Review

Quantifying microbial interactions: concepts, caveats, and applications

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Microbial communities are fundamental to every ecosystem on Earth and hold great potential for biotechnological applications. However, their complex nature hampers our ability to study and understand them. A common strategy to tackle this complexity is to abstract the community into a network of interactions between its members — a phenomenological description that captures the overall effects of various chemical and physical mechanisms that underpin these relationships. This approach has proven useful for numerous applications in microbial ecology, including predicting community dynamics and stability and understanding community assembly and evolution. However, care is required in quantifying and interpreting interactions. Here, we clarify the concept of an interaction and discuss when interaction measurements are useful despite their context-dependent nature. Furthermore, we categorize different approaches for quantifying interactions, highlighting the research objectives each approach is best suited for.

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Introduction

Microbial communities are extremely complex; they are highly diverse species assemblages where the growth and activities of individuals are affected by those of others in the community and by their abiotic environment [1]. Studying such complex systems requires measuring and modeling at the right resolution — one that would not require probing every single cell but would still provide predictive and explanatory power [2]. A common approach that has long been used by ecologists is describing the behavior of a community as arising from a network of interactions between species. This approach has also gained popularity in microbial ecology, partially due to the availability of an array of techniques that enable measuring thousands of interactions in a single experiment [3–5]. However, these technical abilities do not alleviate the conceptual challenges that accompany the study of interactions — what are the proper uses for interaction measurements, how to interpret them, and how to properly quantify interactions. Here, we aim to clarify the utility of microbial interactions, explore various approaches of quantification, and establish a connection between the two.

What do we mean by ecological interactions?

In the most general sense, biological interactions are mutual influences between individuals, where the presence or actions of one individual affect those of another. In ecology, we typically consider interactions as the effects individuals have on each other's survival and reproduction rates [6]. These can be aggregated into interactions between populations, describing how one population affects the overall changes in the growth or abundance of another population. Importantly, such interactions provide a phenomenological description of the effects populations have on each other's growth, regardless of the underlying mechanisms — whether a change in growth is caused by one mechanism or another, the inferred interaction remains the same. Notably, this definition excludes more mechanistic approaches, such as consumer-resource models or metabolic modeling, where species' interactions are not modeled explicitly [7]. Finally, interactions represent causal effects rather than mere statistical relationships (such as correlations in abundances over space or time). Due to the inherent difficulty of inferring causal relationships from observational data (such as co-occurrence analysis) [8,9], here, we focus on interaction measures that rely on direct manipulation of species abundances.

Classic examples of interspecific interactions, such as the fox and the hare or flowers and pollinators, prime us to think of interactions as constant relationships that are intrinsic to the species involved. However, the interaction between two microbes typically involves multiple

Figure 1
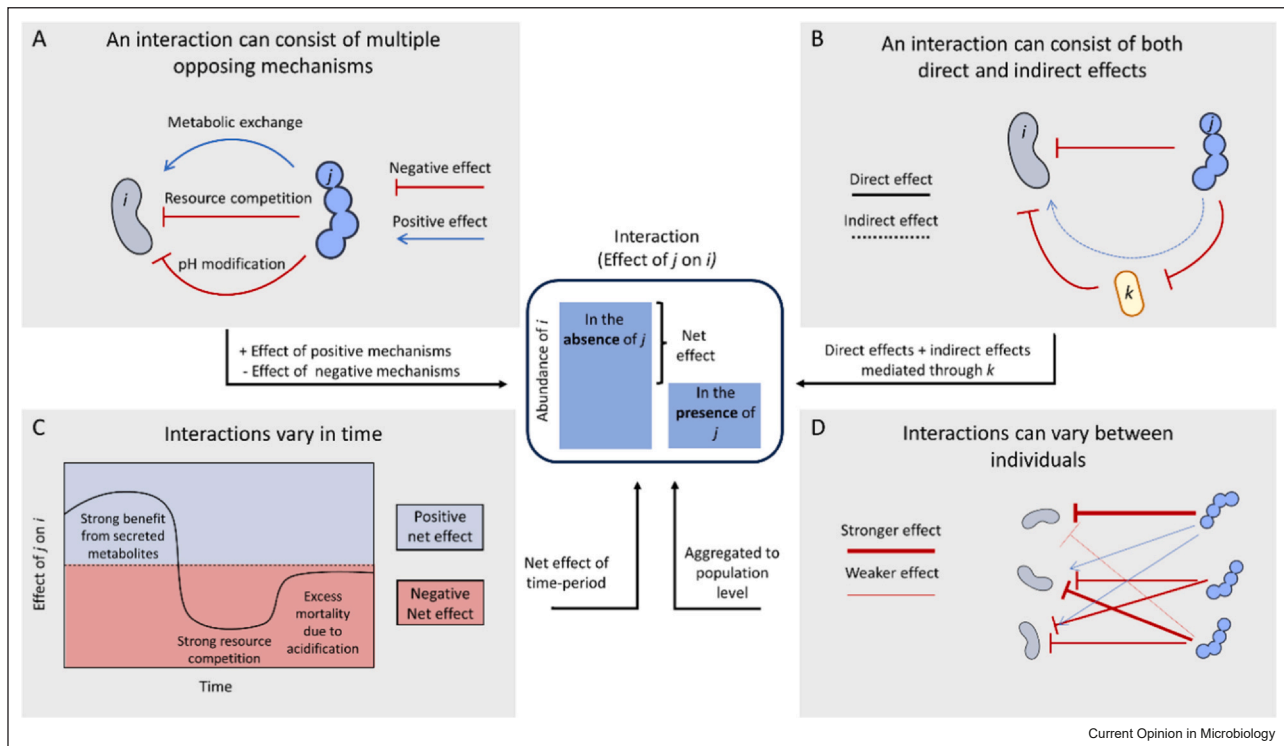
An interaction is the net effect one microbe has on another's reproductive success in a specific time frame and a specific set of abiotic and biotic conditions. **(a)** An interaction can consist of multiple opposing mechanisms, and their effects on reproduction are aggregated. **(b)** In a multispecies community, interactions consist of both direct effects and indirect effects that are mediated through a third species. Indirect effects could be excluded under some experimental setups. **(c)** Due to transitions in physiological states and nonlinear dependencies on abundance, interactions could vary significantly in time but are commonly measured as the net effect over some time interval. **(d)** Interactions could vary significantly between individuals due to genetic or nongenetic variability within each population.

mechanisms, is often mediated by the environment (e.g. consumption or secretion of nutrients), and is highly dependent on species' physiology in the given conditions [1,10,11]. Therefore, the interaction between bacterial species often changes dramatically with time [12], space [13], and species' abundance [14] (Figure 1). For example, the concentration of a single nutrient in the environment could shift facilitative relationships to competitive (or vice versa) [15,16]. The potentially extreme sensitivity of interactions to conditions is further demonstrated by a recent study that found that the temperature at which two species grow before interacting determines which one would be the prey and which would be the predator [17]. Notably, a change in an interaction over time can occur even in the absence of an external change in the environment due to the species' own activities, such as resource consumption or secretions, that change their environment or physiological states. For example, an interaction could be net-positive due to cross-feeding of micronutrients when carbon source is abundant, but shift to net-negative after most carbon source is utilized due to resource competition or accumulation of toxic metabolic by-products (Figure 1c).

Therefore, it would be inaccurate to report 'The Interaction' between two species. Rather, an interaction is the net effect one microbe has on another's reproductive success in a specific time frame and a specific set of abiotic and biotic conditions. This raises the question — if interactions are so contingent on conditions and time over which they are evaluated, is measuring them simply 'stamp collecting'? Or is something gained by doing so?

Why measure interactions?
Predicting composition and function
The ultimate aim of the reductionist approach to community ecology is to predict the dynamics of a community's composition and function based on interactions within the community [18] (Figure 2a). This is most commonly attempted using the generalized Lotka–Volterra model (gLV), which has been shown to provide predictive value in simplified and natural communities [19–22]. However, microbial interactions involve nonlinear and nonadditive mechanisms, such as quorum sensing [14] and diauxic shifts [23], and even seemingly simple mechanisms of resource consumption and

Figure 2
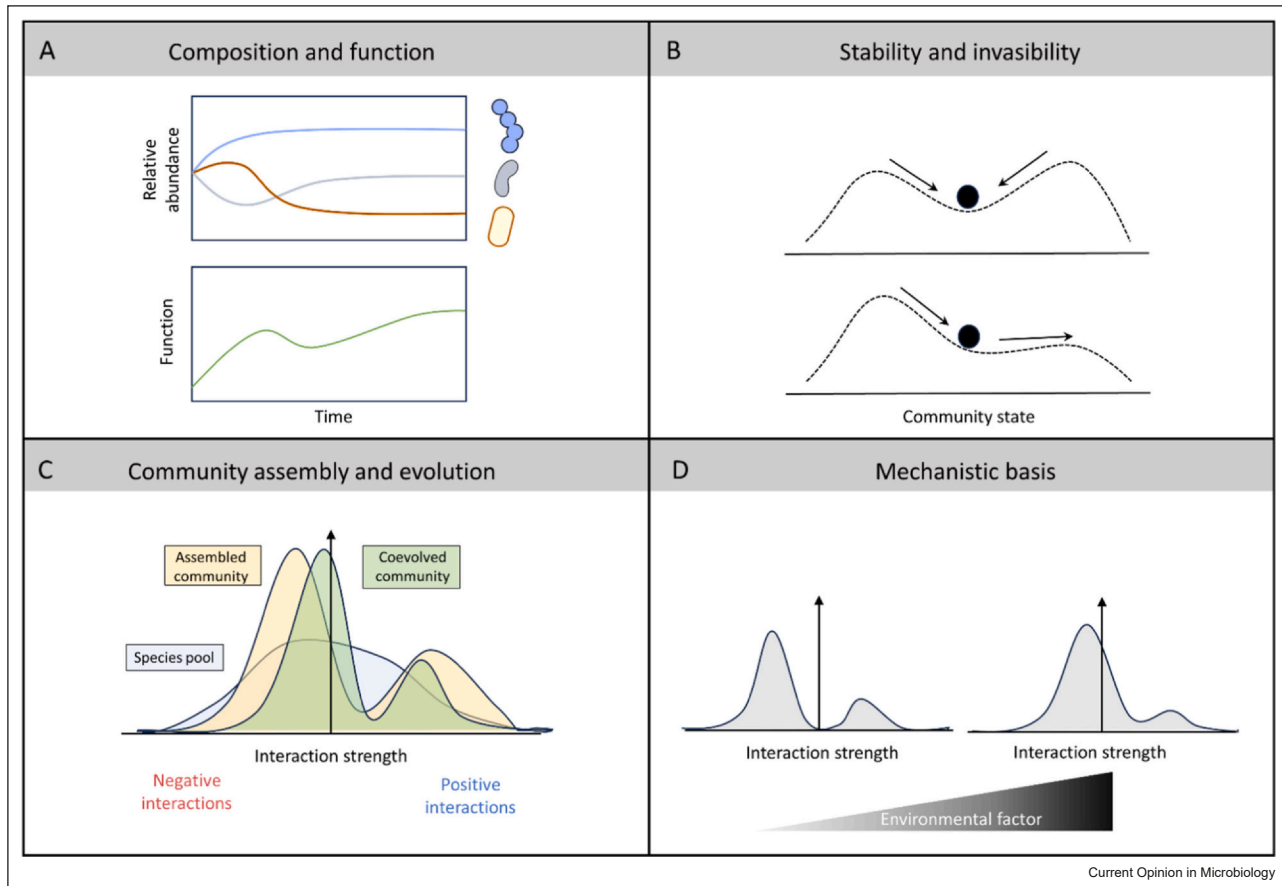
Interactions are used in a range of applications in microbial ecology. **(a)** To predict the composition and functions of communities. **(b)** To study the ability of a community to withstand biotic and abiotic perturbations. **(c)** To gain insight into how communities are formed and how they are expected to evolve. **(d)** To elucidate the mechanistic basis of the effects species have on each other.

secretion may not be well approximated by linear pairwise interactions assumed by the gLV model [24], thus limiting the applications of such an approach.

Even when making detailed predictions about community dynamics and functions is not possible due to such complications, interactions can still provide valuable information. Interactions have long been studied in the ecology of higher organisms, where detailed predictions have not been regarded as the main goal [6]. Rather, these areas have typically focused on more qualitative features of the communities and the forces that shape them. Similar ideas have also recently been applied to microbial communities, as detailed below.

Stability and invasibility

Interactions are central to understanding community stability — whether species will coexist stably, go extinct, or fluctuate in abundance (Figure 2b) [25–28]. Theoretical work has linked community stability with the distribution of types, strengths, and correlations between interactions in a community [28]. For example, intransitive interactions are expected to lead to coexistence-promoting fluctuations [29], and local stability is predicted to decline with interaction strength and the proportion of positive interactions [30], whereas a skewed distribution composed of few strong and many weak interactions is predicted to enhance local stability and persistence [25]. Recent experimental work has validated some of these predictions in microbial communities [26]. Similarly, the ability of communities to resist invasions has long been considered to be related to interactions [31], and recent studies have gained empirical evidence for the connection and advanced our theoretical understanding of it [32,33]. Finally, measuring interactions could also be used in order to identify keystone species that have a high impact on the stability and function of the community [34].

Community assembly and evolution

Species interactions can provide insights into how communities were formed [35,36] and how they are

expected to evolve [37–39] (Figure 2C). For example, theory predicts that correlations between the way a species interacts with other community members could result solely from the assembly of a stably coexisting community, regardless of species-specific traits that drive the interactions [40]. There is also empirical evidence that interactions within microbial communities are not randomly distributed, which may reflect their assembly or evolutionary history: interaction distribution can differ between sympatric and allopatric species [41], and interaction networks are often hierarchical [42].

Evolutionary theory makes predictions regarding how interactions would evolve. These include elucidating the conditions under which evolution leads to increased co-dependency through loss of redundant functions [37], evolutionary arms races [38], or niche differentiation [39]. Several of these predictions have recently been tested experimentally in microbial communities [43–47]. Since interactions affect community structure and function, understanding the feedback between the evolution of interaction and community properties has several implications, including predicting which communities will have their function altered rapidly by evolution and which will maintain it for longer. Such predictions could inform the design of interactions that will select for desired functions, enabling improving community function using artificial selection [48]. However, a more general, predictive understanding of how changes in interactions lead to evolutionary changes in community function is still an ongoing challenge.

Elucidating the mechanistic basis

Measuring interactions can also help elucidate their mechanistic basis, and consistent changes in interactions across abiotic conditions suggest common mechanisms driving the interactions [15,16,49–51] (Figure 2d). For example, facilitation is more commonly observed in anaerobic conditions, and metabolic simulations suggest this is driven by secretion of more energy-rich metabolic by-products [50]; strains that are unable to grow on a specific carbon source are almost always promoted by growing strains, suggesting common cross-feeding interactions [16]; and high carbon source concentrations in unbuffered environment could drive interactions toward stronger competition due to stronger pH modifications [49]. A mechanistic understanding of what drives interactions is crucial for anticipating which interactions are likely to occur in novel conditions or how environmental changes would modulate interactions and may allow linking environmental conditions to interaction-driven community properties, thus alleviating some of the issues regarding the contingency of specific interactions.

## Which information do we need in order to infer interactions?

Since interspecific interactions are the effects species have on each other, measuring them is best achieved by directly manipulating species' presence or abundance and quantifying the resulting changes in abundances or growth rates. Manipulating community composition is straightforward in synthetic communities assembled in the laboratory but is more challenging in self-assembled communities. Ecologists have long manipulated species' abundance in seminatural settings [6] (e.g. by removing or enclosing specific species), but such interventions have thus far not been common in microbial ecology. In contrast, quantifying abundances and growth rates both in laboratory and natural settings is a common task in microbial ecology, and there are many established techniques for doing so [52], although these are more well established for well-mixed conditions and spatially structured communities, such as biofilms, are more challenging to quantify [53]. However, as interactions can vary greatly in time [12], space [13], and between individuals, one needs to consider what is the appropriate measurement technique. Measurement methods range from plating and counting colonies [3], which gives limited resolution as cultures can not be sampled too frequently, to microfluidic devices that enable real-time measurements at the level of a single cell [12,54].

After estimating growth in the presence, and absence, of a partner, there is still a need to decide how to quantify the interaction — that is, which interaction index should be reported and how it should be interpreted. Interaction indices can be classified into four levels — *sign*, *semiquantitative*, *quantitative*, and *parametric* (Figure 3). While it may seem that the more informative level should always be preferred, more qualitative levels are typically easier to obtain and are more robust to technical and biological noise, making them more reproducible and their interpretation more consistent across modeling assumptions. Therefore, we argue that more qualitative measures should be preferred when they suffice to address the research question at hand.

Sign

An interaction could be reported solely as being positive (facilitative) or negative (inhibitory). While simple, the sign of an interaction can hint at the underlying mechanism. For example, a positive interaction changing to a negative one in response to nutrient supplementation suggests cross-feeding [55]. Additionally, the frequency of interaction signs can be related to the evolutionary processes shaping communities. For example, positive interactions are predicted to be scarce in unstructured environments [56]. Finally, the stability of a community can sometimes be inferred solely from interaction signs [30,57].

Figure 3
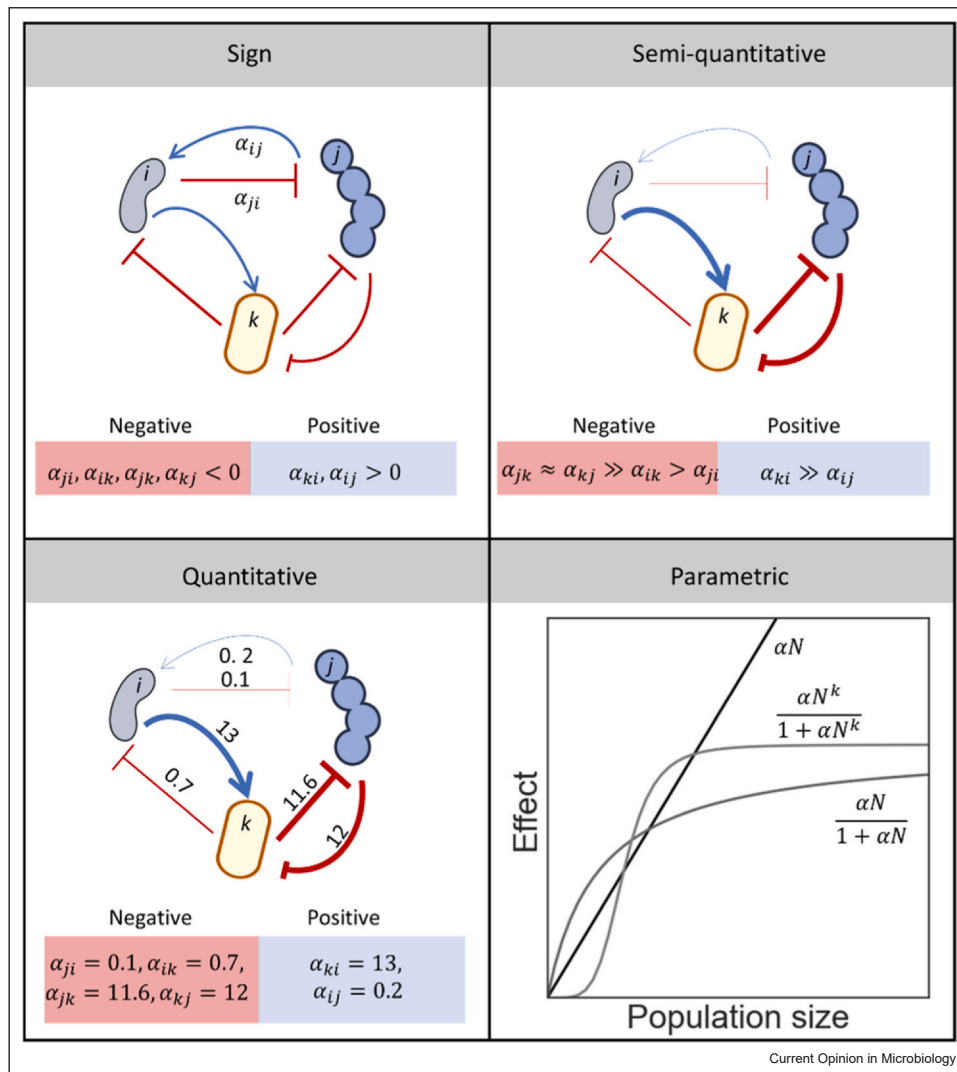
Interaction indices can be classified into four levels. Sign: a qualitative assessment of whether the interaction resulted in a net increase or decrease in reproductive success. Semiquantitative: an estimate of the order-of-magnitude of interaction strength and which interactions are stronger than others. Quantitative: a quantitative estimate that gives an interpretable number. Parametric: a quantitative estimate that corresponds to some parameter in a dynamical model. In this case, the interaction is not a number, but a function. The plot depicts three response functions that are commonly used to describe interactions.

Semiquantitative and quantitative

Ecologists have developed several indices that are commonly used to quantify interaction strength that differ in their interpretation and statistical properties (Box 1). These indices are typically interpreted as an accurate *quantitative* estimate of the effect one partner has on another. For example, an estimated log response ratio of 1 would be interpreted as indicating that the presence of a partner causes the abundance of the affected species to double. Such interpretation could often be important, for example, if researchers wish to understand whether communities adhere to some quantitative stability criteria [57] or to study the additivity

between interactions [58]. However, such interpretation also demands care for the biases that could arise from both measurements and indices (Box 1).

One could also interpret the estimated indices as *semiquantitative*. That is, as a representation of the order-of-magnitude estimate of the interactions' strength. Such interpretation has the benefit of not committing to the accuracy or linearity of the measurement method. Many of the goals we've mentioned above require no more than a *semiquantitative* measurement. For example, measuring how interactions evolve [59] or how they change due to environmental factors [49] typically

Box 1

Generally, interaction indices quantify the effect of one species on another by comparing the growth of the affected species (i) with and without the affecting species (j). *While such indices, including the ones discussed below, typically consider the change in abundances, theoretical models typically consider changes in growth rates, and care should be taken when comparing theoretical predictions to empirically determined interactions.* For simplicity, we consider the case where the comparison is between the affected species' abundance when grown alone (monoculture, M_i) and together with the affecting species (coculture, C_i). However, the indices discussed below also apply when other species are present.

Interaction indices differ in how the comparison between monoculture and coculture is quantified and whether the abundance of the affecting species (C_j) is considered. Therefore, they differ in their mathematical properties, and more importantly, in how they should be interpreted. Below, we discuss the main differences in the context of four commonly used indices (Figure B1): Raw Difference (RD), Paine's Index (PI), Log Response Ratio (LRR) [63], and Relative Intensity Index (RII) [64].

Perhaps, most significant is whether an index is normalized to the abundance of the affecting species (C_j). Non-normalized indices, such as RII and LRR, describe the overall influence of the entire affecting population without considering its size. Normalized indices, such as PI and RD, describe the influence per unit of affecting species and are therefore affected by the way in which abundances are quantified. For example, a normalized index may give the effect per individual, or per unit of biomass, per 16S rRNA count. Notably, indices can readily be modified from population-level to per-capita and vice versa. Some of these modifications are common in the literature. For example, the per-capita version of LRR is often referred to as the Dynamic Index [63]. Nonetheless, deciding whether to use a population-level or per-capita index can have a significant effect on the interpretation of an interaction, as rare species could exert strong per-capita effects, and abundant species, seemingly strongly interacting, could be revealed to have a weak per-capita effect.

Indices also differ in the range of possible values and in how they vary with changes in monoculture and coculture abundances. For example, RII is bound between −1, and 1, while LRR could increase or decrease to infinity. Per-capita indices are typically bound by the minimal value of abundance of the affecting species. Some indices, such as PI, are asymmetrical: PI is bound for negative interactions, but positive interactions could be infinitely strong. This asymmetry makes some sense — the strongest negative interaction is one where only one individual of population *j* is needed in order to drive population *i* to extinction, while any increase in abundance due to a positive interaction is theoretically possible. However, when using PI one should consider its asymmetry when interpreting the results — positive interaction might seem stronger than negative interactions merely due to this property. Many indices, such as PI and LRR, are undefined when the affected species cannot grow alone ($M_i = 0$). Therefore, these indices could not be used to quantify obligate facilitation. Even when populations are small but detectable, these indices should be used with care, as they are highly sensitive to biological and technical noise that occurs in small populations. Finally, some indices correspond to an interaction parameter in a dynamical model when some conditions are met. Both PI and RD are equivalent to a gLV interactions parameter, given that the community has reached a steady state (Supplementary Materials 1).

	Raw Difference (RD) $\frac{1}{C_j} \cdot (C_i - M_i)$	Paine's Index (PI) $\frac{1}{C_j} \cdot \frac{C_i - M_i}{M_i}$	Log-Response Ratio (LRR) $log \frac{C_i}{M_i}$	Relative Intensity Index (RII) $\frac{C_i - M_i}{C_i + M_i}$
Functional dependance on abundances				
Range	$(-\infty, \infty)$	$\left(-\frac{1}{C_j}, \infty\right)$	$(-\infty, \infty)$	$(-1, 1)$
Distribution under uniform abundances				

M_i - abundance of the affected species (*i*) when grown alone
C_i - abundance of the affected species (*i*) when grown in coculture with *j*
C_j - abundance of the affecting species (*j*) when grown in coculture with *i*

So, when should each index be used? When choosing an index, one first needs to acknowledge that there is usually no clear right choice and that it ultimately depends on the nature of the data and question at hand. However, some guidelines could still be given: (i) when communities are far from equilibrium, using an absolute index is likely of limited use as the measured interaction would depend strongly on the choice of initial abundances. Per-capita indices should be less sensitive, at least if the functional response is close to linear. (ii) When one of the organisms grows poorly, it is better to use an index that is defined at zero growth (e.g. RII). (iii) When one aims to use some model (e.g. for predicting community dynamics), it is important to use synthetic index that corresponds to this model.

requires understanding when interactions are stronger or weaker, and the precise magnitude of difference is of less interest. Likewise, some properties of community dynamics, such as time delays or oscillation, can be linked to the topology of the interaction network, regardless of their exact strength [60]. Finally, detecting keystone species can be achieved without exact quantification of interaction strength. While such interpretation of the interaction measurement is generally insensitive to the choice of index, it is sensitive to whether the interaction is quantified as the overall effect of one population on the other or is normalized to the abundance of the affecting species (the per-capita effect of one population on the other; Box 1).

Parametric

When trying to predict how a community changes in time or in response to perturbations in species abundances, one typically needs to quantify interactions in a way that represents specific parameters in a dynamic model — most commonly, gLV (reviewed recently [61]). For this purpose, interactions need to represent the effect of one species on another's growth as a function of their abundances, rather than the overall net effect over some time. Inferring a *parametric* interaction can be challenging, as it requires large amounts of data, typically a time series, as well as selecting the correct parametric model (e.g. whether the effects are linear or saturating) or at least a reasonable approximation thereof. Nonetheless, there are instances where such models have some predictive value [19,20,22]).

Conclusions

Complex systems can be studied at multiple different levels, from the details of their basic building blocks to an effective description of the entire system as a whole. Different levels of description each have their merits and limitations, so choosing the 'the right' level depends on the research's specific aims. Mechanistic approaches such as consumer-resource models [62] or more holistic ones, such as statistical modeling [7], have recently gained traction in microbial ecology. Interactions are an intermediate between mechanistic and holistic approaches that provide some of the intuition about how a systems' properties emerge from its constituents while not requiring as much detailed knowledge about the system as required by more mechanistic models. Therefore, we argue that interactions, if properly quantified and interpreted, remain an essential tool in the arsenal of microbial ecologists.

Data Availability

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

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.

Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mib.2024.102511.

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