Exploring interactions in microbial communities

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Abstract

Most microbial ecosystems cannot be understood without quantifying ecological interactions between their member species. Given the challenges of comprehensively resolving interactions experimentally, a range of prediction methods was developed. Here, we review genome-based prediction methods in particular and discuss their strengths and weaknesses. We then cover different experimental designs to explore microbial interactions and introduce methods to infer interaction signs and strengths from experimental data. Despite the range of available methods to study microbial interactions in silico and in vitro, interactions in a spatial context are still underexplored, and we lack comprehensive interaction databases, which are important gaps to fill in the future.

Introduction

Microorganisms frequently form communities characterized by intricate interaction networks. Thus, to understand and manipulate microbial communities, their interactions have to be resolved. The classical approach to the experimental quantification of pair-wise interactions, introduced by the foundational work of Gause (1), is still widely applied today. It relies on comparing species-specific growth curves in mono- and co-culture, which allows determining whether one species has a positive, negative, or no effect on the growth of the other species and vice versa (Figure 1A). A wealth of interaction prediction and measurement techniques are based on this principle, as we will discuss.

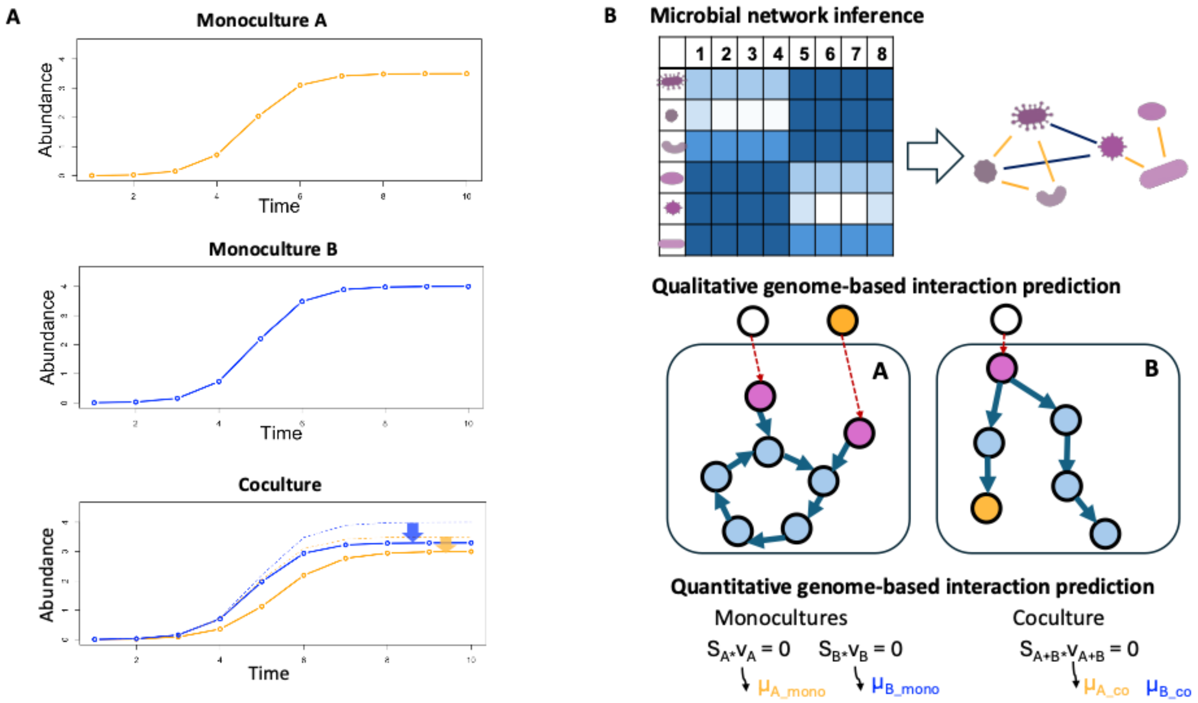

Figure 1: **Interaction quantification and prediction principles.** Panel 1A illustrates the principle of interaction type and strength assessment introduced by Gause. In monocultures, species A and B reach higher maximal abundances than in co-culture (monoculture abundances are indicated in coculture with dashed lines). Thus, species A impacts species B negatively and vice versa, resulting in two negative signs corresponding to competition. The other interaction types (commensalism, mutualism, amensalism, and exploitation) can likewise be detected by comparing mono- and co-cultures. Instead of the maximal abundance, other properties of the growth curve, such as the maximal growth rate, can also be compared. Panel 1B summarizes three ecological interaction prediction methods. In microbial network inference, an algorithm infers a network from taxon abundances measured across samples. Such algorithms usually have a high error rate and often infer undirected networks, which do not allow the detection of interaction types. Qualitative and quantitative genome-based interaction prediction methods are based on metabolic networks constructed from annotated genomes. In qualitative genome-based interaction prediction, a set of seed nodes is computed that represents essential metabolites, which the network cannot produce (purple nodes). In the example, one seed node of species A is produced by species B (orange) and thus can potentially be cross-fed. However, both species also compete for the white seed node. In brief, qualitative interaction prediction infers interactions based on seed node overlap or complementarity. Finally, quantitative genome-based interaction prediction relies on metabolic modeling with Flux Balance Analysis (FBA). In FBA, the metabolic network (encoded as stoichiometric matrix S) serves to compute fluxes through reactions (v), assuming a steady state. One of these fluxes goes through an artificial biomass reaction, thereby representing the growth rate (μ). Consequently, FBA can predict growth rates in monocultures and, in community extensions, also in co-cultures. This allows predicting interaction type and strength based on the principle shown in panel 1A.

How to predict interactions

Since resolving microbial interactions experimentally is challenging, there is an ongoing effort to develop methods to predict them. Here, we will briefly review two main groups of interaction prediction approaches: those based on abundance data and those based on genomes, summarized in Figure 1B.

Nowadays, it is relatively cheap and easy to obtain relative abundances of microbial community members across a large sample number using next-generation sequencing. These large data sets open the door to network inference methods, which aim to predict interactions between taxa given their observed abundance patterns. Microbial network inference algorithms and their strengths and weaknesses have been reviewed elsewhere (2, 3). In general, the accuracy of microbial network inference on simulated data is low (4, 5), and although new inference algorithms are published frequently, a systematic evaluation on biological data is still missing.

As their name suggests, genome-based interaction prediction approaches only require genomic information of microorganisms and focus on metabolite-mediated interactions. They can be further divided into two groups depending on whether they deliver qualitative or quantitative predictions.

Qualitative genome-based prediction of metabolic interactions emerged in the context of the reverse ecology paradigm, suggested by Levy and Borenstein as an umbrella term for genome analyses motivated by ecological questions (6). The core idea of reverse ecology is to predict traits and interactions directly from a species' genome. The first step of genome-based interaction prediction is the construction of a metabolic network from the genome. This step can be automated with tools such as ModelSEED (7), which are fast but usually deliver low-quality reconstructions. The metabolic network can be further refined with additional experiments and manual curation (8). Once a metabolic network is available, its seed metabolites can be computed. Seeds are metabolites that the network cannot produce and which can therefore be interpreted as essential nutrients (9). Given the seed set of an organism, one can check whether another organism can produce these seeds, thereby predicting cross-feeding. Likewise, competition arises when the seed sets of two organisms overlap. Both cross-feeding and competition can be quantified based on the number of seeds that are provided by the interaction partner or that overlap, respectively, and several tools computing cross-feeding and competition scores in this manner are available (10-12). However, there are several caveats: first, it is not easy to ascertain that metabolites that cannot be produced because of absent seeds are indeed essential for growth. Second, a metabolite produced by a potential donor is not necessarily released into the environment or taken up by the recipient.

Quantitative genome-based interaction prediction relies on flux balance analysis (FBA), which computes the distribution of fluxes through all the reactions in a metabolic network under certain constraints and optimality criteria (13). FBA requires an artificial biomass reaction describing biomass formation. The flux through it is interpreted as the growth rate. FBA can be extended to cocultures and thus can simulate the growth of organisms in mono- and co-culture, which allows estimating interaction signs and strengths using Gause's principle. However, FBA makes several assumptions, for instance, that intracellular metabolite concentrations are at steady state and that the cell optimizes its flux distribution to achieve objectives such as a maximal growth rate or ATP production. The steady state assumption is relaxed in dynamic FBA, but optimization is necessary to find a unique solution and is particularly problematic for community variants of FBA. Community-level optimization implemented in tools such as MMinte (14) is not appropriate for exploitative and competitive interactions, whereas optimization at the species level, as in COMETs (15), does not account for community-level optimization that can occur in mutualistic relationships. Flux sampling approaches that bypass optimization altogether are therefore of interest (16).

Finally, both qualitative and quantitative genome-based interaction prediction approaches assume the metabolic network to be accurate and, unless transcriptomics or proteomics data and/or gene regulation are considered, that enzyme-coding genes are expressed and functional.

In the future, reverse ecology methods could consider other traits involved in interactions, in particular toxin production and quorum sensing. The relevance of these traits for interaction prediction could be assessed with machine learning.

Validation of interaction prediction

For several synthetic microbial communities, the interaction network was fully resolved experimentally (17, 18). These data sets, which are also accompanied by abundance data, could serve as biological benchmarks for microbial network inference algorithms. In addition, they could form the basis for interaction databases (19, 20), which are not yet available in a comprehensive form.

Mono- and co-culture data can also be used to assess the accuracy of quantitative genome-based interaction prediction tools. In a recent small-scale evaluation, we found that the accuracy of several FBA-based tools was low when applied to predict ecological interactions with semi-curated metabolic reconstructions (21). More promising results were obtained when predicting interactions between leaf microorganisms with metabolic reconstructions refined with measured carbon utilization profiles (22), illustrating the importance of model curation. A thorough evaluation of quantitative and qualitative genome-based interaction prediction is still missing.

In brief, experimental validation of predicted interactions is still a necessity.

How to measure interactions

A wide array of experimental strategies ranging from mono- and co-cultures to high-throughput platforms, have been developed to explore microbial interaction networks, each with its strengths and constraints. Here, we emphasize the rationale behind their design and the biological insights they provide.

Reductionist Pairwise Systems

Mono- and co-cultures in batch or chemostat setups are foundational tools to probe the nature and strength of interactions under controlled conditions (Figure 2A). Batch cultures are convenient and high-throughput, but they inherently involve changing conditions (e.g., nutrient depletion, waste accumulation, and pH change) that complicate the interpretation of microbial behavior. In contrast, chemostats maintain a constant environment and offer the ability to systematically vary the dilution rate and thus identify maximal growth rates directly, without relying on curve fitting.

To explore interactions without the confounding effect of physical contact, membrane-partitioned systems or spent-media experiments can be used. Membrane setups (23), such as trans wells or dialysis chambers, allow two metabolically active cultures to interact through diffusible molecules in real time, enabling the study of parallel, reciprocal interactions. These systems are ideal for dissecting cross-feeding, toxin exchange, and volatile signaling, while eliminating effects from direct cell-cell contact.

Spent-media assays, by contrast, represent a sequential approach where one species alters the environment, and another is then exposed to the resulting metabolic byproducts (18, 24). This static method is powerful for identifying unidirectional effects, such as metabolic provisioning or growth inhibition, but it neglects temporal dynamics and feedback loops. Both approaches omit physical interactions, which may be essential in structured or host-associated communities, underscoring the importance of complementary methods.

Together, these reductionist approaches offer critical mechanistic insights into microbial interactions, helping to define the ecological and metabolic roles of individual species. However, in a community the outcome of an interaction also depends on whether species are introduced simultaneously (co-assembly) or sequentially (e.g., invasion after steady-state establishment). Invasion assays in particular are useful for assessing the resilience and resistance of established communities, as well as the potential for coexistence in shifting environments. Representative experimental platforms and community assembly strategies are illustrated in Figure 2B.

The Interplay between Microbial Communities and the Environment

Environmental factors such as medium composition, oxygen availability, and nutrient gradients shape microbial interactions. Of note, microorganisms often alter their environment, which in turn can change the sign and strength of their interactions (25, 26). Environmental effects can only be fully controlled in chemostat, since in batch, nutrients are depleted, and toxic metabolites may accumulate.

Scaling Up: High-Throughput and the Challenge of Combinatorics

The exponential growth in potential species combinations poses a significant challenge to traditional interaction studies. With just ten species, there are 120 unique triplets and 1023 combinations in total to consider, making comprehensive testing of large communities impractical. High-throughput designs - based on microfluidics, robotics, microwell plates or nanowell arrays (e.g. (27-29)) - offer a partial solution by enabling parallel testing of thousands of conditions. These platforms have revolutionized our capacity to detect interactions, screen large consortia, and test the impact of environmental factors systematically.

However, the reliability of high-throughput data depends heavily on how microbial growth and interactions are measured. Optical density, a common proxy for growth, is prone to non-linearities at high cell concentrations and may be affected by the small volumes typical in these setups (30). Alternative readouts such as colony-forming units, fluorescence-based reporters, or flow cytometry can offer more accurate insights into cell abundance and activity. Moreover, reactor design (e.g., volume, evaporation resistance, shaking efficiency) can introduce biases that complicate comparisons across platforms or conditions.

Design of Perturbation Experiments

The exploration of interactions between all possible species pairs is often not sufficient to understand the dynamics of the community. Among other reasons, this is due to higher-order interactions (HOIs), in which another species alters the interaction(s) between a set of species (e.g. (31), Figure 2C). To explore the impact of each species on the others in the presence of HOIs, leave-one-out (also named drop-out) experiments are performed by assembling as many sub-communities as there are community members, such that each community member is omitted in one of the sub-communities (e.g. (32)). Leave-one-out experiments can be considered a form of perturbation carried out at assembly. More generally, interactions can be inferred from a microbial community by observing its responses to controlled disturbances (e.g., nutrient shifts, antibiotics). A foundational framework is provided by Bender et al. (33), which distinguishes between two types of perturbations, PULSE (i.e., transient) and PRESS (i.e., sustained), and analyzes how each reveals different aspects of species interactions.

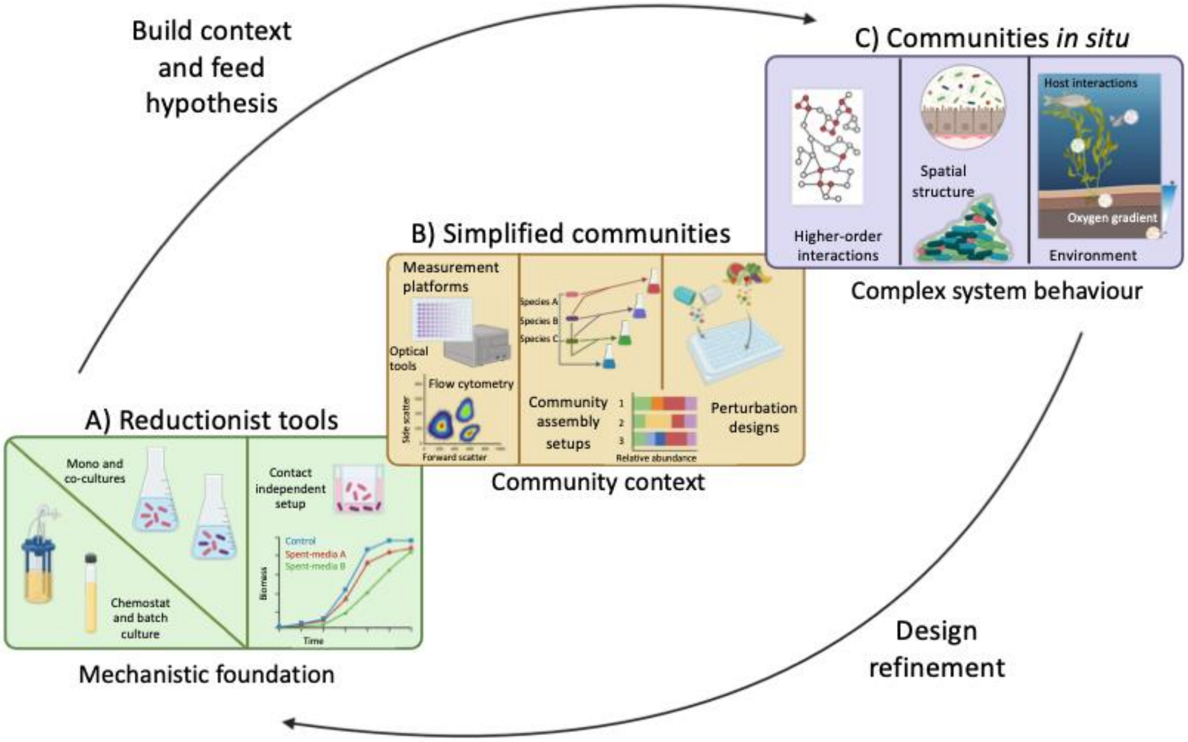

Figure 2. **Overview of experimental strategies to study microbial interactions.** In panel A, reductionist tools provide the mechanistic foundation: mono- and pairwise co-culture designs in chemostats or batch reactors provide controlled conditions to probe species-specific behaviors and quantify interaction strengths and types. Contact-independent setups, such as spent-media or membrane-separated cultures, allow the dissection of diffusible interaction factors. These setups identify growth effects, cross-feeding, or antagonistic metabolite production in a defined, reproducible manner. At the same time, they provide information necessary for the curation of metabolic models (e.g., metabolite production, carbon source utilization). Panel B illustrates the study of simplified communities: building on pairwise insights, defined multispecies consortia (e.g., leave-one-out or co-assembly experiments) allow for testing community-level assembly rules and higher-order interactions. Interactions in a community context are studied through perturbation designs and sequential or simultaneous inoculation setups. Experimental outputs ranging from optical density and flow cytometry to species-resolved sequencing enable systematic exploration of emergent properties such as stability, resistance, and invasion dynamics. In panel C, we portrait the highest level of ecological realism. Here, communities in situ are shaped by processes that are difficult to study in vitro, such as higher-order interactions, spatial structuring (e.g., biofilms, gradients), social interactions between hosts, and history.

These features are difficult to predict from pairwise designs alone and often require community-wide monitoring, spatially resolved tools, or advanced modeling frameworks. The arrows connecting the panels represent the bidirectional flow of information across experimental scales. Figure 2 was designed with BioRender.com.

How to quantify interactions based on measurements

Once growth curves are collected, they can be analyzed using a variety of methods, which broadly fall into two categories: model-free and model-based approaches.

Model-Free Methods for Inferring Microbial Interactions

In model-free methods, interaction signs are typically inferred by directly comparing the maximum (or steady-state) abundances of each species in mono- and co-culture (18, 34, 35). Rate comparisons offer another strategy, where growth rates can be estimated, for example, by measuring the maximum growth rate (36). Another widely used metric is the area under the growth curve (AUC) (18, 37), which captures more growth features, such as growth rate, carrying capacity, and lag phase, compared to maximum abundances or growth rates alone (Figure 3A). Model-free methods are effective at detecting the presence and sign of interactions. While they can offer some insight into the strength of microbial interactions, they provide only indirect information as they do not account for the underlying dynamics.

Capturing Underlying Dynamics Through Model-Based Methods

Model-based approaches account for the underlying dynamics and involve fitting mathematical models to growth curve data. This requires selecting an appropriate growth model (Figure 3B). For mono-cultures, several models are commonly used, including the logistic, Richards, and Gompertz models, among others (38). These models differ mainly in their descriptions of density dependence and whether they explicitly model features such as the lag phase (39). Although many of these models can fit mono-culture data with high goodness of fit, they often yield substantially different parameter estimates, making model selection a challenging step (39). A range of tools is available for fitting growth models to monocultures (Table 1).

For co-culture data, models like the Lotka-Volterra framework are often used (40, 41), typically assuming logistic density dependence. More recent models may include additional parameters, such as lag phase duration or inflection points in the growth curve (42). Some modeling strategies estimate all parameters directly from co-culture data, while others recommend first estimating division rates and carrying capacities from mono-cultures, and then deriving interaction parameters from co-culture dynamics ((42), Figure 3C). Another approach to inferring the sign and strength of microbial interactions in large communities involves analyzing steady-state data from leave-one-out subcommunities ((43), Figure 3D). Instead of exhaustively testing all possible species combinations, an effort that scales exponentially with the number of species, this method requires only a linear number of experiments and employs mathematical modeling to infer pairwise interactions. This approach allows the parameterization of models relying on pairwise interactions in the presence of HOIs, albeit at the cost of a potential failure to accurately describe the dynamics of sub-communities.

A distinct strategy for inferring microbial interactions is based on perturbation experiments. Bender et al. (33) introduced a mathematical framework for estimating interaction coefficients in an S-species community using S PRESS experiments, where each experiment involves removing one species and measuring the steady-state abundances of the remaining species. These measurements are linked to the interaction coefficients through a system of equations (Figure 3E, Supplementary Figure 1A). Similarly, S PULSE experiments involve perturbing one species by adding or removing a small number of microbes and immediately measuring the resulting abundances and their rates of change. The interaction coefficients can then be inferred from a system of equations relating them to these initial post-perturbation dynamics (Figure 3F, Supplementary Figure 1B).

Sensitivity of Model-Based Approaches to Experimental Setup

It is important to note that model-based methods are sensitive to the experimental setup (44). For example, batch culture experiments (45), where nutrients are not replenished, are better captured by metabolite-aware models (46-49). In contrast, traditional Lotka-Volterra models struggle to describe the dynamics of complex communities and metabolite-mediated interactions (50-52). However, such approaches tend to perform well in experimental setups involving chemostats (53) or serial transfers (54), where environmental conditions are more stable. Letten and Ludington showed that microbial community composition is highly sensitive to the resource supply regime: continuous supply (as in chemostats) versus pulsed supply (as in serial dilution) (55). Their simulations highlighted that even modest differences in supply dynamics can lead to drastically different communities, suggesting that the choice between chemostat and serial dilution is not just a practical detail. A recent meta-analysis revealed that more than half of experimental studies aiming to quantify microbial interactions have relied on model-free approaches (44).

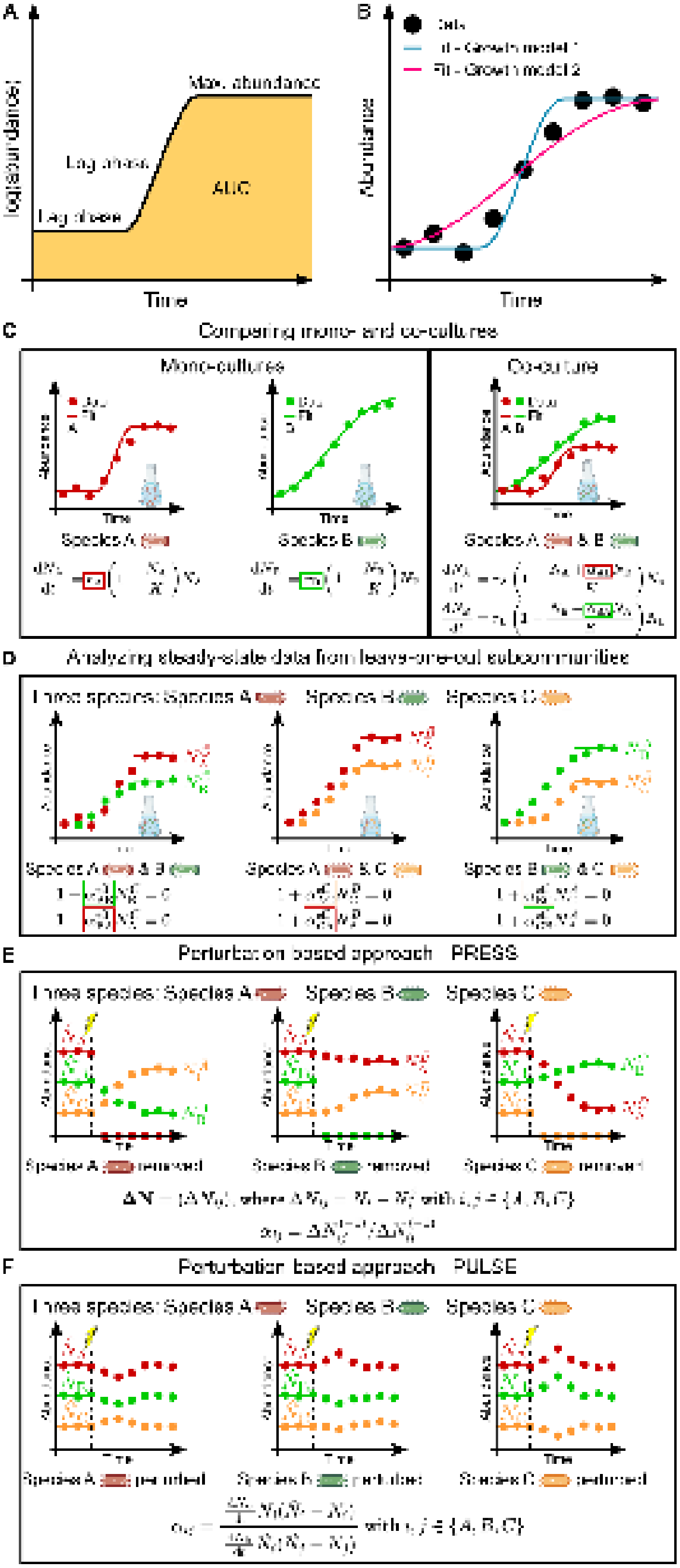

Figure 3: **Overview of interaction inference methods from growth curve data.** Panel A shows a representative growth curve with key metrics: maximum abundance (plateau phase),

growth rate (slope during the exponential phase), lag duration (initial flat phase), and area under the curve (AUC). These metrics, compared between mono- and co-cultures, provide insights into microbial interactions. Panel B shows that fitting different growth models to mono-culture data can yield varying estimates, underscoring the model dependency of growth parameter inference. Panel C illustrates how interaction parameters can be inferred by comparing mono- and co-culture growth curves. Species-specific traits (e.g., growth rate r) are derived from mono-cultures, while interaction coefficients (α) are inferred from co-culture growth curves (42). Panel D presents a leave-one-out approach for estimating (effective) interaction coefficients in an S-species community. By carrying out S experiments, each involving the removal of one species, and measuring changes in steady-state abundances, the impact of each species on the others can be quantified (43). Panel E describes the PRESS experiment, which quantifies microbial interactions in an S-species community through S perturbation experiments, each involving the removal of one species. The difference between pre- and post-perturbation abundances for each species and experiment defines the matrix ΔN, whose inverse yields estimates of interaction coefficients (33). Panel F illustrates the PULSE experiment, where microbial interactions are inferred from transient dynamics following small perturbations (addition or removal of microbes) in each species. Immediately after perturbation, both the rate of change in abundance (dN_i/dt) and the abundance (N_i) are measured, enabling estimation of interaction coefficients (33). In Panels C–F, t denotes time, N_i represents the abundance of species i, and α_{ij} denotes the interaction coefficient between species i and j (i.e., the impact of species j on i). In Panel C, r_i and K refer to the growth rate of species i and carrying capacity, respectively. In Panels D and E, $N_i^{\bar{j}}$ indicates the maximum abundance of species i after the removal of species j. In Panels E and F, $\widehat{N}_i$ represents the maximum abundance of species i prior to perturbation.

Tool name	Implementation	Supported growth models	Pros and Cons	URL and reference
biogrowth	R package	Baranyi, Gompertz, Linear (and variants), Logistic, Richards	Pros: Can take response to environmental factors into account (secondary growth model fit) and can report parameter uncertainty Cons: No graphical user interface and no support for data import	https://CRAN.R-project.org/package=biogrowth (56)
Dashing Growth Curves	Online tool written in python (can be installed locally)	Gompertz, Logistic, Non-parametric (easy linear)	Pros: Graphical user interface, easy data import, manual selection of exponential phase, reports growth curve properties Cons: Only limited number of growth models supported	https://dashing-growth-curves.ethz.ch/ (57)
gcplyr	R package	Non-parametric	Pros: Focus on easy data preprocessing and manipulation, reports area under the growth curve, lag phase, diauxic shifts and several other growth curve properties Cons: No parametric growth models supported	https://mikeblazanin.github.io/gcplyr/ (58)

growthrates	R package	Non-parametric (easy linear, splines), Baranyi, Exponential, Gompertz, Huang, Logistic, Richards	Pros: User-defined growth models and model fitting to multiple data are supported Cons: No graphical user interface and no support for data import	https://cran.r-project.org/package=growthrates
Kinbiont	Julia package	Baranyi, Bertalanffy, Exponential (and variants), Gompertz, Logistic (and variants), Morgan, Richards, Weibull, Stochastic models, ODE models (including gLV and models with resource allocation), Non-parametric	Pros: Data preprocessing, fitting to multiple data, large choice of growth models, support for custom models, machine learning (symbolic and decision tree regression), automated model selection, sensitivity analysis, estimation of confidence intervals for parameters, detection of diauxic shifts Cons: No graphical user interface	https://github.com/pinheiroGroup/Kinbiont.jl (59)

Table 1: **Growth model fitting tools.** The Table provides an overview of selected growth model fitting tools.

Outlook

While considerable progress has been made in characterizing microbial interactions in well-mixed liquid environments, the three-dimensional organization of microbial communities remains one of the most significant knowledge gaps. In natural systems, microbes often grow in biofilms, aggregates, or stratified layers where spatial proximity, diffusion gradients, and attachment surfaces fundamentally shape interaction dynamics (e.g. (60)). Mechanisms like contact-dependent inhibition, quorum sensing, and metabolic cross-feeding are often constrained or enabled by spatial context (61).

Yet, many widely used experimental designs, especially high-throughput systems, implicitly assume a planktonic lifestyle and ignore these spatial complexities. As a result, findings from well-mixed cultures may not translate to structured environments such as host tissues, soil microenvironments, or industrial bioreactors. Addressing this blind spot will require the integration of tools such as confocal and light-sheet microscopy, microfabricated growth chambers, spatial transcriptomics, and 3D modeling frameworks. Ultimately, incorporating spatial structure is essential not only for ecological realism but also for advancing predictive models of community behavior.

Author contributions

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Data availability

No data were used for the research described in this article.

Acknowledgements

We thank all current and former members of the lab of Microbial Systems Biology and Didier Gonze for informative discussions. XZ is the recipient of a scholarship from the China Scholarship Council (Certificate No. 202306350040). AGD has received funding from the European Union’s Horizon Europe research and innovation program under the Marie Skłodowska-Curie grant agreement No. 101169005.

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