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Coexistence Theory for Microbial Ecology, and Vice Versa

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ABSTRACT

Classical models from theoretical ecology are seeing increasing uptake in microbial ecology, but there remains rich potential for closer cross-pollination. Here we explore opportunities for stronger integration of ecological theory into microbial research (and vice versa) through the lens of so-called "modern" coexistence theory. Coexistence theory can be used to disentangle the contributions different mechanisms (e.g., resource partitioning, environmental variability) make to species coexistence. We begin with a short primer on the fundamental concepts of coexistence theory, with an emphasis on the relevance to microbial communities. We next present a systematic review, which highlights the paucity of empirical applications of coexistence theory in microbial systems. In light of this gap, we then identify and discuss ways in which: (i) coexistence theory can help to answer fundamental and applied questions in microbial ecology, particularly in spatio-temporally heterogeneous environments, and (ii) experimental microbial systems can be leveraged to validate and advance coexistence theory. Finally, we address several unique but often surmountable empirical challenges posed by microbial systems, as well as some conceptual limitations. Nevertheless, thoughtful integration of coexistence theory into microbial ecology presents a wealth of opportunities for the advancement of both theoretical and microbial ecology.

1 | Introduction

Sustained improvement in sequencing technologies, database integration, and bioinformatics is accelerating the accumulation of microbial community census data. At the same time, there is growing awareness that these empirical developments have outpaced advancements in our mechanistic understanding of microbial community dynamics (Prosser 2020). To address this imbalance, there has been a recent push to integrate models and frameworks from theoretical ecology into microbial ecology (Picot et al. 2023; Berg et al. 2022; Prosser et al. 2007; Meroz et al. 2024), with theory-driven research already providing deep insights into the assembly (Goldford et al. 2018; Friedman et al. 2017), functioning (Skwara et al. 2023), and stability (Hu et al. 2022) of microbial communities.

Understanding species coexistence and competition has been a central goal of theoretical ecology for over a century (Gause 1935; MacArthur and Levins 1967; Tilman 1982; Chesson, 2000b). With growing insight into the essential role microbes play in regulating organismal and ecosystem health, understanding the causes and consequences of species coexistence has become increasingly relevant to applied and fundamental microbiology. For instance, the maximisation of ecosystem functioning (e.g., starch degradation) in bacterial communities can require the coexistence of specific combinations of species (Sanchez-Gorostiaga et al. 2019), and the evolution of antimicrobial resistance can be shaped by how different environmental conditions influence the coexistence of susceptible and resistant strains (Nev et al. 2020; Letten et al. 2021). A number of different overlapping frameworks have been developed in ecology for understanding species coexistence (Chase and Leibold 2003; Hubbell 2011; Abrams 2022; Clark et al. 2024), some of which (e.g., resource competition theory) are especially well suited to microbial systems (Grover 1997). However, one approach that has become popular among plant and animal ecologists, but that has seen far less uptake in microbial ecology, is so-called "modern coexistence theory" (Chesson, 2000b; Barabás et al. 2018).

The prefix *modern* (dropped in the remainder of the text) is something of a misnomer in the sense that many of the core ideas date back several decades (Chesson and Warner 1981; Chesson 1990, 1994; May 1973) (albeit with continuous development to the present) and that it has not superseded other approaches (Abrams 2022). Nevertheless, coexistence theory's rapid uptake in recent years can be understood as a reaction to the confusing morass of system- and scale-specific coexistence mechanisms that ecologists had offered up by the end of the 20th century (Chase and Leibold 2003; Hubbell 2011; Vellend 2010). It was against this backdrop that John Lawton famously described community ecology as "a mess, with so much contingency that useful generalisations are hard to find" (Lawton 1999). Coexistence theory, developed primarily by Peter Chesson and colleagues (hence also referred to as "Chesson's coexistence theory") emerged as a unifying framework that brought a welcome degree of order to the study of species coexistence (Chesson, 1994, 2000b; Barabás et al. 2018; Adler et al. 2007). More specifically, coexistence theory provides a quantitative framework for sorting the many low-level (e.g., system-specific) coexistence mechanisms into a small number of high-level umbrella categories. As such, it concentrates and links many disparate processes, by showing how, for example, temporal fluctuations in temperature, nectar chemistry and rainfall, can mediate coexistence in aquatic plants, floral yeasts and annual grasses, respectively, via the same underlying mechanism (Armitage and Jones 2019b; Letten et al. 2018; Angert et al. 2009).

The purpose of this review is to highlight the wealth of opportunities for applications of coexistence theory in microbial ecology, and the equally rich potential for testing coexistence theory with microbial systems. We begin with *"A coexistence theory primer"*, which provides a brief summary of the fundamental concepts of coexistence theory, but point readers wishing to go deeper to existing reviews detailing the mathematical intricacies of coexistence theory and guidelines for its empirical implementation. Next in *"Empirical applications"* we present the results of a systematic review of existing empirical studies employing coexistence theory in microbial systems. Then in *"Mutual benefits of coexistence theory and microbial ecology"* we discuss how coexistence theory is well suited to tackling a range of fundamental and applied problems in microbial ecology, and conversely how the tractability of microbial systems makes them ideal for testing and developing emerging branches of coexistence theory. Finally, in *"Cautionary notes"* we identify some of the empirical challenges and fundamental limitations of coexistence theory, especially in the context of microbial systems. Indiscriminately applying coexistence theory as an additional "off-the-shelf" analysis risks losing sight of the ecological questions and processes of interest. Nevertheless, thoughtful integration of coexistence theory and microbial ecology holds great promise for generating new insights in both fields.

2 | A Coexistence Theory Primer

The framework of coexistence theory is best understood as two related approaches for expressing competitors' invasion growth rates as tractable combination of mechanisms fundamental to coexistence. Here, invasion growth rates are a species' (or genotype's) long term average growth rate when introduced at very low density into an established (resident) community of potential competitor species at equilibrium or within a dynamic attractor. If invasion growth rates are positive for all species within a community, then they are said to be able to coexist. The two main threads of coexistence theory use this critical information recovered from invasion growth rates to (1) quantify competitors' niche differences and fitness differences (Figure 1D), or (2) quantify competitors' responses to average environments and fluctuations around this average (Figure 1E). From a practical perspective, the first thread uses the concepts of niche and fitness differences to predict if competitive exclusion or coexistence will occur, and the second thread uses a partitioning approach to quantify how different mechanisms are promoting or preventing coexistence. In this primer section, we introduce the main concepts and jargon of coexistence theory, using microbial examples as illustrations throughout, but we point readers to: Spaak and Schreiber 2023 for a more detailed overview of niche and fitness differences, Barabás et al. 2018; Johnson and Hastings 2023 for a rigorous account of the partitioning approaches, and Godwin et al. 2020; Ellner et al. 2019 for detailed practical guidelines on applying coexistence theory.

The first thread provides a higher-level framing of the requirements for coexistence along two fundamental axes. Specifically, stable coexistence among species depends on niche differences exceeding any inherent fitness differences (Chesson, 2000a, 2013; Letten et al. 2017; Spaak and Laender 2020). Niche differences capture the extent to which competitors use different resources (e.g., consumption of citrate versus glucose by different strains of *E. coli* in the long-term evolution experiment (Blount et al. 2008)) or are targeted by different predators or pathogens (e.g., specificity of bacteriophages to different bacterial strains or species (Koskella and Brockhurst 2014)). In contrast, fitness differences reflect how well adapted competitors are to their shared environment. It follows that if two or more species are characterised by large fitness differences, they can only coexist if their niches are sufficiently differentiated (Figure 1D). Conversely, when species have small fitness differences, even small niche differences can facilitate coexistence (note that a combination of infinitesimal fitness *and* niche differences can yield so-called neutral coexistence (Adler et al. 2007)). In the jargon of coexistence theory, a process that increases niche differences is referred to as a stabilising mechanism, while one that reduces fitness differences is referred to as an equalising mechanism (Chesson, 2000a, 2003).

The second thread of coexistence theory is concerned with identifying the contributions of different stabilising mechanisms to coexistence and partitioning them into additive terms reflecting various types of fluctuation-independent and fluctuation-dependent mechanisms (Chesson 1994; Barabás et al. 2018; Ellner et al. 2019; Letten et al. 2018; Johnson and Hastings 2023). Fluctuation-independent mechanisms promote coexistence in a constant environment, while fluctuation-dependent mechanisms

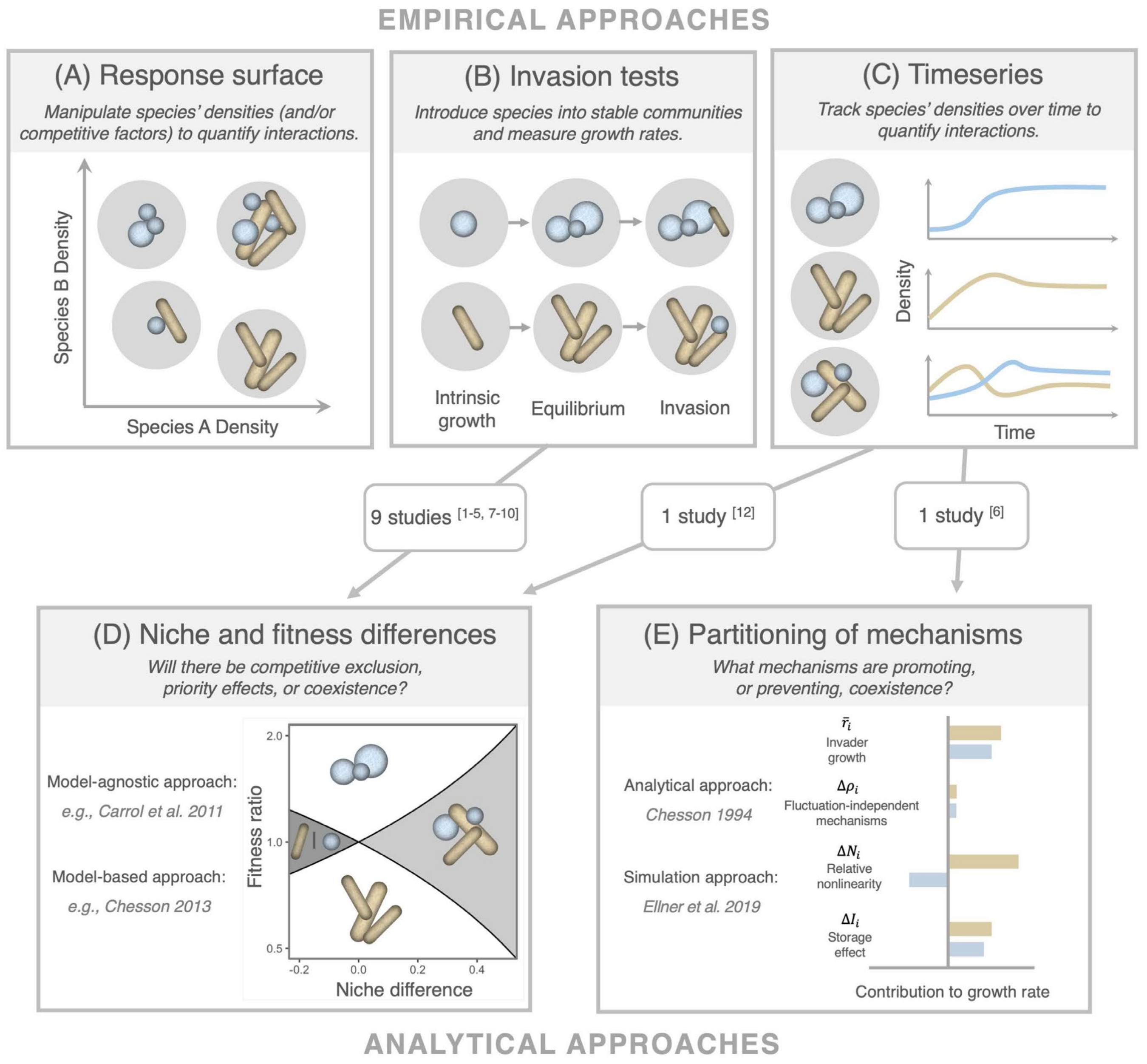

FIGURE 1 | Experimental approaches that can be used to acquire the necessary data for coexistence theory (A–C) and the two threads of coexistence theory that can be used to analyse the data (D, E). The arrows between panels show the number of studies in our systematic review that used that combination of empirical and analytical approaches. The numbers in superscripts refer to row numbers of Table 1. (A) Response surface designs, which can not always be applied in microbial systems (see *Empirical challenges*), manipulate species' densities to parameterize population models. Alternatively, competitive factors (e.g., resource concentrations) can be manipulated to parameterize mechanistic models (e.g., consumer-resource models). (B) Invasion tests can be performed to test the mutual invasion criterion (i.e., coexistence predicted if all species can invade the community from low densities). (C) Timeseries datasets can be used to parameterize population dynamics models or to test coexistence theory predictions. (D) The most common analytical approach for applying coexistence theory is quantifying niche and fitness differences, either directly from the data through model-agnostic approaches or from parameterized population models using mathematical approaches. Communities can then be placed in a "coexistence plane" with axes defined by niche and fitness differences where different regions indicate either competitive exclusion (pale grey zones on top and bottom), stable coexistence (mid grey zone on the right), or priority effects (dark grey zone on the left). (E) Coexistence theory can also be used to partition the effects of different coexistence mechanisms either using analytical techniques or using simulation-based approaches. In this hypothetical example, there is stable coexistence between the blue and yellow species (both have positive invasion growth rates) primarily due to large contributions from fluctuation-dependent mechanisms.

act through environmental fluctuations to strengthen, weaken, or even reverse coexistence outcomes. The emphasis on stabilising mechanisms (niche differences) reflects the prepotent role they play in mediating stable coexistence; stable coexistence is impossible in the absence of a stabilising mechanism. The extreme (and arguably implausible) case of near-zero fitness and niche differences leads to a neutral scenario, where long-term exclusion remains inevitable. However, disturbances that keep

TABLE 1 | The 12 empirical coexistence studies using microbial systems identified by our systematic review.

Study	Taxa	Empirical approach	Analytical approach	Model	Pairs	Replicates	Units
Narwani et al. 2013	Algae	Invasion	ND-FD	None	28	3	168
Tan et al. 2016	Bacteria	Invasion	ND-FD	None	6	3	57
Zhao et al. 2016	Bacteria	Invasion	ND-FD	None	5	3	180
Tan et al. 2017	Bacteria	Invasion	ND-FD	None	1	5	60
Tan et al. 2017	Bacteria	Invasion	ND-FD	None	6	6	78
Letten et al. 2018	Yeast	Timeseries	Partitioning	Consumer-resource	6	4	160
Gallego et al. 2019	Cyanobacteria	Invasion	ND-FD	None	15	3	126
Grainger et al. 2019b	Yeast	Invasion	ND-FD	None	6	8	576
Li et al. 2019	Bacteria	Invasion	ND-FD	None	24	3	540
Jackrel et al. 2020	Algae	Invasion	ND-FD	None	6	3	72
Zufiaurre et al. 2021	Protists	Proxy	ND-FD	None	n/a	2	22
Hogle et al. 2022	Protists	Timeseries	ND-FD	Lotka-volterra	1	4	24

Note: Empirical Approach describes what type of experimental design was used; mutual invasion tests ("invasion"), densities tracked over time ("timeseries"), or empirical studies where natural communities are observed and proxies are used to estimate niche and fitness differences ("proxies"). For *Analytical Approach*, "ND-FD" indicates studies that quantified niche and fitness differences and "partitioning" indicates studies that quantified the relative contributions of different coexistence mechanisms to invasion growth rates. *Model* indicates the population model used, *Pairs* records the number of species pairs for which coexistence was estimated, *Replicates* records the number of replicates per species pair (for each treatment), and *Units* records the number of distinct experimental units used in the coexistence part of the study.

communities away from their equilibria can either temporarily support persistence or accelerate exclusion. Nevertheless, most mechanisms will also have an equalising component, as these two axes are not orthogonal (Song et al. 2019). Under the standard partition, all fluctuation-independent mechanisms (including resource, predator and pathogen partitioning) are grouped into a single category. The fluctuation-dependent mechanisms capture the effects of both temporal *and* spatial heterogeneity on coexistence. The two main types of fluctuation-dependent mechanisms are the *storage effect* and *relative nonlinearity of competition*, both of which have temporal and spatial analogues (a third, exclusively spatial, mechanism is termed fitness-density covariance (Chesson, 2000a; Barabás et al. 2018)).

The temporal storage effect can facilitate coexistence when species exhibit different growth responses to density independent environmental factors (e.g., temperature or pharmaceutical antibiotics) that vary across space or time (Chesson 1994; Letten et al. 2021). The emphasis on variation in density-independent environmental factors, which are not directly influenced by the focal species, is a distinguishing feature of the storage effect. In the jargon of coexistence theory, these species-specific responses can lead to density-dependence in environment-competition covariance (Chesson 1994; Yuan and Chesson 2015; Barabás et al. 2018). This is simply to say that when a species is rare in the system it will experience little covariance in the strength of competition and environmental favourability across space or time (i.e., it experiences little competition when the environment is favourable), which allows it to take full advantage of good conditions. Conversely, when a species is dominant in the system it will experience high positive covariance between these two factors, which should limit its ability to take advantage of favourable conditions. Provided rare species are also able to buffer the negative effects of high competition during unfavourable periods (e.g., via dormant life stages such as bacterial endospores or via overlapping generations), this process can underpin species coexistence.

In contrast with the storage effect, relative nonlinearity relies on species exhibiting trade-offs in their per capita growth responses to shared factors that mediate competition, such as nutrient resources, which fluctuate through time (note that although there is a spatial analogue of relative nonlinearity, we focus on the more canonical temporal mechanism here) (Armstrong and McGehee 1980; Chesson 1994; Yuan and Chesson 2015; Barabás et al. 2018). These fluctuations may be driven endogenously (e.g., cyclic bacteria-phage dynamics) or exogenously (e.g., resource pulsing in the animal gut). The classic trade-off permitting coexistence via relative nonlinearity is between gleaners and opportunists, which are better competitors in continuous versus fluctuating resource environments, respectively (the name relative nonlinearity refers to the required difference in the nonlinearity of the per capita growth responses that permit this trade-off) (Yamamichi and Letten 2022). Microbes that are adapted to constant, oligotrophic environments are referred to as gleaners, while microbes that thrive under feast-famine resource regimes are referred to as opportunists. Alongside the trade-off, stable coexistence via relative nonlinearity additionally requires that each species, when resident, induces feedback on the magnitude of fluctuations in a direction that favours its competitor (Chesson 1994; Yuan and Chesson 2015; Barabás et al. 2018). For example, for the coexistence of gleaners and opportunists, the former needs to increase temporal resource variability relative to that imposed by the opportunist, and vice versa. These negative feedbacks typically emerge as a natural byproduct of each strategist's per capita growth response, but it is also possible for positive feedbacks, and hence priority effects, to occur when competitors change the temporal pattern of resource availability in a direction that benefits them (relatively speaking) (Ke and Letten 2018).

These methods for partitioning invader-resident growth differences into an additive sum of contributing mechanisms have also been generalised into a method for assessing the role of any interspecific difference on coexistence. This has been called "trait-decomposition" in recent literature and quantifies the sensitivity of invasion growth rates to interspecific differences in model parameters such as nutrient uptake rates or mortality rates, all else being equal (Ellner et al. 2019).

Finally, it is worth emphasising at this stage that most applications of coexistence theory to date have focused on pairwise interactions. However, it has recently been demonstrated in microbial systems that pairwise coexistence alone does not always predict community-level coexistence in species-rich systems (Chang et al. 2023). Emerging theory is attempting to extend the general concepts discussed above to systems with three or more species (Chesson 2018; Spaak and Schreiber 2023), but these theoretical developments have not yet been empirically validated. As we shall argue below, however, while the diversity and complexity of microbial systems pose a challenge to traditional implementations of coexistence theory, it also presents a valuable opportunity for further theory development.

3 | Empirical Applications

A variety of experimental and observational approaches have been used by researchers to confront the predictions and inference of coexistence theory with empirical data. Experimental approaches for applying coexistence theory can be broadly categorised into three groups: response surface experiments (Figure 1A), invasion tests (Figure 1B), and timeseries (Figure 1C). In a response surface experiment, densities of species are systematically manipulated and the subsequent changes in population dynamics are observed (Inouye 2001; Hart et al. 2018). Alternatively, the underlying competitive factors (e.g., resource concentration or the abundance of shared predators) might be manipulated to parameterise mechanistic models of ecological dynamics (e.g., consumer-resource models) (Letten et al. 2018). In invasion tests, species are introduced at low densities into stable communities and coexistence is predicted if all species can invade (mutual invasability criterion) (Grainger et al. 2019b). A common form of invasion test that focuses on pairs of species involves growing one species (the "resident") until it reaches a steady state followed by the introduction of a second species (the "invader") at low density. Performing two of these tests with species alternating as invader and resident is an efficient way to obtain data—intrinsic growth rates, carrying capacities in monoculture, competition coefficients—that can be interpreted through the lens of coexistence theory (Carroll et al. 2011). Finally, in timeseries experiments, repeated sampling of a community (and sometimes also of monocultures) is used to track species' densities through time. These three experimental approaches can be used independently or in combination to obtain the relevant metrics of coexistence theory (e.g., invasion growth rates, competition coefficients, fitness differences and niche overlap), but they can also be used to fit population models to provide broader insights into a system's dynamics.

The obtained estimates of invasion growth rates or model parameters may then be used to predict (or explain) coexistence based either on the partitioning of fitness and niche differences in equilibrium systems (Figure 1D) or on the partitioning of different fluctuation-dependent and -independent mechanisms (Figure 1E) (Godwin et al. 2020; Ellner et al. 2019). Most empirical research employing coexistence theory has been conducted in plant systems, with few studies in microbial systems; of the 49 studies that quantified niche and fitness differences reviewed by Terry and Armitage (2024), 39 focused on plant communities and just six studied microbial communities.

To confirm the rarity of empirical explorations of coexistence theory in microbial systems, we performed a systematic review (see Supporting Information) that identified studies in two ways. First, we screened 174 empirical coexistence studies compiled in previous reviews of the field (Buche et al. 2022; Terry and Armitage 2024; Hawlena et al. 2022) and found nine studies that applied coexistence theory to microbial systems. Second, we performed an additional search of the literature in May 2024 and used a machine learning framework to screen abstracts (Schoot et al. 2021). From an initial search that returned 2164 records, we found just 12 relevant studies (Table 1), including all nine relevant studies that had been identified from the previous reviews.

This low number of studies precludes a formal analysis, but it does demonstrate how rarely coexistence theory has been applied in microbial systems. The 12 studies were published between 2013 and 2022 (with no obvious increase over time) and they used bacteria ($n = 5$), phytoplankton ($n = 3$), protists ($n = 2$), or yeasts ($n = 2$). Most studies (9/12) used mutual invasion tests (Figure 1B) and species sensitivity to competition (sensu Carroll et al. 2011) to calculate niche and fitness differences. Two studies tracked the density of monocultures and multi-species communities over time (Figure 1C) and fitted population models to the timeseries data to either quantify niche and fitness differences (Hogle et al. 2022) or to quantify the relative contributions of different coexistence mechanisms (Letten et al. 2018). No studies used the response surface approach (Figure 1A) which dominates plant and animal empirical coexistence study designs (Terry and Armitage 2024), and one study observed natural communities and used "performance proxies" (sensu Grainger et al. 2019b for niche and fitness differences (Zufiaurre et al. 2021)).

Some studies focused on the coexistence of just one pair of species (e.g., Tan et al. 2017a; Hogle et al. 2022), while other studies investigated the coexistence of over 20 pairs of species (e.g., Li, Li et al. 2019; Narwani et al. 2013). The tractability of these microbial systems results in the numbers of experimental units—defined as the smallest division of the experimental setup that can be independently manipulated and measured (e.g., microbial cultures, microcosms, or individual wells)—far exceeding typical animal or plant experiments, with some studies having more than 500 units (e.g., Li et al. 2019; Grainger et al. 2019a). This increase in experimental units was not used to increase the replicates per treatment to gain more robust predictions (across the 12 relevant studies the maximum number of replicates was eight and the median was three). Instead, the additional experimental units were used to increase the number of species pairs examined (e.g., Li et al. 2019; Narwani et al. 2013) or to test the effects of additional factors such as the effect of environmental variables (e.g., Letten et al. 2018; Grainger et al. 2019a), trait diversity (e.g., Hogle et al. 2022), or evolutionary history (e.g., Zhao et al. 2016; Tan et al. 2017). The low number of relevant studies limits our ability to synthesise these previous experiments beyond this summary of their practical details. With such a wide range of communities studied, including nectar yeasts, freshwater green algae and predatory protists, and such a wide range of coexistence mechanisms explored, from resource fluctuations and spatial heterogeneity to intraspecific trait diversity and trait variation, it is hard to glean general insights from these dozen studies. However, we use these pioneering studies as examples in the sections below to illustrate the insights and challenges that arise when combining coexistence theory and microbial systems.

4 | Mutual Benefits of Coexistence Theory and Microbial Ecology

To facilitate closer integration of coexistence theory and microbial ecology, in this section we explore the mutual benefits between the two areas of research. We first highlight how coexistence theory can provide a fresh perspective on key challenges in the study of microbial communities. We then argue that microbial communities provide unique opportunities for novel tests of coexistence theory that would otherwise be intractable (i.e., in macro-organisms).

4.1 | How Coexistence Theory Can Benefit Microbial Ecology

Many mechanisms, some general to all ecological systems (e.g., resource and natural enemy partitioning) and others more specific to microbial systems (e.g., cross-feeding, horizontal gene transfer), have been proposed to explain the high levels of microbial diversity commonly encountered in metabarcoding studies (Thompson et al. 2017). Coexistence theory can help microbial ecologists move beyond verbal theories and intuitions for how microbes coexist by quantifying the relative importance of these mechanisms and by relating different mechanisms to each other. Coexistence theory might be leveraged to synthesise disparate models in microbial ecology, and it holds particular promise for guiding the growing interest in how spatio-temporal variability impacts microbial communities. The remainder of this section will use three high-level examples to outline how different aspects of coexistence theory described in the primer section—(i) niche and fitness differences, (ii) fluctuation-dependent mechanisms of coexistence and (iii) trait-decomposition simulations—can shine light on open problems in fundamental and applied microbial ecology.

A common approach taken by microbial ecologists (albeit one that is not unique to microbial ecology) is to infer species interactions and community assembly processes from the observed covariances between taxa in metagenomic/metabarcoding inventories (Armitage and Jones 2019a). This includes characterising communities as being under stochastic versus deterministic regulation, depending on the functional (or phylogenetic) distribution of co-occurring taxa (Stegen et al. 2012). Although this data undoubtedly provides a valuable starting point for more mechanistic predictions, inference of assembly processes from observational data is well known to be fraught (Barner et al. 2018), not least because of the confounding effects of spatial and temporal scale dependence (Blanchet et al. 2020; Armitage and Jones 2019a). Viewed from the perspective of niche and fitness differences, it is apparent that all assemblies must fall along a continuum from stochastic (weak niche *and* fitness differences) to deterministic (strong fitness differences *or* strong niche differences), and that the magnitude of niche and fitness differences is going to be highly dependent upon the spatial and temporal scale at which they are evaluated. However, it is likewise unclear how the traditionally pairwise niche and fitness difference metrics relate to observed covariation among species in complex multispecies communities (Chang et al. 2023). Thus, we envision at least one way in which these approaches can complement one another. By tracking experimental or observational low density growth rates derived through metagenomic, metabarcoding, or plate count timeseries, we can begin to relate stabilising niche differences (a key requirement of invasion growth rates) to the topological properties of a community's statistical correlational structure or its position along a predicted niche-neutral continuum. By benchmarking the inferential accuracy of these methods in experimental microbial communities in which each species' invasion abilities are already known, we can identify the inferential ability of such approaches and their specific failure modes.

Understanding the impact of environmental fluctuations on eco-evolutionary dynamics is another increasingly active area of inquiry in microbial ecology (e.g., Abreu et al. 2020; Nguyen et al. 2021; Shibasaki et al. 2021). Microbial systems are rarely homogeneous, particularly at the temporal and spatial scales relevant in applied microbiology (Nguyen et al. 2021). This spatio-temporal variability—from periodic antibiotic dosing to drying and wetting of soils, to daily and seasonal fluctuations in temperature and light—undoubtedly plays a critical role in the eco-evolutionary dynamics of microbial systems. As opposed to most (but not all, e.g., Ontiveros et al. 2023) commonly applied ecological theory that assumes homogeneous environments (Meroz et al. 2024; Picot et al. 2023), coexistence theory offers a mathematically rigorous framework to systematically study the role of fluctuating environments and to partition the relative effects of different variation-dependent mechanisms on microbial coexistence. Furthermore, the stabilising effects of spatio-temporal fluctuations may be leveraged to help engineer microbial communities for the optimisation of ecosystem function (Letten and Ludington 2023). Functional landscape approaches can be used to predict which combinations of species have the maximum performance for a given function across different applications from food and beverage production to waste management (Sanchez-Gorostiaga et al. 2019; Sanchez et al. 2023). However, these optimal communities may not have sufficient niche differences to overcome any fitness differences under standard conditions, so their coexistence might require the introduction of temporal or spatial fluctuations, which could be designed based on coexistence theory predictions. If, on the other hand, fluctuations are an unavoidable feature of the process, they will need to be considered in the design of microbial consortia. Using coexistence theory to better understand fluctuating environments therefore has the potential to advance both basic and applied microbial ecology.

As is common in many nascent fields, microbial ecology has seen a wide proliferation of alternative hypotheses and theories, with limited conceptual integration between them to date. Consider two popular theories concerning microbial coexistence: the seed bank and kill-the-winner models (Thingstad and Lignell 1997; Winter et al. 2010; Lennon and Jones 2011; Lennon et al. 2021). The seed bank model posits that persistent populations of dormant or sporulated microbial cells can respond rapidly to transiently favourable environmental conditions and buffer their populations against losses they would incur if otherwise active and competing for resources (Lennon and Jones 2011; Lennon et al. 2021). Although it has been loosely linked with the temporal storage effect in the past, recent work shows that microbial dormancy may also mediate coexistence via relative nonlinearity of competition when dormancy is adaptive under periods of resource starvation (cf. density-independent stressors such as temperature, osmostic stress or antimicrobials) (Letten et al. 2024). The "kill-the-winner" model, on the other hand, is a proposed fluctuation-independent mechanism by which lytic bacteriophage viruses—each specific to a single strain of bacterial competitor—act to suppress the abundance of otherwise competitively dominant bacteria (e.g., by virtue of being better resource competitors) (Thingstad and Lignell 1997). It is reasonable to assume that trade-offs between growth and dormancy, and growth and phage resistance, frequently occur side by side, but we are aware of no efforts to theoretically synthesise them and assess their joint contributions to microbial coexistence. By unifying existing models of kill-the-winner and seed bank dynamics into a single community dynamics model, growth rate partitioning approaches could be used to evaluate their relative contribution to community average stabilisation and fitness equalisation (Ellner et al. 2019). This "trait-decomposition" approach would not only provide high-level inference on the importance of these two prominent microbial coexistence mechanisms, but would also allow for the further partitioning of the fluctuation-independent (e.g., classic resource competition vs. trade-offs in phage susceptibility) and fluctuation-dependent contributions (i.e., temporal storage effect vs. relative nonlinearity).

4.2 | How Microbial Ecology Can Benefit Coexistence Theory

The development of coexistence theory has undoubtedly outpaced its empirical validation (Godwin et al. 2020; Terry and Armitage 2024). This lag is at least in part due to the comparatively technical nature of early presentations of the theory, but it is also due to the challenges of testing the theory in plant and animal systems. Most empirical coexistence studies of animal and plant communities are conducted over a single generation due to logistical constraints (Terry and Armitage 2024, but see Terry 2025), which means coexistence theory can only be used to produce (error-prone) predictions rather than to explain observations. However, the short generation times and rapid dynamics of microbes allow for direct observations of coexistence, competitive exclusion, or priority effects (i.e., through invasion tests or timeseries). As such, with microbes, it is much easier to both make and test predictions informed by coexistence theory. This tight connection between theory and experiments places microbial systems in an excellent position to rigorously test and to develop emerging theory on a range of themes in coexistence theory, including, but not limited to, spatiotemporal fluctuations as coexistence mechanisms, evolutionary effects on coexistence and coexistence in diverse multispecies communities.

The challenges of manipulating environmental variability at meaningful temporal and spatial scales in animal and plant systems have hindered empirical research on fluctuation-dependent mechanisms of coexistence—e.g., the storage effect and relative non-linearity of competition. Firstly, as environmental fluctuations must occur on timescales longer than generation times to act as stabilising mechanisms, studies of temporal storage effects and related mechanisms in plant and animal systems have typically relied on simulation approaches with parameterised models (e.g., Armitage and Jones 2019b; Hallett et al. 2019). In contrast, empirical tests of fluctuation-dependent coexistence mechanisms that require multi-year experiments for annual plants can be done in days for bacteria. It is unsurprising then that the few direct empirical tests (cf. simulation-based predictions) of fluctuation-dependent coexistence have mostly been carried out in microbial systems (e.g., Descamps-Julien and Gonzalez 2005; Jiang and Morin 2007; Ellner et al. 2016; Letten et al. 2018). Secondly, removing or manipulating spatial heterogeneity in plant and animal experimental systems is often not feasible. Experimental arenas for microbial communities, on the other hand, can be easily modified to create or remove spatiotemporal variability in environmental conditions (e.g., using a chemostat). One of the few studies that has empirically quantified spatial coexistence mechanisms found a higher diversity of *Pseudomonas* strains in static (heterogenous) compared to shaken (homogenous) environments when dispersal between environments was permitted (Tan et al. 2017). Modified chemostats with gradients of environmental conditions (i.e., "gradostats", *sensu* Lovitt and Wimpenny 1981), complex artificial "micro-landscapes" (Larsen and Hargreaves 2020), or biofilm model systems (O'Brien and Fothergill 2017) all offer more sophisticated and highly controllable approaches for testing the effects of spatial heterogeneity on species coexistence.

Amidst growing interest in the role of rapid evolution in regulating community dynamics and coexistence on ecological time scales (Pastore et al. 2021; Hiltunen et al. 2017; Wittmann and Fukami 2018; Yamamichi and Letten 2021), several recent studies have shown that evolution can shift niche or fitness differences through changes in species traits and competitive abilities. However, with only a few exceptions (e.g., Bernhardt et al. 2020) these are limited to studying the sorting of standing genetic variation rather than *de novo* evolution (Sakarchi and Germain 2023; Hart et al. 2019). The rapid generation times and large population sizes of microbes permit observation of evolutionary impacts on species coexistence over relatively short timescales. In two rare examples identified from our systematic review, Zhao et al. (2016) studied coexistence between bacteria evolving in sympatry and allopatry, while Hogle et al. (2022) observed community dynamics and species coexistence over 70 generations of ciliates (a study that would have taken an annual plant ecologist 70 years). Furthermore, precise genetic manipulation of microbial populations (e.g., transfer of antimicrobial resistant genes) could deepen our understanding of the molecular basis of traits that shape species coexistence. Expanding coexistence studies into microbial systems also has the potential to refine our understanding of the processes responsible for the persistence or loss of allelic diversity in evolving populations, as the framework's ideas naturally map to population genetic processes such as clonal interference and negative frequency-dependent selection (Maddamsetti et al. 2015; Letten et al. 2021).

The small-scale nature of microbial systems also allows the use of far more experimental units than realistically achievable in animal and plant study systems. This is to say that the "combinatorial explosion problem", where the number of species combinations increases exponentially with species richness, is far less of a limiting factor in microbial systems. Indeed, Li et al. (2019) used over 500 experimental units to quantify niche and fitness difference between 24 pairs of bacterial species. Higher samples sizes can also help to overcome challenges related to parameter estimates and prediction uncertainty that hinder plant and animal studies (Terry and Armitage 2024; Armitage 2024). High-throughput robotic or droplet-based systems, which can rapidly assemble and observe the dynamics of many different microbial communities (e.g., Diaz-Colunga et al. 2024; Kehe et al. 2019), could be especially powerful tools for testing emerging theory on coexistence in species-rich systems that requires vast numbers of replicates to combat the combinatorial explosion problem. Many features of microbial systems—prevalence of positive interactions, high diversity, multistability, spatial structure—are on one level major barriers to the implementation of traditional coexistence theory, which is best suited for studying simple pairs of competing species. Indeed, in the next section we discuss these features in detail and frame them as challenges. However, these complexities of microbial systems can also be viewed as an opportunity for further theory development. Recent advances in coexistence theory, such as the development of niche and fitness difference metrics that accommodate positive interactions (Spaak and Laender 2020; Koffel et al. 2021) and the use of permanence theory to study coexistence in species-rich communities (Spaak and Schreiber 2023), arguably can only be robustly tested in microbial systems.

5 | Cautionary Notes

There are inherent features of microbial systems that introduce novel challenges for applying coexistence theory. Moreover, this framework is not a silver bullet for investigating all problems related to species coexistence. As such, awareness of the constraints and limitations in implementing coexistence theory is essential for its effective use in microbial ecology.

5.1 | Empirical Challenges

While the fast growth and short generations of microbes are a convenience in many circumstances (see above), they can also present unique challenges for the empirical application of coexistence theory. For response surface experiments, where species' densities must be finely controlled (Inouye 2001), the fast dynamics of microbial systems are potentially prohibitive. It is therefore unsurprising that our systematic review found no studies applying this approach. Response-surface experiments manipulating competitive factors such as resource concentrations do, however, offer great potential for applying coexistence theory in microbial systems. For mutual invasion tests, where a key assumption is that the resident community is at equilibrium when the invader is introduced, batch culture experiments are inappropriate as they do not allow community dynamics to establish a stable equilibrium. High-frequency serial transfer, or better still, chemostat systems should instead be used to ensure that resident communities are indeed at equilibrium (Picot et al. 2023), so that invader growth rates can be accurately estimated. For timeseries approaches in microbial systems, it may be important to consider eco-evolutionary dynamics when fitting population models to estimate species interaction coefficients. Rapid evolution may cause species interactions to change over time, and dramatic shifts in the population structure of a given species can even occur between different growth stages (Zambrano et al. 1993).

Microbial communities also generally exhibit much higher phylogenetic and biochemical diversity than animal and plant communities (Prosser et al. 2007). While understanding the coexistence of two macroorganisms (e.g., endangered wolves and common coyotes) is often critical for conservation and ecosystem management, it is less common for the coexistence of just two microbial taxa to have significant implications in applied settings (but see, for example, the balance of Bacterioidetes and Firmicutes in the mammalian gut). As such, the diversity of microbial communities may seem at odds with coexistence theory, which has traditionally used species pairs as the focal study unit. Indeed, the concepts of niche and fitness differences were originally formulated for two competitors from the same trophic level, and the limitations of invasion analysis when studying more than two species have long been acknowledged (MacArthur and Levins 1967; Spaak and Schreiber 2023). Extensions of coexistence theory to multi-trophic systems, or to systems with three or more competitors from the same trophic level, are therefore at the forefront of theoretical work on coexistence theory (Chesson 2018; Spaak and Schreiber 2023; Song and Spaak 2024; Ranjan et al. 2024), and are likely to be important for microbial applications of this framework.

Quantifying microbes poses other challenges that animal and plant ecologists, who can count their study species with the naked eye, typically don't have to worry about. Colony counting is time consuming, sensitive to researcher variability, and it can be challenging to find the narrow dilution windows that produce useful results. It is particularly tricky for multi-species counts, where researchers are restricted to studying species that have distinct colony morphologies (Castledine et al. 2024) and large differences in species' abundances may require multiple platings to detect rare species. One way to remedy this is to employ selective markers such as antibiotic resistance to quantify rare invaders. Optical density is a coarse measure of population abundances and requires innovative solutions (e.g., selective growth conditions, multi-wavelength measurements) when quantifying species in co-culture. Adding fluorescent labels on plasmids is another option, but plasmids can be lost or transferred between species, and there can be associated fitness costs of carrying the plasmid. There are also devices that spatially isolate strains in permeable growth chambers which make it possible to quantify competition for a common resource without the need for markers (Ahn et al. 2006), though they remain underused. Sequence-based count methods can be expensive and—unless tools like qPCR or ddPCR are used—produce relative rather than absolute abundances. Inferring all the parameters of population models (e.g., Lotka-Volterra) cannot always be done with relative abundance data alone (Remien et al. 2021). Although key metrics of coexistence theory such as invasion growth rates and (relative) competition coefficients can still be obtained, integrating relative abundances into coexistence theory workflows requires careful consideration.

High frequencies of non-competitive or context-dependent interactions (Kehe et al. 2021; Momeni et al. 2017; Kost et al. 2023) are additional features of microbial communities that complicate the application of coexistence theory. Facilitation can be challenging to model with traditional tools (e.g., Lotka-Volterra) and most definitions of niche and fitness differences—including the most popular metrics such as Chesson's classic square root equations (Chesson 1990, 2013; Chesson and Kuang 2008) and the sensitivity to competition formula of Carroll et al. (2011)—do not account for positive interactions between species (Spaak and Laender 2020). However, such interactions are seemingly ubiquitous in microbial communities, with ample evidence of cross-feeding (Bello et al. 2021), multi-species biofilm formation (Nadell et al. 2016), and sharing of antibiotic resistance genes (Ellabaan et al. 2021). For example, it would be challenging to conduct reciprocal invasion tests when one of the taxa is auxotrophic and therefore reliant on cross-feeding interactions to maintain a positive growth rate. Non-competitive interactions are not beyond the scope of coexistence theory—new metrics for niche and fitness differences that can accommodate positive interactions have recently been developed (Spaak and Laender 2020; Koffel et al. 2021)—but they are nonetheless difficult to integrate with traditional approaches. Further, interspecific interactions among microbes have been shown to readily switch signs from facilitative to competitive depending on the local environmental context (Hoek et al. 2016; Meroz et al. 2024). Indeed, the sign flexibility of interspecific interactions in microbes merits further study, as it may be one pathway from which fluctuation-dependent mechanisms can emerge even in the absence of external environmental variation.

5.2 | Limitations and Conceptual Mismatches

Several of the empirical challenges discussed above can be addressed—at least conceptually—through recent extensions to the original theory. However, there are some limitations of the theory that must be treated with caution. One of the most obvious, which has received considerable attention in the ecological literature, is that invasion analysis does not always predict coexistence. The analytical approaches of applying coexistence theory (Figure 1D,E) are built upon the assumption that mutual invasibility predicts coexistence. However, it can be shown that there are cases where all species can invade but will not ultimately coexist or where not all species can invade even if they could all coexist (Barabás et al. 2018; Johnson and Hastings 2022; Clark et al. 2024). As complex ecological dynamics, such as Allee effects, multistability which is seemingly common in microbial systems (Chang et al. 2023; Lopes et al. 2024), and stochasticity (both environmental and demographic), can break the simple logic of invasion analysis, all results should be interpreted with at least some caution. However, even in multispecies systems, invasion growth rates of one species into a mixed consortium can still be broadly informative (Chesson 2018; Schreiber et al. 2023; Clark et al. 2024).

Another arguable shortcoming of coexistence theory (or at least how it is commonly implemented) is the (over)emphasis placed on qualitative (e.g., coexistence vs. exclusion), rather than on the quantitative, community states. Often, however, relative abundances of coexisting species are an equally, if not more, important response variable (Abrams 2022). If multiple species coexist but one is far more abundant, the community may function like a monoculture. For instance, lakes dominated by toxic algal blooms often have communities where cyanobacteria, diatoms and green algae are all coexisting, but where the cyanobacteria dominate by orders of magnitude (Scheffer et al. 1997; Grover 1997; Jankowiak et al. 2019). Similarly, predicting if a pathogenic microbe can coexist with (or be excluded by) beneficial microbes may not be as useful as predicting the abundance of the pathogen (Letten et al. 2021). If the pathogen coexists with other microbes but is held at low density (i.e., below the minimal infective dose), it may be of no material consequence for the host. Nevertheless, with the data required to evaluate qualitative community states informed by coexistence theory, it is usually possible to quantify other response variables of interest (e.g., relative abundance at equilibrium, invasion growth rates or time to extinction (Clark et al. 2024)) that may be especially relevant in more applied contexts.

Finally, quantifying niche and fitness differences in isolation only tells us *if* species coexist but not necessarily *how* they coexist. Niche and fitness differences summarise the combined effects of potentially many underlying mechanisms, so focusing solely on these properties risks obscuring underlying ecological processes. This is particularly true if phenomenological models (e.g., Lotka-Volterra competition) rather than more mechanistic models (e.g., resource-consumer models) are used to estimate niche and fitness differences, which have been the dominant empirical application of coexistence theory to date (Chesson 2013; Godwin et al. 2020). Indeed, phenomenological approaches, such as the widely used generalised Lotka–Volterra model, are far more common than mechanistic approaches in the microbial literature (Picot et al. 2023). The field of microbiology, however, has a rich history of precisely identifying and manipulating the biochemical processes underlying an organism's ecology, resulting in the development of metabolic models capable of predicting microbial growth from first principles (Delattre et al. 2019). Incorporating these more sophisticated models into the coexistence framework will be challenging but likely fruitful, expanding our ability to make mechanistically informed manipulations of diverse microbial systems in applied contexts.

6 | Conclusions

With our systematic literature search finding limited uptake of coexistence theory in microbial ecology to date, we hope that the observations and opportunities outlined in this review can act as a stimulus for closer cross-pollination between these two fields. This includes leveraging coexistence theory to foster rigour and synthesis in the study of coexistence and community assembly in microbial systems, as well as taking advantage of the high-throughput of microbial systems to push the frontier of coexistence theory forward. Echoing broader concerns in microbial ecology (Prosser 2020), it is, of course, important that coexistence theory is not merely appended to descriptive studies to provide an illusion of theory-driven research. The ever-expanding framework of coexistence theory will only benefit microbial ecology if it is applied appropriately and is used to inform study design as well as data analysis. Nevertheless, thoughtfully incorporating coexistence theory into microbial ecology offers numerous opportunities to explore new research avenues, driving progress in theoretical and microbial ecology alike.

Author Contributions

James A. Orr: conceptualization (equal), data curation (lead), formal analysis (lead), methodology (equal), project administration (equal), visualization (lead), writing – original draft (lead), writing – review and editing (equal). **David W. Armitage:** conceptualization (equal), methodology (equal), writing – original draft (supporting), writing – review and editing (equal). **Andrew D. Letten:** conceptualization (equal), formal analysis (supporting), funding acquisition (lead), methodology (equal), project administration (equal), supervision (lead), writing – original draft (supporting), writing – review and editing (equal).

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The code and data used and generated during the systematic review are available at https://doi.org/10.5281/zenodo.14759136.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.