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Microbial interactions in theory and practice: when are measurements compatible with models?

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Most predictive models of ecosystem dynamics are based on interactions between organisms: their influence on each other's growth and death. We review here how theoretical approaches are used to extract interaction measurements from experimental data in microbiology, particularly focusing on the generalised Lotka–Volterra (gLV) framework. Though widely used, we argue that the gLV model should be avoided for estimating interactions in batch culture — the most common, simplest and cheapest in vitro approach to culturing microbes. Fortunately, alternative approaches offer a way out of this conundrum. Firstly, on the experimental side, alternatives such as the serial-transfer and chemostat systems more closely match the theoretical assumptions of the gLV model. Secondly, on the theoretical side, explicit organism-environment interaction models can be used to study the dynamics of batch-culture systems. We hope that our recommendations will increase the tractability of microbial model systems for experimentalists and theoreticians alike.

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Introduction

In microbiomes, a fair rule of thumb is that function follows composition and composition follows interactions [1,2]. For example, suppose that a patient is administered an antibiotic that kills a specific microbe in their gut community. Though not directly impacted by the antibiotic, other community members that were competing for limited resources with the target will likely thrive, while those that relied on them for secreted metabolites will collapse. Ultimately, this can lead to substantial restructuring of the community and loss of community members other than the target. This in turn can lead to dysbiosis for the patient, in the worst cases creating pathogenesis [3,4].

Such unintended consequences result from interactions, the effect of one species on the growth and death of another. As this example illustrates, these interactions can lead to complex changes to the long-term composition of the community and its stability in response to perturbations [5]. One of the principal efforts in microbiology has therefore been to catalogue the vast diversity of interaction outcomes [6], motivated by the goal to ultimately engineer stable microbiomes with desired properties. Yet, measuring these interactions and using them to build predictive models is not always straightforward in practice [7,8]. Historically, much of the theoretical ecological literature on macroflora and fauna has had interactions at its core, and the different measures of interaction strengths and their application to theoretical models have been extensively reviewed in the non-microbial context [9–11]. Two such approaches have been directly imported into the microscopic world, and are now widely used in microbial ecology [12,13]: the generalised Lotka–Volterra (gLV) and Consumer-Resource (CR) frameworks (Box 1). The question that we address in this review is whether the approaches used to couple these mathematical models to experimental interaction data from microbial communities have a valid theoretical basis.

Collecting experimental data

The first stage in the process of quantifying interactions is to collect data that contain interaction-related information from the community in question. Complex

Box 1 Modelling ecological dynamics in microbial ecosystems.

Several frameworks exist for simulating the ecological dynamics of microbial communities. Here, we briefly outline two of the most common modelling approaches used to explore experimental data, which have recently been more extensively reviewed elsewhere [12,13]. First, the gLV framework [5,30]:

$$\frac{dS_i}{dt} = S_i\left(R_i + \sum_j a_{ij}S_j\right). \quad (1)$$

Here, a_{ij} represents the interaction from species j to species i, which can be positive (j increases the growth rate of i), negative (j decreases the growth rate of i) or zero (no effect of j on i). These interactions are assumed to be direct (e.g. predator-prey-type relationships) and constant. R_i represents the intrinsic growth rate of i in isolation at low population sizes.

A second framework is that of CR models [31–36], in which interactions arise from reciprocal feedbacks between the growth of microbes and their resulting impacts on their environment. The CR framework can be generalised to consumers as well as producers, and resources as well as inhibiting substrates [36], in which case they may be called organism-environment models. For consistency with previous literature, however, we continue to call them CR models here, keeping the more general form in mind.

CR models can usually be broken into so-called 'impact' and 'sensitivity' functions, which determine respectively the effect of organisms on their environment and their growth rate in a given environment. Explicitly, the growth rate of a species i in a given environment $\boldsymbol{C}$ (where the elements C_k indicate the concentrations of different resources, toxins, pH etc.) is given by the sensitivity function g_i

$$\frac{dS_i}{dt} = S_i g_i(\boldsymbol{C}, \boldsymbol{q}_i), \quad (2)$$

where $\boldsymbol{q}_i$ represents the function parameters, such as the maximal growth rates, yield coefficients and so on. Similarly, the environment itself is affected by the collection of all species j via the impact functions $\boldsymbol{f}_j$

$$\frac{d\boldsymbol{C}}{dt} = \boldsymbol{\sigma} + \sum_j S_j \boldsymbol{f}_j(\boldsymbol{C}, \boldsymbol{p}_j), \quad (3)$$

where $\boldsymbol{p}_j$ represents the parameters of the impact function and $\boldsymbol{\sigma}$ represents sources and sinks of resources into and out of the system.

Under certain conditions, these two frameworks can be shown to be closely related. For example, in chemostat-type systems with quickly equilibrating resources, CR models map directly onto the gLV equation [31,32,35]. In general however, they result in distinct dynamics.

communities are typically sampled from the environment directly, but for finer control of conditions, communities are also often studied in the lab using one of three culturing methods: batch cultures (e.g. [14,15]), serially transferred batch cultures (e.g. [16,17]) or chemostats (e.g. [18]). While batch culture allows the study of short-term community dynamics in an environment with finite resources, the latter two system types allow investigation of equilibrium properties.

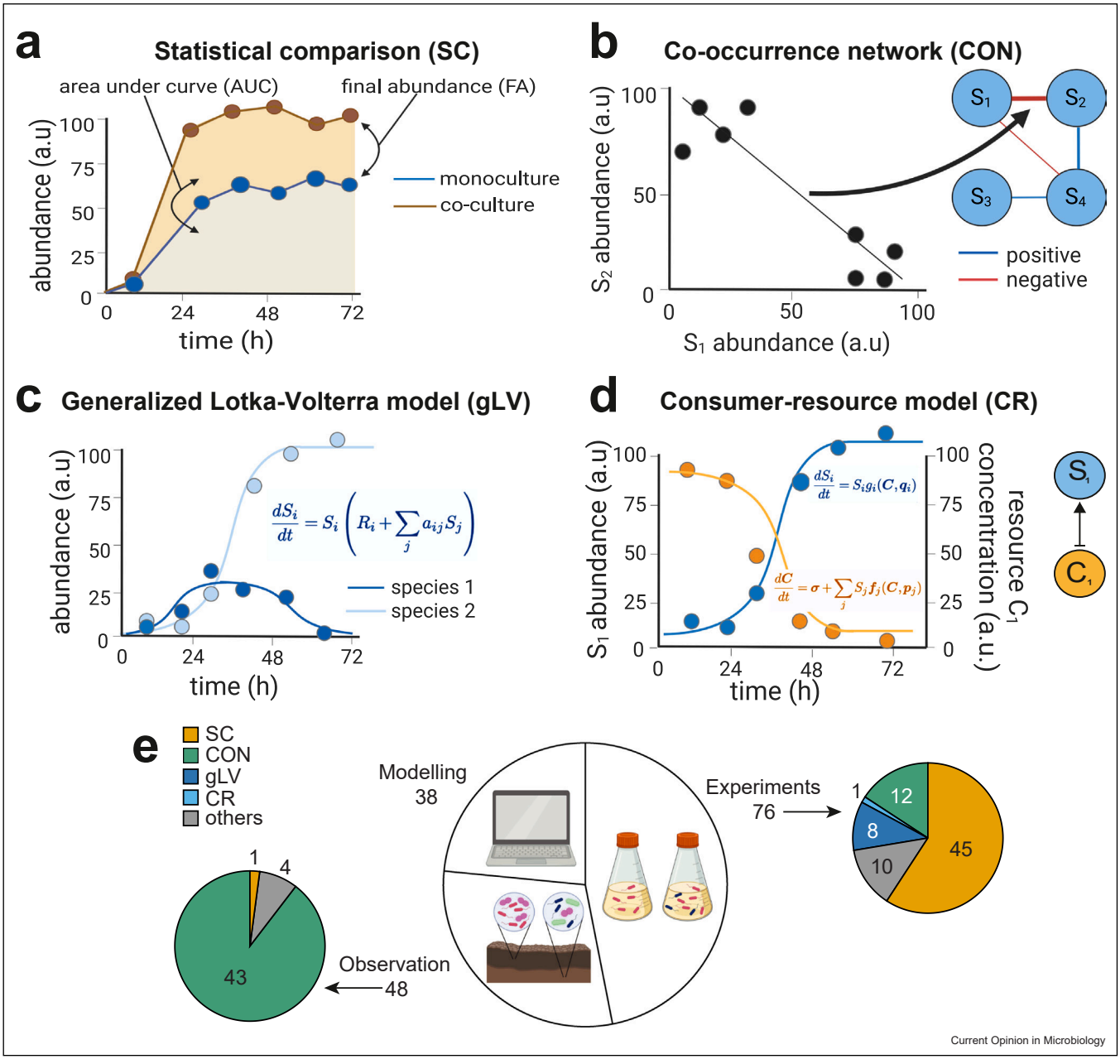
Interaction estimates are made using measurements of these communities, typically based on the population sizes of different community members, which can be measured by 16S amplicon sequencing (e.g. [16]), colony counting on selective media (e.g. [19]), microscopy (e.g. [20]) or quantitative Polymerase chain reaction (PCR) (e.g. [21]). Beyond these general approaches, many innovations are helping to increase the number of interactions measured per experiment [16,22,23]. Other approaches aim to improve the specificity of interaction measurements by culturing species in each other's spent media (e.g. [17,24–26]) or on opposite sides of a permeable membrane [27,28]. Alternatively, single-cell growth rates can be measured directly by combining microfluidics and microscopy [14]. One can also avoid quantifying population sizes altogether and instead rely on indirect measures of community function, such as respiration rates [19,26,29]).

Extracting interactions from data

Once data about a community have been collected, the next step is to pull some measurement of the constituent interactions out of it. The first type of approach is to remain agnostic to any particular model of the dynamics of interactions and define an interaction using some summary statistic, typically comparing population abundance curves of monoculture and co-culture conditions (statistical comparison (SC), Figure 1a). Interactions can then be defined as the difference between final abundances (FA) [22,24,29], or the area under the abundance curves (AUC) [19,24]. Alternatively, correlations can be measured between the abundances of species pairs, which is known as a co-occurrence network (Figure 1b, CON). Such correlations are sometimes interpreted as species interactions. As the issues with this approach have been discussed elsewhere [37–39], we do not discuss it further here.

While statistical approaches are broadly applicable, they are blind to ecological theory and do not directly translate into model parameters. A second broad approach that overcomes this issue is therefore to parameterise a mathematical model of community dynamics directly from community data. The most popular of these approaches uses the gLV framework (Figure 1c, Box 1). As in the case of SCs, one way of parameterising the gLV equation is to culture a species alone and in co-culture

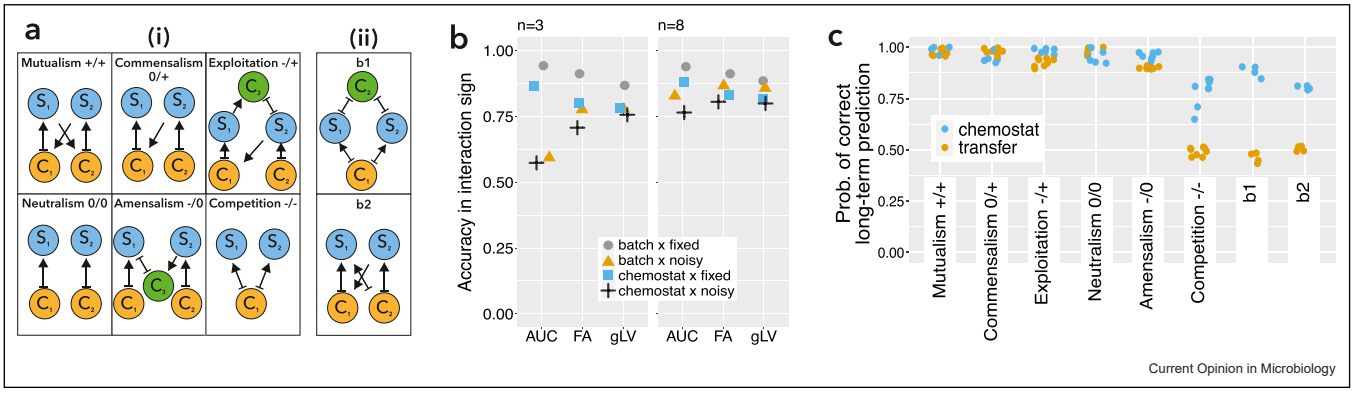
Figure 1



Summary of interaction measurement approaches. **(a)** SC of FA (at time point 72 in this figure) or areas under growth curves (AUC, shaded areas) between mono- and co-cultures. **(b)** A CON can be built by measuring correlations between the abundances of a species pair. **(c)** Parameters of a gLV model (R_i, a_{ij}) can be estimated using a fitting algorithm. This describes interactions between species S phenomenologically (see Box 1). **(d)** Parameters of a CR model (functions gi and f_j) can be estimated. Here, interactions between species S are mediated by chemical compounds C in the environment (see Box 1). **(e)** We classified previous studies of microbial interactions into three categories: experiments (laboratory cultures and/or manipulation of culture conditions), observations (collecting natural samples without manipulations) or modelling (i.e. in silico systems). Observation and experimental studies are then further categorised into 5 different approaches for extracting interactions (illustrated in panels **(a)–(d)**): SCs, CON, gLV models, CR models and others. Created with Biorender.com.

with a partner species, and then fit the interaction parameters to the abundance data [15,40]. Another approach applicable to longitudinal clinical and in vivo datasets is to estimate the gLV parameters by fitting them to the dynamic abundance data of all species in the community simultaneously [41–43]. Alternatively, a CR framework can be used (Figure 1d, Box 1), where parameters representing substrate uptake and production rates are

Figure 2



Benchmarking of interaction estimation approaches. **(a)** We defined eight CR networks (technically organism-environment networks, as species can be producers or consumers and compounds can be resources or toxins, see Box 1 for benchmarking interspecies interaction measurement approaches. Blue, orange and green circles represent species, resources and toxins, respectively. Arrowheads represent how each agent affects the dynamics of another: A → B represents that A has a positive effect on B, while C ⊣ D means that C has a negative effect on D. These networks are divided into two classes: in class (i), the signs of the effective interspecies interactions are fixed. In class (ii), the signs are dependent on the relative concentrations of the substrates. **(b)** We evaluated the accuracy of three methods for predicting the sign (positive, neutral or negative) of two-species interactions for the class-a networks: SCs using the area under the growth curves (AUC) or FA, or fitting of abundance timecourses to the gLV model. In each method, we evaluated the accuracy over the number of replicates (n), whether the initial abundances are fixed or noisy, and whether we simulated batch cultures or chemostats. **(c)** The probability that gLV interaction estimates correctly predict the long-term co-existence outcomes (i.e. how often the presence or absence of two species at t = 720 in gLV matches the presence or absence in the CR models) in simulated serial-transfer experiments (yellow, parameterised by batch-culture gLV fits) and chemostats (blue, parameterised by chemostat gLV fits) given the interaction networks in panel (a). Each circle differs in the number of replicates and whether the initial condition is fixed or noisy (class (i) networks only).

fitted to experimental measurements of species abundances and substrate concentrations. Such approaches can themselves adopt differing levels of detail, ranging from coarse-grained approaches in which groups of functionally related metabolites are pooled together [44] to fine-grained approaches in which the entire suite of metabolic processes of every organism in the community is accounted for [45]. In- between, explicit simulation of a specific subset of metabolic mechanisms that mediate a given interaction can provide a balance between accuracy and tractability [46,47]. The emergent properties of metabolic models with varying levels of detail have recently been reviewed elsewhere [13].

In a semi-systematic literature review by keyword search (Supplementary Note 1, Figure 1e) [48], we classified the approaches of 162 studies investigating microbial interactions as experimental (laboratory cultures and/or manipulation of culture conditions), observational (collecting natural samples without manipulations) or computational (i.e. in silico systems). Typically, observational studies relied upon CON, while in experimental studies, interactions were more commonly derived from SCs. Fitting a gLV model to the data is slightly less common than CON, and CR modelling is used only in one study (Figure 1e).

Benchmarking the approaches

Given these different approaches for estimating interactions, which are the most effective? To answer this question, we need to consider both aspects of community behaviour that interaction measurements are intended to provide information on: the short-term effect of species on each other's growth and the resulting long-term effect on community composition. To this end, we generated in silico simulations of two-species communities based on the CR networks depicted in Figure 2a (see also Supplementary Notes 2 and 3). We estimated the effect of one simulated species on the growth of another to be positive, negative or neutral from the dynamics of population abundances under batch-culture and chemostat-like conditions. We compared how often the following three methods can predict the species interactions outlined in Figure 2a (excluding b1 and b2): using SCs (AUC or FA, Figure 1a) or fitting the parameters of a gLV model. All approaches in all culture conditions were broadly accurate at assigning interspecies interaction signs, especially when large numbers of replicates (n = 8) were available (Figure 2b).

Does the accuracy of these interaction sign measurements translate into accurate predictions of long-term co-existence? As statistical methods do not have such

explicit predictive power, we addressed this question for the gLV framework. Our fitted gLV parameter estimates were used to predict long-term community composition in serial-transfer and chemostat-like conditions (Figure 2c). Taking the CR outcome as the 'ground truth' (generated by running the CR networks in Figure 2a), the long-term dynamics of serially transferred competing species — likely representative of many natural situations [29] — were quite hard to predict using gLV. This is probably because the relative strength of competition (i.e. a_{ji}/a_{ii}) is critical to determining co-existence outcomes [49]. In addition, the long-term prediction accuracy of the two scenarios where interaction sign is context-dependent (b1 and b2 in Figure 2a) was also quite low. In these cases, the gLV model failed to predict the long-term community composition when the model estimated species interactions to be competitive (Figure S2), and — as in the purely competitive scenario — the predictability was lower in the serial transfer than in the chemostat condition.

Pitfalls of interaction extraction using generalised Lotka–Volterra

It is very tempting to use the gLV framework for interaction extraction. We have a deep theoretical understanding of the model (e.g. we can analytically predict co-existence outcomes), no knowledge of the mechanisms underlying interactions is needed (e.g. the role of substrate concentrations in mediating growth rate impacts) and it tends to have few parameters (two per interaction). Yet, we have already found that the gLV model often fails to predict community co-existence in our simulated data (Figure 2c). What is causing this failure, and what does this tell us about the use of gLV models for interpreting interactions?

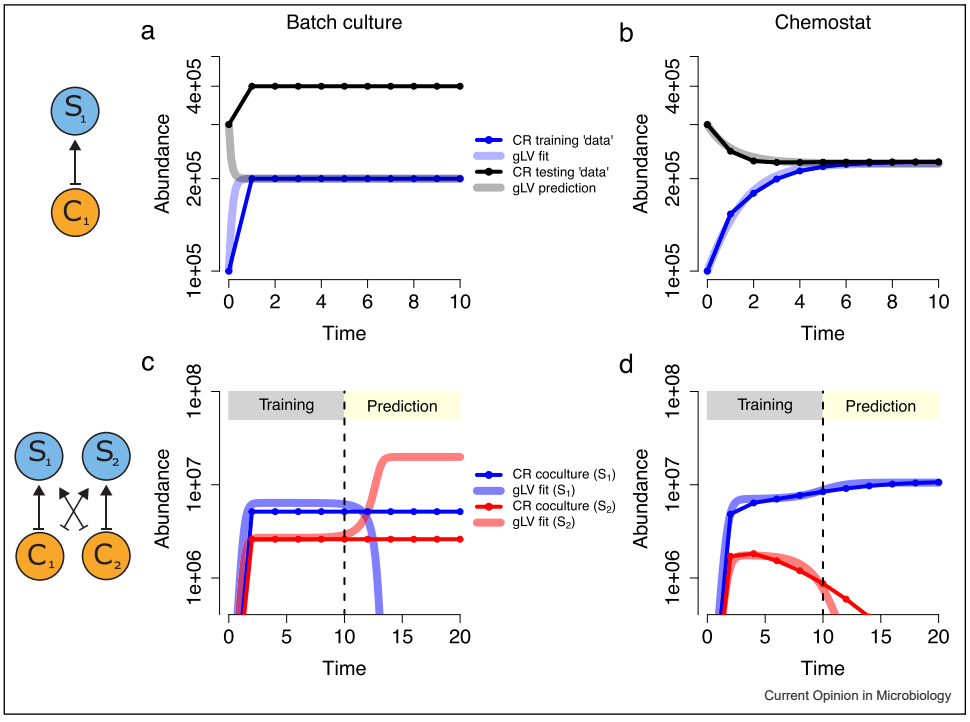
We will address this question by focusing on batch-culture data, which was the most widely used approach in our review of the literature (40 out of 76 experimental studies in Figure 1e use batch culture, of which 6 fit gLV to abundance dynamics). Both gLV dynamics and batch-culture CR dynamics share the functional form of a logistic curve, with populations reaching some saturating abundance after an initial exponential transient. One can therefore often obtain a very close fit between a batch-culture timecourse and either model. However, this apparent relationship between the two frameworks is deceiving. Fundamentally, the mechanism behind the steady state in the two systems is different: in the CR framework, it arises because resources in the system are depleted and growth rates approach zero, while in the Lotka–Volterra framework, it is assumed that (finite) growth rates are balanced by equal and opposite mortality rates — that is, that the gLV steady state is a dynamical equilibrium. Although this may seem a subtle distinction, it leads to qualitatively different predicted behaviours of the two systems.

One example of such a difference results when comparing batch monocultures initialised with different starting densities of bacteria (Figure 3a). In CR-type models, higher initial bacterial abundances lead to higher steady-state abundances [50]. On the other hand, the gLV framework assumes a constant long-term carrying capacity for a given set of parameters (although parameter changes coupled to environmental changes can be incorporated [51,52]). Consequently, simulations initialised at low and high abundances equilibrate at the same level, with the population shrinking ('dying' on net) if it begins above the carrying capacity. Even stronger differences are observed in communities. In Figure 3c, the gLV model is fitted to the early part of simulated growth curves from a batch-culture competition scenario (Figure 2a). Although the models almost match within the fitting window, beyond this point, the gLV dynamics lead to the extinction of one of the species as the fitted interaction parameters correspond to an unstable equilibrium. Ultimately, this difference between the two systems arises because the steady state is assumed to be dynamic in the case of the gLV framework and static in the case of the CR framework.

These issues have led to a number of controversies around the adequacy of batch-culture data for parameterising gLV models [15,40,53–55]. To assess how well a pairwise gLV model can reflect CR dynamics, Momeni et al. [55] simulated CR dynamics in a number of ecological systems and compared the results to gLV fits, similarly to the approach we adopt here. They demonstrated that pairwise gLV modelling is often unsuitable, particularly when resources are consumable rather than renewable. In their 2019 study, Ram et al. [40] developed a method to infer pairwise interactions from mono- and pairwise co-culture data in batch-culture systems. They first fitted monoculture data to a logistic model to obtain growth parameters such as the intrinsic growth rate of strains, then fitted a gLV competition model on total abundance data to infer competition coefficients. However, attempts by Balsa-Canto et al. [53] to reproduce their method showed that it is highly dependent on the experimental design and wrongly predicts which species win in competitive scenarios, likely due to the factors outlined above.

All is not lost however, as the gLV framework can be compatible with systems that reach a dynamic steady state [31,32,35]. This is satisfied in continuous cultures (e.g. chemostats or serial-transfer systems), in which continual dilution of cells acts as an effective mortality rate and resources are constantly renewed. In Figure 3b and d, we show that in chemostats, the CR and gLV frameworks both agree on the dependency of the dynamics on the initial conditions and on the longer-term outcome of co-cultures. Serial-transfer systems may also be reasonably approximated by the gLV equation, at

Figure 3



Batch-culture growth is incompatible with gLV fitting, whereas continuous cultures such as chemostats are compatible. **(a)** Using batch monoculture growth data (dark-blue line, 'CR training data'), we can fit a gLV (logistic) growth curve (transparent blue, 'gLV fit'). This fit is however entirely specific to the given initial resource and population-size conditions. For instance, starting from a higher initial abundance should lead to a higher yield in batch culture (black line, 'CR testing 'data', simulated from a mechanistic CR model in batch culture), while with the fit parameters, the gLV prediction leads to the same carrying-capacity equilibrium, consistent with the nature of the model (transparent black line, 'gLV prediction'). **(b)** Instead of a batch culture, we simulate a chemostat monoculture in which different initial conditions lead to the same equilibrium. Hence, the fit made on one of the initial conditions is accurate in predicting the dynamics starting from another initial condition. **(c)** Even on a given specific initial condition, gLV fitting can capture unstable dynamics by overfitting on the transient phase. Here, we fit both batch monocultures (not shown) and co-cultures of two species (blue and red dotted lines) to estimate the gLV coefficients in the initial time period ('training', light grey). However, when we extend the simulation time ('Prediction', light yellow), the gLV equilibrium is unstable and leads to the extinction of one of the two species (this is because the intraspecific competition is weaker than interspecific competition). In the extended simulated batch culture, the two species remain at the stationary phase at their maximum abundance, without additional death. **(d)** With a continuous culture, the gLV model accurately predicts what happens after the 'training phase' and leads to correct predictions regarding the co-existence or extinction of species at equilibrium.

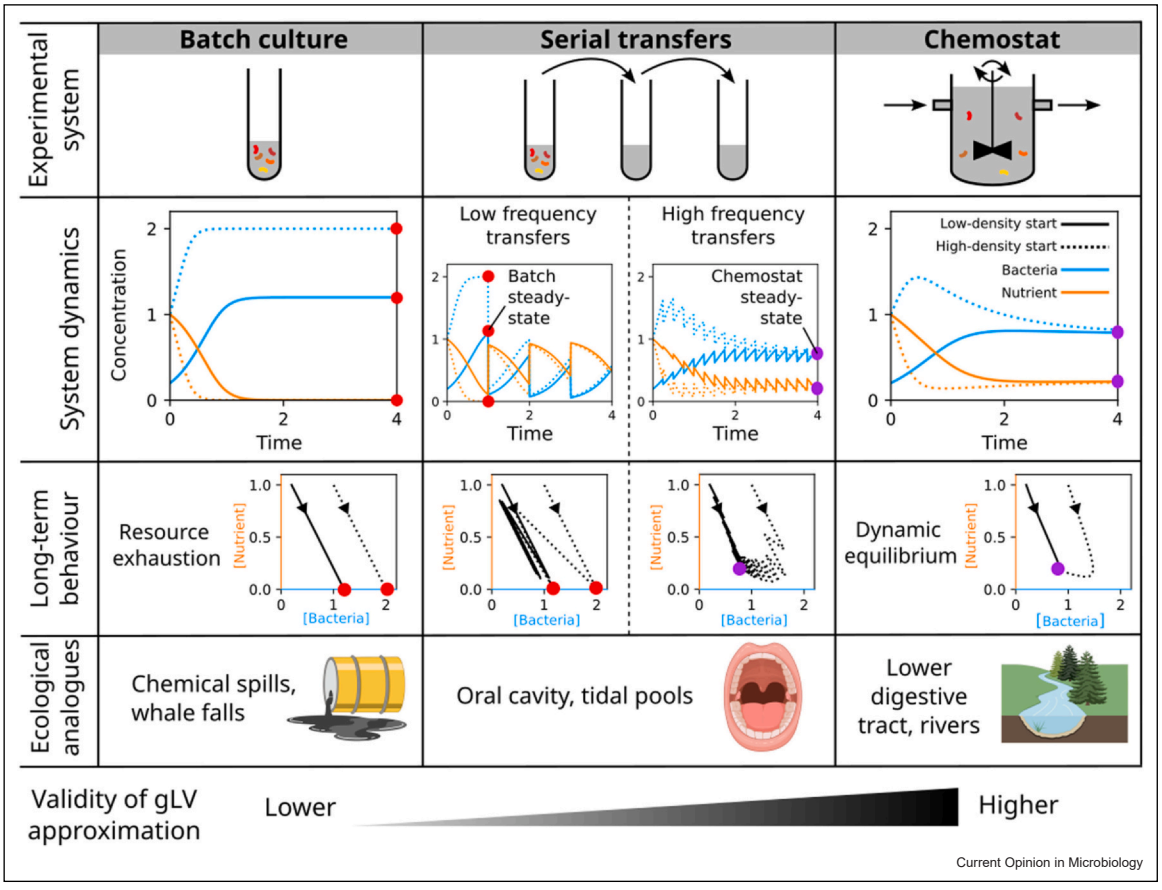
least at rapid dilution frequencies [56]. However, in these cases, it is best to fit the gLV equation to the global dynamics of the entire serial-transfer experiment. As we discuss in Supplementary Note 4 and Figures S3–S6, fitting to a single dilution round can lead to inaccuracies such as the incorrect prediction of the winner of a competition scenario. Whether or not the gLV framework can always correctly capture the dynamics of continuously fed systems is yet to be confirmed, but there is no reason in principle to avoid using gLV for a chemostat-like system such as the mammalian gut.

Are there viable alternatives to generalised Lotka–Volterra?

If the gLV framework is not always appropriate for determining interactions, how do the other methods compare? SCs have the advantage that no underlying model needs to be defined that generates the observed microbial dynamics, and so no parameters need to be estimated either. One downside is that they depend on arbitrary experimental decisions, such as the length of a batch-culture experiment. However, a bigger issue is that unlike the gLV framework, they cannot generate predictions as they are purely descriptive.

As discussed in Box 1, CR models, or organism-environment interaction models generally, offer an alternative dynamical framework for representing microbial ecosystems that can be used for predictive purposes. The strongest assumption that they make is that all interactions are mediated by the uptake and production of chemical substrates. They therefore cannot incorporate direct effects between microbes such as predation [57] or contact-dependent interactions [58,59]. Nevertheless, in a well-mixed community where interactions are chemically driven, a CR model can capture the context

Figure 4



The validity of the gLV framework varies for different types of ecological systems. In the 'system dynamics' section, we show timecourses of a simple CR simulation with a single bacterial species and a single nutrient cultured in a batch-culture system, a serial-transfer system or a chemostat. All serial-transfer and chemostat systems are diluted at the same rate over long periods of time. In the 'long-term behaviour' section, we show phase portraits representing the long-term dynamics of the nutrient and bacterial abundances from the same simulations. In each case, we show separate simulations for systems initialised with high and low densities of bacteria. These converge for all systems other than batch cultures, either on the same cycle (serial transfers) or the same steady state (chemostats). Red points indicate the steady-state outcome of the batch-culture system, while purple points indicate that of the chemostat. Images in the Ecological analogues section created with Biorender.

dependency of interactions [19,60]. Another important phenomenon that can be captured is that of higher-order interactions, the change in pairwise interactions caused by the addition of new species to the community [61–63]. This makes them a powerful tool for more flexibly predicting long-term dynamics.

The downside of CR models is that parameterising them relies on knowing the concentrations of chemical substrates that are supplied by the experimenter, or that are taken up or produced by the microbes. Quantifying rates of microbial substrate uptake and production can be quite challenging compared with measuring interactions directly [46]. However, if more species are added to the system, the number of parameters scales only linearly for CR models, but quadratically if pairwise interactions need to be measured (i.e. in gLV). Parameterising CR models can also be aided by using chemically defined growth media, conducting metabolomics analyses [44,64] or by building metabolic models for the interacting species and using them to predict substrate consumption and production [12,13].

Under certain circumstances, CR models can still be accurately parameterised without time-series data of all substrate concentrations. For example, knowledge of initial substrate concentrations and the time at which growth ceases are sufficient for estimating consumption rates [65]. Similarly, if it is known that one substrate is rate-limiting (e.g. a sole carbon source), other unknown ones may be negligible. Chemostats are also very convenient for parameterising these models, as one can estimate consumption rates based on the steady-state populations at a known dilution rate [66,67]. Ho et al. [68] have even shown that a CR model with randomly sampled parameters can recapitulate the statistical

properties of real microbiomes (see also [69]). Despite these encouraging findings, though, exactly how much information regarding the chemical environment is needed to correctly parameterise a CR model is an important open question.

Conclusions and recommendations

In this review, we have shown that the gLV framework can be more or less applicable to understanding microbial ecosystems driven by CR dynamics, depending on the system in question. We summarise these arguments in Figure 4. In systems that resemble batch culture, the assumptions of the gLV framework are broken as the steady state that is approached is not dynamic: perturbing the abundance of one species has no effect on the growth rate of the others. By contrast, chemostat-like systems generally stabilise around a dynamical equilibrium that resembles that of the gLV framework. Bridging batch cultures and chemostats, serial-transfer-type systems effectively form a continuum from low- to high-frequency transfer rates, with batch- and chemostat-type systems forming limiting cases at either end. In cases where transfers occur frequently relative to the growth rate of the community, these systems stably oscillate around the dynamic equilibrium of the chemostat and a gLV model may be appropriate. However, increasingly long transfer windows can profoundly influence the co-existence outcomes of such communities in a way not captured by the assumptions of the gLV framework [56]. Precisely when the gLV framework can reliably predict long-term outcomes is something we only partially address here (Figure 2c), and would be important to explore further.

Ultimately, the ideal choice of interaction measurement depends upon both these considerations of the experimental setup and the end to which the measurement will be applied. In many cases, such as detecting overlap of substrate use by two species, a relatively straightforward approach such as pairwise batch-culture experiments combined with a statistical interaction measurement will be entirely sufficient. We hope that the principles that we have laid out here will help guide the design process of experimentalists, as well as highlight some critical properties of microbial systems that theoreticians should take into account when modelling such communities.

Data Availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mib.2023.102354.

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- • of special interest
- •• of outstanding interest.

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