of microbial communities is widespread. Human-associated microbiota impacts health, environmental microbes determine ecosystem sustainability, and microbe-driven industrial processes are expanding. This broad range of applications has led to a wide range of approaches to analyze and describe microbial communities. In particular, theoretical work based on mathematical modeling has been a steady source of inspiration for explaining and predicting microbial community processes. Here, we survey some of the modeling approaches used in different contexts. We promote classifying different approaches using a unified platform, and encourage cataloging the findings in a database. We believe that the synergy emerging from a coherent collection facilitates a better understanding of important processes that determine microbial community functions. We emphasize the importance of close collaboration between theoreticians and experimentalists in formulating, classifying, and improving models of microbial communities.

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Modeling Microbial Communities: A Call for Collaboration between Experimentalists and Theorists

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Abstract: With our growing understanding of the impact of microbial communities, understanding how such communities function has become a priority. The influence of microbial communities is widespread. Human-associated microbiota impacts health, environmental microbes determine ecosystem sustainability, and microbe-driven industrial processes are expanding. This broad range of applications has led to a wide range of approaches to analyze and describe microbial communities. In particular, theoretical work based on mathematical modeling has been a steady source of inspiration for explaining and predicting microbial community processes. Here, we survey some of the modeling approaches used in different contexts. We promote classifying different approaches using a unified platform, and encourage cataloging the findings in a database. We believe that the synergy emerging from a coherent collection facilitates a better understanding of important processes that determine microbial community functions. We emphasize the importance of close collaboration between theoreticians and experimentalists in formulating, classifying, and improving models of microbial communities.

Keywords: microbial communities; mathematical modeling; community ecology; interspecies interactions; mechanistic modeling; phenomenological modeling

1. Introduction

Biology traditionally investigates the complex, unique, case-particular phenomenology of the natural living world. This focus on exceptional instances has inadvertently limited the efforts, or perhaps the desire, compared to other scientific disciplines, to identify general and overarching principles. Throughout the history of modern science, interactions between the abstract, generalized way of Mathematics, and the detailed, case-oriented way of Biology have been of a tumultuous nature. Despite this history of mismatch in perspectives, the importance and potential impact of works merging these disciplines are broadly accepted.

Microbes are among the primary forces that have shaped life on Earth. In the context of biological research, microbiology has historically emerged as an overarching common ground among life-science disciplines. Microbes are ubiquitous; they are therefore an object of interest for research ranging from detailed organ-specific physiology to large-scale ecological issues. Microbiology also harbors a proven potential to direct and propel technical and conceptual proceedings in a variety of contexts (biomedical, agricultural, and industrial, among others) and has been central to the development of many of the most essential experimental tools in modern biology (from PCR [1] to CRISPR [2–4]), and for constructing miniature models of ecological and evolutionary processes [5–12]. Microbiology was among the first biological disciplines to embrace an interdisciplinary approach through the research by Esty and Meyer on *Clostridium botulinum*, in 1922 [13]. In their work, the dynamics of bacterial population growth

were described on a semi-logarithmic scale in relation to the environmental temperature. The work is still employed to this day in the monitoring of food safety during the canning process. It is, to the best of our knowledge, among the first examples of how mathematics can effectively be an accessory to biology and produce highly impactful science. It is our belief that rapid progress is facilitated when theorists and microbiologists systematically coordinate their efforts, and we will argue our point approaching several topics that we believe to be of interest to theorists and experimentalists, both in Academia and in Industry.

This paper is divided into two main parts. The first part introduces a brief classification of mathematical models. It is meant as a brief overview of some of the models that have effectively complemented experimental research in the field of microbiology, and highlights where the efforts of theorists have focused so far. A section is also dedicated to the history of the modeling of the rumen bacterial community, which is a striking example of how complementation with mathematics can advance research in microbiology.

The second part discusses the philosophical differences that underlie how experimental research is approached by biologists and physicists, and how these differences often hamper interdisciplinary cooperation in the process of model development. We will advocate for research applicability as the focus around which experimentalists and theorists can more easily set aside their differences and effectively coordinate their efforts, especially in the process of microbial community assembly. The outstanding issue of model validation is also discussed. Finally, we speculate the potential impact of a unified catalog of modeling approaches in microbiology to make modeling more accessible to experimentalists and to inspire future research directions.

2. Background: Past Experiences in Modeling Microbes in Communities

2.1. Mathematical Modeling of Microbial Assemblies: An Overview

A mathematical model is defined as an equation, or a set of equations, that attempts to explain instances of reality in a simplified manner, utilizing only a system's most pertinent properties [14]. A scientific theory is founded when a mechanistic explanation is given for a set of observed natural phenomena. In the physical sciences, hypotheses are often converted into mathematical statements, and models are assimilated into the experimental process. In biology, however, theory is rarely the ground on which hypotheses are formulated, and mathematical models are oftentimes developed as the aftermath of a mass of data [15,16].

For instance, Pearson and colleagues developed a theoretical framework for the theory of evolution [17], Lotka & Volterra produced models for theoretical ecology that described competition and prey-predation [18], and Kermack & McKendrick created some of the first epidemiological mathematical models [19]. Such efforts, and many others of their kind, have been instrumental in advancing their respective fields [16]. Nonetheless, the development of new mathematical models in biology is often treated with skepticism. This skepticism is in part instigated by an uncertainty in the usefulness of a new modeling framework. Does the framework capture the crucial aspects of the biology? Does it address the important questions faced by researchers in the field? Is the model simple enough to inspire insights into important processes? Is the model general or is it specific to the details and nuances of a particular biological phenomenon? These questions naturally arise when studying microbial communities as well, and reflect the intrinsic trade-offs of each modeling framework as discussed below.

Thornley and France [20] outlined the basic principles of modeling, classifying models as: (1) dynamic or static; (2) deterministic or stochastic; and (3) mechanistic or empirical [15,20]. These categories are not mutually exclusive, and published models are often of a hybrid nature [20]. In the rest of this section, we will describe examples of methodologies that have been applied to microbiology. We will also illustrate the chronological progression of modeling, from basic input-output

(empirical) to more comprehensive mechanistic models, by describing the advances made in modeling anaerobic fermentation by rumen bacterial communities.

2.1.1. Metabolic Models

The foundation of the metabolic model is an interconnected network of potential reactions leading to the outputs/products of interest. Genome sequencing data allow researchers to determine an organism's metabolic potential [21]. Whenever genomic data is attainable for a species of concern, annotation of the pathways can outline a comprehensive metabolic network eventually refined by the addition of biochemical data from the literature [22]. The resolution of the model can range from a single metabolic pathway [23] to the whole primary metabolism [24]. Stoichiometric data applicable to the previously established gene products/reactions are then assimilated into the model. The resulting stoichiometric matrices relate the flux rates of enzymatically-driven reactions to time derivatives of metabolic concentrations [21]. This type of model can then be used for Flux Balance Analysis (FBA) [21] and allows investigators to correlate a genotype to its phenotype (in an individual cell or in a community) through the derivation of metabolic fluxes [25–27].

Under-determination is a common issue that arises in initial metabolic models. When a model contains more reactions than metabolites, the observed outputs are not enough to fully constrain the model parameters. In order to rectify under-determination, biological constraints representing realistic cellular limitations are often imposed [28]. These constraints include, but are not limited to: physiochemical, spatial, topological, environmental, and regulatory [21].

Once stoichiometric data and related constraints are overlaid onto a metabolic network, FBA can aid in understanding how metabolic fluxes contribute to cellular physiology. FBA applies linear optimization techniques in order to determine the resulting steady-state fluxes [21]. Frequently applied objectives include: the maximum growth rate, maximum biomass production, and minimization of nutrient uptake. No single objective likely describes the flux states of a biological system in all environmental conditions. Therefore, meaningful objectives must be determined for each modeling scenario [21,29]. For example, Schuetz and colleagues tested a constraint-based stoichiometric model for *Escherichia coli* in six different environmental conditions and identified two objectives that described the fluxes in all conditions tested [29].

2.1.2. Kinetic Models

Initial descriptions of complex microbial communities utilized coarse-grained 'black-box' approaches (limited to inputs and outputs, with no intermediate mechanisms included). Black-box approaches apply empirical parameters to describe the basic kinetic function of community dynamics [30]. In general, kinetic models describe the growth of bacterial cultures through the use of empirically-derived equations that incorporate the concentration of the limiting substrate and the growth (or uptake) rates corresponding to that concentration [31]. Monod and Michaelis-Menten equations are two commonly-used kinetic equations expressing cell growth and substrate uptake, respectively, based on a single growth-limiting substrate and enzyme-catalyzed uptake [25,32]. Empirically-derived equations are useful for predicting the rate of an enzymatically-driven process when substrates are abundant and end-product concentrations are constant. However, unlike in a model, a biological system often contains low concentrations of a substrate, and end-products can accumulate, thus inhibiting the reaction; this aspect is not taken into account by Michaelis-Menten-based models which treat every reaction as irreversible.

In order to rectify the limitations of these equations, Hoh and colleagues designed a kinetic model which takes into consideration rate-limiting factors and thermodynamic theory [33]. The model requires the following assumptions: (1) a reaction that has reached equilibrium cannot proceed in any direction due to the lack of a driving force (change in Gibbs free energy); (2) a reaction that is only slightly displaced from its equilibrium will proceed at a reduced rate compared to a reaction that is further away from equilibrium; (3) the model is free of any additional empirically

measured parameters, excluding the organism-specific reaction rates incorporated into the original Michaelis-Menten kinetic equations.

2.1.3. Spatial Models

Metabolic and kinetic models describe many of the major factors that drive microbial community dynamics (growth rate, substrate uptake, and metabolite production). In comparison, spatial considerations have received relatively little attention, mostly to keep the models simple. However, there are many microbial systems for which these factors are essential in defining the dynamics within a community [34]. Microbes often exist in complex, spatially structured communities such as biofilms. In this type of association, spatial features cannot be neglected.

The first efforts to develop a microbial biofilm model revolved around growth balance [35]. These types of models were initially one-dimensional and incorporated reaction-diffusion equations for nutrients and other cell-produced compounds [35,36]. In time, models have increased in dimension (2D and 3D) and have made use of individual-based modeling (IBM) [37,38] to more concisely describe the heterogeneous behavior commonly observed within a biofilm [35,39]. Although growth remains the primary focus of many biofilm models, other factors such as quorum sensing [35,40] and biofilm mechanics [35,41] have also been represented. To elucidate the features of a microbial biofilm model, the 3D simulation of a biofilm on porous media [42–44] or in unsaturated soil [45] has been considered. In both cases, the focus is on the effect that biofilms have on the hydraulic properties of soil. Graf von der Schulenburg et al. [42] modeled the velocity, pressure, nutrient concentration, and biomass distribution of a biofilm using a biofilm IBM previously established for a 2D model [46], complemented by parameters for fluid velocity, pressure, and solute concentration. Complementary to this work, Rosenzweig et al. [45] developed a channel-network model to describe the effect that biofilm spatial distribution has on soil hydraulic properties. Essential parameters that have been considered are time-dependent flow, substrate transport, and biofilm growth under various soil saturation conditions [45]. Simpler models of spatial structure have also been used to capture how the organization of cells influences range expansion [47,48], intercellular interactions [49,50], or access to environmental resources [51,52]. Even without invoking details such as biofilm mechanics, cell adhesion, or cell differentiation, these models were still useful in teaching us about how spatial structure might affect microbial communities.

2.1.4. Microbial Population Models

Population level modeling efforts have been thoroughly summarized in a recent review [34]. Here, we mention their salient traits.

Population modeling is based on one of two alternative approaches: bottom-up or top-down. In bottom-up approaches, the lower level is described in order to predict the outcome at the higher level. As an example, an IBM may characterize a microbial system using individual interactions/characterization [53,54]; these individuals can be single cells, species, or groups of microbes within a particular spatial and/or temporal context. Population level information emerges as a natural byproduct of the IBM's description [34]. IBMs are inherently more complex and case-specific, but offer highly descriptive predictions and are more suitable for modeling heterogeneity.

Conversely, top-down approaches, such as the use of Population Level Models (PLMs), describe population level changes. In contrast to IBMs, time and space are often considered continuous. PLMs can be based on either ordinary differential equations (ODEs) or partial differential equations (PDEs), depending on the spatial structure requirements of the model [55,56]. ODEs are most often applied and assume that the environmental space is homogenous. However, if spatial structure is a required aspect of the model, different ODEs can be assigned to each different 'compartment' (e.g., spatial compartment, species compartment, phenotype compartment). By assessing each compartment according to its own parameters, it allows for a more accurate assessment. In general, PLMs are simpler models with fewer input requirements leading to significantly easier analyses [34].

2.2. Empirical and Mechanistic Models: From Observations to General Principles

Computational models can also be categorized as either empirical (phenomenological) or mechanistic. Empirical models fit a set of parameters (with a presumed relationship) to the experimental data relevant to the particular system of interest [15,57]. Empirical models (also called "reverse" models [58]) thus often have narrow applicability and offer limited explanatory power outside their "training" scope. However, they are more manageable than their mechanistic counterparts and often prove useful in driving the experimental branch of studies on complex microbial systems by providing a trajectory for developing hypotheses [59]. At large, empirical modeling methodologies follow an iterative cycle of development, utilization, and refinement, which entails the continual input of experimental data followed by further regression analyses [15,17,60]. Thus, a model can evolve from a simplified to an increasingly more complex product as more data are acquired and incorporated.

A mechanistic model (also called a "forward" model [58]) is derived from assumed or known principles of nature and not from a set of experimental data [15]. A mechanistic model with well-founded principles is a powerful tool applicable to studies beyond the scope of its original dataset. In the 20th century, the advent of molecular biology lifted the curtain on the mechanisms underlying many biological processes, granting a new level of depth to phenomenological data [61–64]. Today, biologists advance into the unprecedented age of 'big data'. Many current modeling efforts have shifted to methodologies that allow for the incorporation of such data; FBA in community scale metabolic models is a good example [65].

2.3. Modeling Microbial Anaerobic Fermentation in the Rumen

Empirical and mechanistic models are distinct in many of their general characteristics. However, as a model develops over the years, this distinction blurs. Nascent models often begin as simple phenomenological descriptions of a microbial system; however, as knowledge of the system accumulates and gets refined, by incorporating more data (genetic, kinetic, etc.), the model gradually shifts towards a mechanistic semblance. A good example of this process is the mathematical modeling of anaerobic fermentation by the rumen microbial community.

The ruminal microbiota is a complex system, deeply intertwined with the health of its host [66,67]. In order to establish how an animal's diet affects its ability to produce milk, gain mass, or generate offspring, scientists must first elucidate how usable nutrients, such as volatile fatty acids (VFA), are produced within the rumen. The three main VFAs (acetate, propionate, and butyric acid), produced through the microbial fermentation of carbohydrates, are the primary sources of energy for ruminants. In order to characterize the relationship between diet/feed components and their respective fermentation products, many scientists have turned to modeling.

Early empirical models: In 1989, a publication by the National Research Council characterizing the nutrient requirements of dairy cattle, incorporated mathematical equations into the Cornell Net Carbohydrate and Protein System (CNCPS) to account for varying microbial growth yields [60,68]. The methods set forth by Murphy and colleagues applied to anaerobic fermentative communities within the rumen, and enabled investigators to directly relate fermentation products to diet composition [68]. Murphy et al. based their model on mathematical equations first established by Koong et al. [69] for sheep feeding on white clover; and like Koong, this model utilized stoichiometric measurements of major metabolic pathways in order to determine relative concentrations of VFAs, methane, and carbon dioxide in the presence of various digestible feed fractions [68,70].

Refining the assumptions on conversion efficiency: Although these initial models provided a framework for understanding the role of microbial communities in ruminant nutrition, their strictly empirical inputs left them unreliable for feeds that differed significantly from the ones used to derive the stoichiometric coefficients [70]. In response, a more dynamic model proposed by Argyle and Baldwin [71] incorporated equations allowing for the adjustment of the stoichiometric coefficients depending on the ruminant pH. This led to more reliable predictions for all energy sources and resulted in the overall improvement in model performance [60].

In parallel to the dynamic model proposed by Argyle and Baldwin [71], other modifications were being made that addressed a number of inconsistencies found between simulated and observed data due to overgeneralized metabolizable energy (ME) terms. Up to this point, modeling techniques applied to anaerobic fermentation and rumen microbial communities relied on constant efficiencies of ME, i.e., the efficiency of conversion (from catabolically-produced compounds to body/milk fat, for example) was assumed to be the same for all products. However, research has shown that the efficiency of conversion varied between individual nutrients, which leads to discrepancies between modeled and experimental outcomes [60]. For instance, the conversion efficiency for acetate is 78–80%, while the efficiency for VFAs is a significantly higher 95–97% [60].

Incorporating thermodynamic considerations: According to the second law of thermodynamics, a reaction will not proceed if the reactants are limited compared to the products [70]. More recently, thermodynamics has been assimilated into both metabolic and kinetic models of the anaerobic fermentation of microbial communities [28,72]. In the rumen, the concentration of many reactants (i.e., glucose) is oftentimes low. Thus, the incorporation of thermodynamic considerations is essential for achieving a precise characterization of low-abundance compounds. The aforementioned work by Hoh [33] made a significant contribution in this direction.

Furthermore, a dynamic model for glucose fermentation was developed by Kohn and Boston [70] in which the efficiency of glucose fermentation is established for each metabolite individually (56% efficiency for acetate, propionate, and butyrate; 70% efficiency for methane), and the initial concentrations of metabolites are set to physiologically relevant levels. This model also incorporates an ionophore effect by considering how acid production leads to increased energy expenditure by the bacteria in order to maintain internal ion concentrations. In order to determine conversion efficiencies, the Gibbs free energy maximum efficiency (threshold free energy), the point at which the reaction is as close to equilibrium as it can possibly get, is calculated for each metabolite. By considering the threshold free energy for each individual end-product, the model increased the simulation accuracy by eliminating unfavorable forward reactions at points of equilibrium. To further enhance the accuracy of the model, continual infusion of glucose is simulated into the system, while VFAs and methane are removed at a constant fractional rate to better reflect what occurs within the rumen. The result of such a model is a mechanistic explanation for previously observed conflicts between the modeling results and the experimental data.

Current diet evaluations for dairy cattle are still based on ME (i.e., net energy), and lack any consideration for VFAs and their effect on energy allocation [66]. However, Ellis and colleagues [73] demonstrated that taking a more mechanistic approach proves to be more accurate than the currently utilized energy evaluations for agricultural animals. The biggest challenge in building a model more reflective of experimental data is the implicit inaccuracy in VFA concentration predictions and how this relates to the chemical compounds within various ruminant feeds.

Incorporating meta-omic data, a step towards causality: Although the incorporation of 'omics' data (i.e., genomic, metagenomics, transcriptomic, proteomic) into rumen microbial models remains somewhat uncommon, a number of studies have queried genomic and metagenomic data to better understand the rumen microbial community [74]. For example, microbiologists have sought to unveil the microbes, and their associated enzymatic repertoires, responsible for fiber degradation in the rumen. To do this, the genomes of established fibrolytic organisms, such as *Fibrobacter succinogenes* and *Ruminococcus albus*, were screened for their fiber degradation potential [74–77]. These studies provided insight into the genetic potential of the rumen microbial community, and also facilitated the use of plant lignin manipulation techniques to improve the efficiency of fiber digestion in ruminants. Such studies are regarded as great contributions to our understanding of the mechanisms behind ruminant fiber degradation, which could be further improved through metagenomic analyses of the rumen community [74].

The concepts that stemmed from these works have some degree of universality in microbiology, and have been applied to other communities, including biotechnological systems such as wastewater

treatment, bioremediation, organic acid biosynthesis, etc. In an attempt to improve upon current fermentation mixed-culture models, Rodriguez and colleagues [78] developed a mechanistic model in which product formation, thermodynamic, and pH considerations are incorporated. The authors argue that since bioreactors operate at or near thermodynamic equilibrium, the microbial diversity of the system can be neglected. Therefore, in this model, the culture is treated as a single microbe capable of catalyzing most major fermentation pathways resulting in ethanol, weak organic acid, hydrogen, biomass, and CO_2 outputs. The model is built upon a metabolic network of the major reactions for glucose fermentation, and is constrained by thermodynamic considerations (i.e., change in Gibbs free energy). The bioenergetics of the system are also considered in terms of both pH and the intracellular concentration of acidic compounds.

3. Reaching out across Disciplines

3.1. Our Message for Theorists: There May Be No Elegant Solution

In this section, we reach out to theorists who are willing to approach, or have already approached, the field of microbiology. This is a time in science when multidisciplinary efforts are encouraged, and rightly so. Biology needs the support of physicists, chemists, mathematicians, and all others willing to research the living world. This is especially true for microbiology, a discipline currently under the spotlight, on the verge of being the focus of many research projects. After all, there is a general feeling that this may well be the "Microbial Century" [79].

It has recently been stated [80] that, in modern biology, impending issues that need prompt intervention are the fragmentation of life sciences and the lack of coordination among research endeavors. In this context, we believe that, if not smoothly integrated into the research effort, the modeling of microbial communities may just add another partition to the ensemble. Our lab is made of theorists and experimentalists. To us, it is very evident how different the approaches to research can be for professionals with different backgrounds. This distance is often rooted in deep differences, almost deontological, on what is an insightful scientific question, and on what would be a satisfactory answer to that question. Nevertheless, this distance must be bridged. The contribution of theorists to microbiology is sorely needed. Biologists often cannot have the competence to critically take part in the formulation of a mathematical model, or even critically evaluate the work of those who develop mathematical models. To them, mathematics is still alienating and unfamiliar. Microbiologists are no exception: they are experimentalists by formation and, maybe more importantly, vocation. The bench biologists will often have a hard time in fully understanding a mathematical model, which to their eyes may appear non-intuitive and off-target in relation to their immediate research needs. This is not a novel issue. J.D. Murray has written about the importance of "easing" biologists into mathematics in his seminal textbook: *Mathematical Biology: An introduction* [81]: "The best models show how a process works and then predict what may follow. If these are not already obvious to the biologists and the predictions turn out to be right, then you will have the biologists' attention. (. . .) The use of esoteric mathematics arrogantly applied to biological problems by mathematicians who know little about the real biology, together with unsubstantiated claims as to how important those theories are, do little to promote the interdisciplinary involvement which is so essential". Theoretical modeling should be smoothly integrated in the process of microbiological research, lest biologists may feel their discipline is being usurped from them. Less dramatically, they may simply acknowledge and accept that certain modeling-oriented research directions, while within the microbiology field, will just be out of their area of expertise. Over time, this may lead to an extreme specialization and fragmentation of research competencies. This happens already in many branches of biology (physiology or cancer research come to mind) where experts' focus on specifics hardly leaves any room for employing mathematical models that are based on general principles. We thus run the risk of severing any exchange between experimentalists and theorists.

The living world is recalcitrant to be framed in a synthetic mathematical representation. The physicist or mathematician eager to contribute to this representation will have to resist the understandable temptation of approaching biology as they would thermodynamics, or electrical engineering. Biology is not an exact science or, at least, if there is exactness to it, our current knowledge is not yet in the condition of appreciating it (i.e., data will be noisy). Professional exchange between biologists and other groups of researchers has always had a love/hate nature; this has been especially true with physicists. In 1993, W. Daniel Hillis [82] efficiently surmised the clash between these two categories of scientists, a conflict deeply rooted in the founding principles and practices of their respective disciplines. Hillis pointed out that Biology is not endowed with the power of prediction, and even the synthesis of Darwinian evolution theory gives its best at describing phenomena, not so much at predicting them: "Biologists are annoyed when they sense that physicists blame this on biologists themselves, rather than on the inherent difficulty of the subject matter. (. . .) Biological systems are multi-causal, poorly partitionable and, let's face it, messy. Biological systems have a beauty of their own, but often it is a beauty of complexity and richness, rather than the stark simple reductionist elegance of physics." Indeed.

Experimentalists broadly accept that evolution is the only way through which biology makes sense (T. Dobzhansky [83]), and theorists may find that evolution has nothing to do with "stark simple reductionist elegance." It actually piles up "un-elegant" outcomes by the score. Evolution does not walk the line of extraordinary, efficient solutions. It is the progressive adaptation of fallible living systems along flickering environmental conditions. It is the struggle of the living in coping with their environment through progressive adaptation, based on and constrained by preexistent anatomical structures, in no small part driven by chance. It is a work of tinkering and make-do [84]. Photosynthesis, the pillar of many trophic chains on this planet in the last 400 million years, is a good example. The photosynthetic process, despite hundreds of millions of years of evolution, is still running on very low general efficiency rates: about 2–3% of the overall exploitable light energy [85]. Yet, Mother Nature kept her job whereas no engineer could have.

The mathematician/physicist that plans on tackling biology must keep these aspects in mind, and be ready to accept that sometimes there may be no elegance to be sought, no essentiality to be spotted. Of course, that is not to say that there are no simple general principles in Biology. Sometimes finding a simple description is a matter of perspective. Take central limit theorem as an example. A simple description may adequately represent the combined effects of many random unknown causes. However, finding a simple model that captures important features of interest is far from trivial amid the chaos of messy biological mechanisms. The history of encounters with non-intuitive, complex systems has made biologists suspicious of simple models. To say it with Hillis [82]: "Physicists have learned the lesson that a very simple theory of what is going on is often correct. Biologists have learned the opposite lesson: simple mathematical theories of biology are usually wrong."

3.2. What Experimental Microbiologists Need from Theorists: A Focus on Applications

Even though the details of specific research would be different from case to case, we believe that the following thought process, in mentioning shared features among many questions of interest, will be relevant to other researchers. As microbiologists, we often intend to employ bacteria to address a real-life issue, which could be of biomedical, environmental, or industrial concern. Essentially, we want one or more bacterial strains to employ their genetic potential for our contingent need.

This is, for instance, what currently happens in our lab: we intend to address a well-known environmental issue, specifically, the mycotoxin contamination of food commodities. Mycotoxins are fungal secondary metabolites of an unclear biological purpose [86], responsible for a vast array of pathologies (including cancer) when eaten and assimilated by mammals [87]. Mycotoxins are highly present in cereals and dairy products, exceptionally stable even at extreme environmental conditions, and very hard to denature without aggressive, chemical means. The burden of mycotoxin contamination may amount yearly to billions of dollars both for industrial and medical issues [88].

From scientific literature, and general wishful thinking, we expect that there must be a way to effectively tackle mycotoxin contamination through bioremediation by bacteria. We could identify a list of different strains up to the task. Also, from the literature, we know that different kinds of mycotoxins will often co-occur on the same substrate [89]. We are thus interested in devising a viable, efficient microbial community capable of degrading mycotoxins in the specific environmental conditions of the food production chain. In a nutshell, we are in no different predicament than most applied microbiology labs: we want to craft a community to address a specific issue. Microbiology harbors immense potential for application in all areas of biological research. Simplifying their diversity in form, these applications may oftentimes be categorized as no more than two main processes: the production or degradation of chemical compounds. In this context, what use could experimentalists have for a mathematical model?

It is our opinion that theorists need to focus on the mechanisms that will allow experimentalists to tinker with the potential of microbes, prioritizing the experimental outcome over the mechanistic insight that makes such an outcome possible. That is not to say that mechanistic insight is unnecessary. Mechanistic insight is the essence of real knowledge, but it is also a massive undertaking. In complex systems (i.e., in the real world), true mechanistic insight might be at the moment beyond our technical, or even intellectual, possibilities. This is of course not a certainty, but pursuing such an ambitious goal headlong may not be wise.

The main current challenge of modeling microbial communities is that it is unclear how much knowledge about the mechanisms is required to give us enough predictive power for functions of interest. The current trajectory of approaches is based on identifying and characterizing the activities of individual species (traditionally in monoculture assays), and then combining them to form a model of the community. Is such an approach necessary? We don't know. Is it sufficient? Unclear. A strictly mechanistic approach requires the modeler to incorporate known processes into the model, hoping that, if this is at all achievable, the formalization of such models explains how a community of different members functions. The achievement would be enormous and laudable, but could prove unrealistic and, to some extent, unnecessary. It is more pragmatic to only focus on the product we are interested in, often a specific community function or property, such as the rate of degradation of an environmental toxin, or the coexistence of community members. Modelers would be speaking the language of most biologists if they focused their efforts for the sake of experimental application. Applicability is what drives most experimentalists. In turn, experimentalists can help modelers in their search for the "proper level of abstraction" by focusing on specific communities with well-defined functions and relative characterizing traits. An understanding of the founding principles, and its mathematical synthesis, will come through the synthesis of well-characterized particular cases. But even if not, we would still be endowed with well-characterized particular cases.

3.3. What Theorists Need from Experimental Microbiologists: Data, Possibly in a Specific Form

In the general spirit of establishing a coordinated effort in microbiology, we believe it would be useful to encourage biologists to make their raw data of published work available in an open database. This would be similar to how next generation sequencing raw data are required to be available on public domains for other researchers to access them. This would give modelers the chance to find the mathematical rationale behind works that have independently achieved experimental success. In doing so, they will be able to provide opinions on what future experiments they believe would be insightful to refine the different aspects of the model. This in turn will allow coordination with biologists to further develop our understanding of the observed systems.

There may even be a specific journal dedicated to publishing mathematical modeling papers based on data coming from past experimental publications of applicative relevance and insight. In 2015, Quincey Justman wrote an editorial on Cell Systems to introduce Math | Bio [90], a novel journal founded on a very intriguing premise: to publish papers containing no data, but rather a mathematical argument. Justman is inspired by John J. Hopfield's paper on kinetic proofreading, published in

1974 in PNAS, at a time when, Justman argues, interesting ideas were enough to deserve publication. Math | Bio aims to throw ideas into the fray for biologists to pick them up and test them, if they feel they are insightful and potentially game-changing. We find this idea to be very precious and farsighted. In a much more trivial manner, it could be reversed and applied to microbiology research. In many instances, data is already available (and expanding) for researchers to be put into a mathematical framework. If modelers "adopted" a laboratory or a specific research topic, they might give new insight to published experimental results and, at the same time, provide unexpected inputs on future directions. Even though modeling is part of the current research activities in our lab, we would still love to be "adopted" to facilitate this process.

3.4. Microbial Communities Assembly: An Opportunity for Theorists and Experimentalists to Work Together

To show how research applicability could drive cooperation between theorists and experimentalists, we believe community assembly is fertile ground. In devising experimental research built around applicability, one of the first decisions to make is whether to focus on optimizing a single species for the function of interest, or employ a community of multiple species. Single species have the advantage of being easier to identify and handle in a laboratory environment. Additionally, using a single species makes processes such as artificial selection and data analysis more expedited and easily interpretable. After all, the complications of culturing communities are vast and, sometimes, hardly addressable. Cultivability is a constant issue in microbiology and, in the economy of a natural community, the loss of significant, unculturable strains can largely hamper the desired community function in controlled experimental conditions. Thus, the process of modeling itself, which often relies heavily on data acquired under controlled and monitored conditions, is made easier in in vitro conditions. Nonetheless, we believe that the successful cultivation of a community, even the most essential, is the premise for the most interesting research. From a purely speculative standpoint, the study of community-driven traits (inter-specific cross-talk, microenvironment modifications, ecological interactions) is among the most intriguing topics for present day microbiologists; also, in terms of the application potential of the findings to come, a community, once established and applied for the purpose of bioremediation/biosynthesis/biomedical needs, is likely to be more reluctant to perturbations than any species taken singularly.

To make an exemplificative argument, if we value bioremediators in terms of the genes they bear, we can consider the community as a scaffold that harbors a wide inventory of genetic potential, much wider than what a single-species bioremediator could. Being able to craft stable communities will thus grant much more potential in terms of the amplitude of applicability, and such is the general indication that comes from recent experimental findings [91]. Moreover, a more in-depth formalization of the principles underlying community assembly has been deemed essential by researchers that focus on the highly intriguing field of Synthetic Ecology. We quote from Johnson et al. [92]: "A deeper understanding of the biochemical causes of metabolic specialization could serve as a foundation for the field of synthetic ecology, where the objective would be to rationally engineer the assembly of a microbial community to perform a desired biotransformation."

3.4.1. An Intriguing First Step: Coexistence Theory

In this theoretical context, it is important to point out general concepts around which the process of mathematically describing community assembly would revolve. We believe a clear outline can be found in the principles that constitute the coexistence theory. Coexistence theory is a theoretical framework of concepts that describe and formalize principles that allow a community to retain or lose its identity. In other words, it describes the forces behind coexistence (not surprisingly) and provides insight on how to achieve a successful assembly of communities. It can thus be of great use for the general purpose of most microbiologists.

As outlined by HilleRisLambers et al. [93], the main concepts relative to community assembly are not numerous, and coexistence is described as depending on niche and fitness differences.

1. Relative fitness differences: outline the outcome of competition among species in the absence of stabilizing differences.
2. Stabilizing niche differences: when a species is self-limited by the environmental context rather than by competitors. It is a force in favor of community diversity.
3. Competitive exclusion: happens when relative fitness differences are stronger than stabilizing forces, and the relatively less fit disappears from the community over time.
4. Stable coexistence: Diversity is sustained and stable over time. Stabilizing forces play a greater role than fitness differences.

The task in hand is to experimentally examine which of these processes apply to a particular case of interest and how. Currently, there might not be enough precedence to formulate a systematic protocol for identifying and characterizing the impact of these factors. Nonetheless, as more instances are being examined, we see a hopeful perspective in a future not too far away. Minty's validated model on cross-regnum consortia for isobutanol production, or Zuroff's work on a community for ethanol production from cellulose, are examples notable in their thoroughness [94–97].

3.4.2. The Outstanding Issue of Model Verification

The task of verifying what models are suitable for representing microbial communities, while challenging, is absolutely necessary. Without this verification and refinement step, the cloud of doubt about the relevance of models will keep experimentalists suspicious of all modeling results.

There are still many open questions about the validity or relevance of common assumptions used in modeling microbial communities. As an example, consider the use of Lotka-Volterra (L-V) models for simulating microbial communities. Being the most popular platform for modeling communities, L-V models abstract all the interactions between species into pairwise fitness effects [98–101]. This is motivated by the historical precedence of community studies on prey-predation food webs [102–104] or plant-pollinator mutualisms [105–107]. The relative success of L-V models in the past to represent such communities has established this platform as the go-to model for ecological networks. Additionally, the mathematical tractability of the model gave it a central role in theoretical studies of community stability [99,100,108–110]. This further secured the position of L-V models in theoretical ecology. When simulating microbial communities, this history has been used as justification to extend the same modeling framework to represent microbial interactions. However, pairwise fitness models may not always accurately capture common situations in which multiple diverse interactions are present or when compounds mediating the interactions are shared among multiple species [111]. Identifying and recognizing such limitations allow us to use the very useful L-V modeling platform when it is applicable. We thus advocate for dedicated research to clarify the limitations and range of applicability of common modeling platforms.

Another fundamental assumption in almost all community modeling frameworks is the additivity assumption [111]. For simplicity, it is often assumed that in communities, the effects exerted on an individual or a population by different factors can be superimposed in an additive manner. There are of course many examples to the contrary. The presence of non-additive effects in fact has been widely recognized in ecological modeling under the umbrella of indirect interactions, nonlinear interactions, or higher-order interactions [101,112–114]. Researchers have even rigorously examined whether or not additivity assumption holds for examples in the utilization of resources from the environment [115,116]. Several studies on the combined effects of antibiotics have also shown synergy (or antagonism) between them, showing inhibition effects stronger (or weaker) than what is expected based on an additive model [117–124]. Nevertheless, when it comes to modeling communities, because the extent and prevalence of deviations from additivity is not established, models almost unanimously drop back to assuming additivity. Systematic work is needed in this area to clarify when and under what conditions such an assumption is acceptable.

Performing the necessary work to support and justify model assumptions requires not just the will of researchers, but also the support of the community, including peers, publishers, and funding agencies. Exploring uncharted territories and coming up with new hypotheses using a theoretical platform sounds more exciting, and is often rewarded and recognized as being innovative. This bias comes at a cost: the necessary steps of verifying the basic assumptions of such models are considered "less exciting." The unfortunate outcome of this trend is that a body of theoretical work will develop, without a clear understanding of the conditions under which those findings are relevant. In turn, when experimental data outside the range of applicability of such models deviate from predictions, it will be considered a failure of the theory, widening the divide between theoreticians and experimentalists.

We believe it is time to give the field of model-verification the attention it deserves. Groundwork verification efforts should be treated as independent research contributions on their own, rather than side-notes. There are examples in other fields, where the importance of such groundwork efforts has been recognized. A notable recent example is the reproducibility project in cancer biology to evaluate the reproducibility of previous reports [125–127]. Support from researchers, funding agencies, and publishers in this case shows an exemplary instance in which the scientific community is rallying behind a necessary groundwork. The field of microbial community modeling can certainly benefit from a similar attitude.

3.5. Compiling What Is Known, Clarifying the Assumptions, and Making Models Accessible

When experimentalists devise a novel research plan, it goes through a phase of information gathering that precedes the formalization of the details of the research. In this context, we believe the mathematical model would ideally be of assistance in between the preliminary process of information gathering and the beginning of the experimental phase itself: a good model would outline what variables of the system are more likely to be influential, which is invaluable information. Screening prior research to identify such a model, even if it existed, is not a streamlined process, and surprisingly so. After all, wouldn't it be easier for experimentalists, in deciding what model would be most appropriate for their system, to refer to previous reports and studies in related, well characterized, even if not similar, situations? Unfortunately, a database of microbial interactions and previous modeling efforts currently does not exist, to the best of our knowledge.

Models are most often based on phenomenological data pertaining to a specific biological process, and focus on a single instance within that process: they are hardly approached by researchers not already within that specific field. Proceedings in microbiology, as previously stated, are relevant to many disparate scientific disciplines. Nonetheless, at present, it is normal to assume that a hypothetical model based on a set of microbiological data relevant, for instances, to the field of Transfusion Medicine, is unlikely to be of interest to an environmental engineer interested in bacteria-mediated wastewater management. Yet, if the process under investigation is general enough (dynamics of microbial cell diffusion, for instances), one cannot decidedly rule out a fruitful cross-disciplinary cooperation. To make a more specific example, albeit outside the field of microbiology, a model that described fungal hyphal development, distilled to its essence, could more generally be viewed as a model describing the growth of apically polarized cells. As has already been observed [128,129], this implies that the mechanics relevant to hyphal growth could also be descriptive of neuronal outgrowth: neurons are *de facto* apically polarized cells. Pointing out that, in the right context, hyphal development can be representative of neuronal development is no trivial intuition. In doing so, researchers in the field have essentially observed that Mycology and Neuroscience may happen to cross paths, and we believe that mathematical analysis would be the main way to substantiate this link. Finding a mathematical synthesis for this and other kindred observations is also our best bet towards the mechanistic description of nature. At present, poor communication across disciplines is holding everyone back. If we facilitate the process of bringing together professionally distant researchers,

we will probably find ourselves with similar observations. We may even find our example of the link between Transfusion Medicine and Environmental Engineering not to be so far-fetched.

If there was a public platform that collected works on modeling biology (i.e., works that attempt to distill phenomena to their essence), and that platform could intuitively be browsed by experimentalists, the situation may change. The platform would be a tool where mathematical models are uploaded, along with their respective publication. It would require the participation of both modelers and experimental biologists. The role of modelers would be to upload their work clearly stating the purpose for which the model was developed. They would also be required to provide a user-friendly graphical user interface (GUI), bearing in mind that an experimentalist with very little experience in programming and mathematical analysis is their target user. The user would be put in the condition to easily identify, through the GUI, the variables included and the entity of their effect on the model output.

The key aspect would be to make the model approachable through different research queries of interest to the broadest spectrum of researchers. Models should be sorted through the main processes they describe, the outputs for which they were devised, and with all the variables included. Examples of processes could be: diffusion, cell growth, cell-cell interaction, motility, mutation, biotransformation, and artificial or natural selection, etc. Examples of variables could be: temperature, pH, oxygen concentration, species involved, culture conditions (liquid culture or agar plate), resource availability (rich, minimal, or restrictive, medium), etc. All these elements should also be catalogued by the widest number of biologically relevant terms they could represent in a variety of biological contexts: metabolite diffusion in one context could equate to disease spread in a different context.

Resorting to this platform would be advantageous for both theorists and experimentalists.

For theorists: It would be a rare opportunity to unleash their models in the wilderness of research for other scientists to test them, as they may be representative of more than the one biological context that they originally described. We believe this would be a precious shortcut to make the cross-context (mechanistic) traits of the model emerge, and would facilitate the identification and formalization of the relations among those contexts. At the cost of the supplementary work of providing a user-friendly GUI for their models, theorists would have a lot to gain and nothing to lose from this initiative.

For experimentalists: The experimentalist would approach the platform in search of inspiration in devising his/her experimental plan, gaining insight on which approach is more likely to be successful. After all, examining collected instances in one place offers synergy for interpreting the observations and uncovering patterns. The biologist will have the opportunity to get in touch with one or more publications of models that have dealt with the more (if applicable) similar conditions and premises of his/her own system, and receive a conceptual synthesis of which variables led to which results, gathering some guidance on how to proceed for his/her experiments.

We believe the literature harbors a plethora of models that can be useful to researchers in other fields, but those researchers may never become aware of the existence of such models. A lot is to be gained by facilitating and encouraging communication through the lens of mathematical representation. Actively pursuing the identification of common mechanisms across the different branches of biology holds great potential for life sciences in general, and microbiology in particular.

4. Conclusions and Final Remarks

To summarize, we believe that the great potential harbored by microbes can be unleashed through a close collaboration between theoreticians and experimentalists. Mathematical modeling is the vehicle towards this objective that requires investment and cooperation by both sides. Here, we have compiled suggestions to facilitate this cooperation between researchers from different backgrounds and disciplines. These suggestions come from experiencing interdisciplinary research within our own lab. In our opinion, there is a need to be aware of the differences among researchers from different disciplines, their outlooks, and their interests. To collaborate and cooperate, we need to make adjustments to accommodate these difference. A theoretician may have to balance the

generality-realism trade-off and focus on functions and properties of practical applicability to experimentalists. An experimentalist, in turn, may have to adjust their experiments to collect and compile the data in a form that will be readily usable by theoreticians. Communications is key in this bilateral exchange and compromise. We advocate for practices that facilitate this communication: we encourage experimentalists to compile an easily accessible database of their data for theoreticians and encourage theoreticians to make their modeling frameworks welcoming to experimentalists. Finally, we propose a coordinated effort by the scientific community to lower the barriers between disciplines by focusing on processes and commonalities, built around the common language of mathematical modeling. The outcome will be a better understanding and an elevated intuition of microbial processes for both theoreticians and experimentalists, with a tremendous impact on applications from human health to industrial biotransformation to ecosystem sustainability.

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