Coexistence theory for microbial ecology, and vice versa

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Data availability statement: The code and data used and generated during the systematic review are available at `https://github.com/jamesaorr/coexistence-microbes`.

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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 encompasses a body of theory for disentangling the contributions of different fluctuation-independent (e.g., resource partitioning) and fluctuation-dependent (e.g., environmental variability) mechanisms 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 heterogenous 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.

Introduction

Sustained improvement in sequencing technologies, database integration, and bioinformatics are 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, Shibasaki, Meacock, & Mitri, 2023; van den Berg et al., 2022; Prosser et al., 2007; Meroz, Livny, & Friedman, 2024), with theory-driven research already providing deep insights into the assembly (Goldford et al., 2018; Friedman, Higgins, & Gore, 2017), functioning (Skwara et al., 2023), and stability (Hu, Amor, Barbier, Bunin, & Gore, 2022) of microbial communities.

Understanding species coexistence and competition has been a central goal of theoretical ecology for over a century (Gause, 1935; MacArthur & 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 maximization of ecosystem functioning (e.g., starch degradation) in bacterial communities can require the coexistence of specific combinations of species (Sanchez-Gorostiaga, Bajić, Osborne, Poyatos, & Sanchez, 2019), and the evolution of antimicrobial resistance can be shaped by how different environmental conditions influence the coexistence of susceptible and resistant strains (Nev, Jepson, Beardmore, & Gudelj, 2020; Letten, Hall, & Levine, 2021). A number of different overlapping frameworks have been developed in ecology for understanding species coexistence (Chase & 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 amongst plant and animal ecologists, but that has seen far less uptake in microbial ecology, is so-called 'modern coexistence theory' (Chesson, 2000b; Barabás, D'Andrea, & Stump, 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 & 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, its rapid uptake in recent years can be understood, at least in part, as a reaction to the confusing morass of system- and scale-specific coexistence mechanisms that theoretical and empirical ecologists had offered up by the end of the 20th century. 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, HilleRisLambers, & Levine, 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 & Jones, 2019b; Letten, Dhami, Ke, & Fukami, 2018; Angert, Huxman, Chesson, & Venable, 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 (see Barabás et al., 2018; Godwin, Chang, & Cardinale, 2020; Spaak, Ke, Letten, & De Laender, 2023; Chesson, 2018; Ellner, Snyder, Adler, & Hooker, 2019; E. C. Johnson & Hastings, 2023). 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 study 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.

A coexistence theory primer

The framework of coexistence theory is best understood as two related approaches for expressing competitors' invasion growth rates as a small 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 critical information recovered from these invasion growth rates are generally either: 1) competitors' niche differences and fitness differences; or 2) competitors' responses to average environments and fluctuations around this average.

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, Ke, & Fukami, 2017; Spaak & De 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, Borland, & Lenski, 2008)) or are targeted by different predators or pathogens (e.g. specificity of bacteriophages to different bacterial strains or species (Koskella & 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 stabilizing mechanism while one that reduces fitness differences is referred to as an equalizing mechanism (Chesson, 2000a, 2003).

The second thread of coexistence theory is concerned with identifying the contributions of different stabilizing 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; E. C. Johnson & Hastings, 2023). Fluctuation-independent mechanisms promote coexistence in a constant environment, while fluctuation-dependent mechanisms act through environmental fluctuations to strengthen, weaken, or even reverse coexistence outcomes. The emphasis on stabilizing mechanisms reflects the prepotent role they play in mediating stable coexistence; stable coexistence is impossible in the absence of a stabilizing mechanism, and sufficient stabilization may depend on environmental fluctuations. Nevertheless, most mechanisms will also have an equalizing component, as these two axes are not orthogonal. 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 (hence they are also commonly referred to as variation-dependent mechanisms). 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 fluctuate across time (Chesson, 1994; Letten et al., 2021). The emphasis on variation in density-independent environmental factors, which to a first approximation are not 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 & 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, 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 or overlapping generations), this process can drive the requisite negative frequency dependence underpinning species coexistence.

In contrast with the storage effect, relative nonlinearity relies on species exhibiting trade-offs in their per capita growth responses to competitive factors (cf. density-independent environmental factors), 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) (Chesson, 1994; Yuan & Chesson, 2015; Barabás et al., 2018). These fluctuations may be driven endogenously (e.g., cyclic predator-prey dynamics) or via exogenous resource pulsing (e.g., in the animal gut). The classic trade-off permitting coexistence via relative nonlinearity is between gleaner and opportunists, which tend to be better competitors in continuous versus fluctuating resource environments (the name relative nonlinearity refers to the required difference in the nonlinearity of the per capita growth responses that permit this trade-off) (Yamamichi & Letten, 2022). Alongside the trade-off, stable coexistence via relative nonlinearity additionally requires that each species feedback on the magnitude of fluctuations in a direction that favours its competitor (Chesson, 1994; Yuan & Chesson, 2015; Barabás et al., 2018). For example, for coexistence of gleaners and opportunists, the former needs to increase temporal resource variability relative to that imposed by the opportunist. 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 which favours their own competitive ability (Ke & 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).

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. Typically this involves the direct measurement of invasion growth rates via mutual invasion tests (Figure 1B), or the parameterization of dynamical models (e.g., Lotka-Volterra or consumer-resource models) using timeseries datasets (Figure 1C) or "response surface" experiments that manipulate species' densities (Figure 1A) (Grainger, Levine, & Gilbert, 2019; Terry & Armitage, 2024). 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 system (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 & 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 Supplementary Materials) that identified studies in two ways. First, we screened 174 empirical coexistence studies compiled in previous systematic reviews of the field (Buche, Spaak, Jarillo, & De Laender, 2022; Terry & Armitage, 2024; Hawlena, Garrido, Cohen, Halle, & Cohen, 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 (Van De Schoot et al., 2021). From an initial search that returned 2,164 records we found just 12 relevant studies (Table 1), including all nine relevant studies that had been identified from 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, Cardinale, & Nisbet, 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, Hepolehto, Ruokolainen, Cairns, & Hiltunen, 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 & Armitage, 2024), and one study observed natural communities and used "performance proxies" (sensu Grainger, Levine, & Gilbert, 2019 for niche and fitness differences (Zufiaurre et al., 2021).

Some studies focused on the coexistence of just one pair of species (e.g., Tan, Rattray, Yang, & Jiang, 2017; Hogle et al., 2022), while other studies investigated the coexistence of over 20 pairs of species (e.g., Li, Tan, Yang, Ma, & Jiang, 2019; Narwani, Alexandrou, Oakley, Carroll, & Cardinale, 2013). The tractability of these microbial systems result in the numbers of independent replicates far exceeding typical animal or plant experiments, with some studies having more than 500 experimental units (e.g., Li et al., 2019; Grainger, Letten, Gilbert, & Fukami, 2019). 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, Letten, et al., 2019), trait diversity (eg., Hogle et al., 2022), or evolutionary history (e.g., Zhao, Zhang, & Zhang, 2016; Tan, Rattray, et al., 2017).

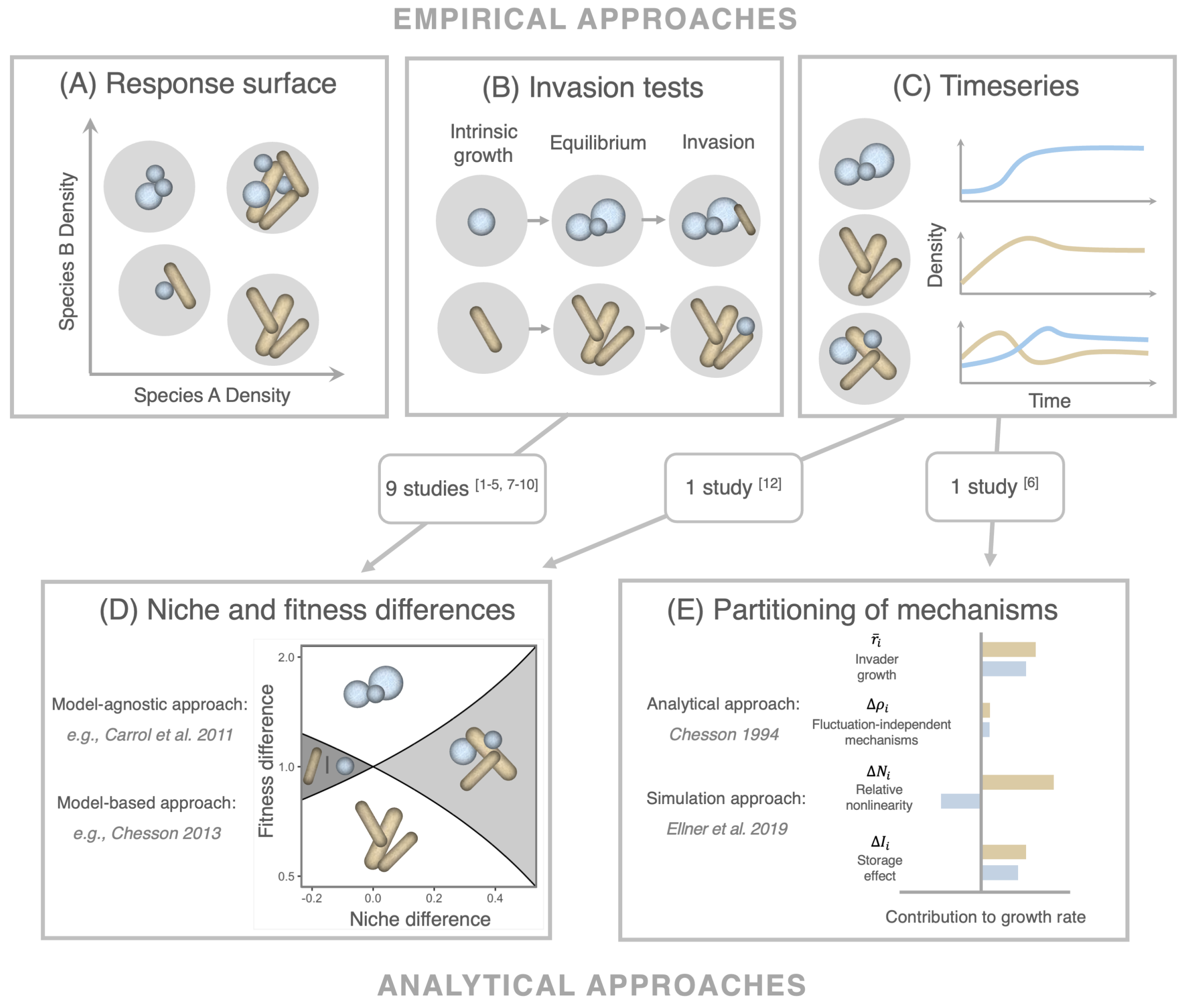

Figure 1: Approaches to data acquisition (A-C) and analysis (D-E) taken in empirical applications of coexistence theory. The arrows between panels show the number of studies in our systematic review that used that combination of empirical and analytical approaches. The numbers in superscripts refer to row numbers of Table 1. **(A)** Response surface designs, which can not always be applied in microbial systems (see emphEmpirical challenges), are used to parameterize population 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 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.

Table 1: The twelve empirical coexistence studies using microbial systems identified by our systematic review. *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.

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, Rattray, et al., 2017	bacteria	invasion	ND-FD	none	1	5	60
Tan, Yang, & Jiang, 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, Letten, et al., 2019	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

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).

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 common 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 species coexist by quantifying the relative importance of these mechanisms and by relating different mechanisms to each other. Coexistence theory might be leveraged to synthesize 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 & Jones, 2019a). This includes characterizing communities as being under stochastic versus deterministic regulation, depending on the functional (or phylogenetic) distribution of co-occurring taxa (Stegen, Lin, Konopka, & Fredrickson, 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, Coblentz, Hacker, & Menge, 2018), not least because of the confounding effects of spatial and temporal scale dependence (Blanchet, Cazelles, & Gravel, 2020; Armitage & Jones, 2019a). Viewed from the perspective of niche and fitness differences it is apparent that all systems 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. 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, Andersen Woltz, Friedman, & Gore, 2020; Nguyen, Lara-Gutiérrez, & Stocker, 2021; Shibasaki, Mobilia, & Mitri, 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 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 stabilizing effects of spatio-temporal fluctuations may be leveraged to help engineer microbial communities for the optimization of ecosystem function (Letten & 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 & Lignell, 1997; Winter, Bouvier, Weinbauer, & Thingstad, 2010; Lennon & Jones, 2011; Lennon, den Hollander, Wilke-Berenguer, & Blath, 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 & 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 under periods of resource starvation (cf. density-independent stressors such as temperature, osmostic stress or antimicrobials) (Letten, Yamamichi, Richardson, & Ke, 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 & 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 stabilization and fitness equalization (Ellner et al., 2019). This "trait-decomposition" approach (recently developed by Ellner et. al (2019)) 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).

How microbial ecology can benefit coexistence theory

The development of coexistence theory has undoubtedly outpaced its empirical validation (Godwin et al., 2020; Terry & 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 & Armitage, 2024, but see Terry, 2024), 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 has 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 stabilizing mechanisms, studies of temporal storage effects and related mechanisms in plant and animal systems have typically relied on simulation approaches with parameterized models (e.g., Armitage & Jones, 2019b; Hallett, Shoemaker, White, & Suding, 2019). In contrats, 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 & Gonzalez, 2005; Jiang & Morin, 2007; Ellner, Snyder, & Adler, 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 spatio-temporal variability in environmental conditions (e.g., using a chemostat). One of the few studies that has empirically quantified spatial coexistence mechanisms used found higher diversity of *Pseudomonas* strains in static (heterogenous) compared to shaken (homogenous) environments when dispersal between environments was permitted (Tan, Rattray, et al., 2017). Modified chemostats with gradients of environmental conditions (i.e., "gradostats", *sensu* Lovitt & Wimpenny, 1981), complex artificial "micro-landscapes" (Larsen & Hargreaves, 2020), or biofilm model systems (O'Brien & 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 (Lankau, 2011; Hiltunen, Kaitala, Laakso, & Becks, 2017; Wittmann & Fukami, 2018; Yamamichi & 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 sorting of standing genetic variation rather than *de novo* evolution (Sakarchi & Germain, 2023; Hart, Turcotte, & Levine, 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, Zhao et al., (2016) studied coexistence between allopatrically and sympatrically evolved pairs of bacteria, 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, Lenski, & Barrick, 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. High-throughput robotic or droplet-based systems, which can rapidly assemble and observe the dynamics of many different microbial communities (e.g., Diaz-Colunga, Catalan, San Roman, Arrabal, & Sanchez, 2024; Kehe et al., 2019), would be especially powerful tools for testing emerging theory on species coexistence in diverse communities where indirect interactions can significantly complicate predictions of species coexistence (Spaak & Schreiber, 2023).

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.

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 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. 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 for 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 population structure of a given species can even occur between different growth stages (Zambrano, Siegele, Almirón, Tormo, & Kolter, 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 & Levins, 1967; Spaak & 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 & Schreiber, 2023; Song & Spaak, 2024; Ranjan, Koffel, & Klausmeier, 2024), and are likely to be important for microbial applications of this framework.

High frequencies of non-competitive or context-dependent interactions (Kehe et al., 2021; Momeni, Xie, & Shou, 2017; Kost, Patil, Friedman, Garcia, & Ralser, 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; Chesson & Kuang, 2008; Chesson, 2013) and the sensitivity to competition formula of Carroll et al. (2011) – do not account for positive interactions between species (Spaak & De Laender, 2020). However, such interactions are seemingly ubiquitous in microbial communities with ample evidence of cross-feeding (Dal Bello, Lee, Goyal, & Gore, 2021), multi-species biofilm formation (Nadell, Drescher, & Foster, 2016), and sharing of antibiotic resistance genes (Ellabaan, Munck, Porse, Imamovic, & Sommer, 2021). 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). 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 & De Laender, 2020; Koffel, Daufresne, & Klausmeier, 2021) – but they are nonetheless difficult to integrate with traditional approaches. 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.

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 limitations, 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 and E) are built upon the assumption that mutual invasibility predicts coexistence. However, it is easy to imagine cases where all species can invade but will not ultimately coexist or where not all species cannot invade even if they could all coexist (Barabás et al., 2018; E. Johnson & Hastings, 2022; Clark et al., 2024). Although complex ecological dynamics (e.g., Allee effects) and stochasticity (both environmental and demographic) can break the simple logic of invasion analysis, even in multispecies systems, invasion growth rates have been shown to be broadly informative (Chesson, 2018; Schreiber, Levine, Godoy, Kraft, & Hart, 2023; Clark et al., 2024).

Another shortcoming of coexistence theory is the (over)emphasis it has traditionally placed on qualitative (e.g. coexistence vs exclusion), rather than 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, Rinaldi, Gragnani, Mur, & van Nes, 1997; Grover, 1997; Jankowiak, Hattenrath-Lehmann, Kramer, Ladds, & Gobler, 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 the 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 always 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, common uses of coexistence theory – specifically the quantification of niche and fitness differences

– tells us *if* species coexist but not necessarily *how* they coexist. Niche and fitness differences summarize the combined effects of 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 mechanistic models (e.g., resource-consumer models) are used to estimate niche and fitness differences, which has been the dominant approach of applying coexistence theory to date (Chesson, 2013; Godwin et al., 2020). Indeed, phenomenological approaches, such as the widely-used generalized 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, Desmond-Le Quéméner, Duquennoi, Filali, & Bouchez, 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.

Conclusion

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 stimulus for closer cross-pollination between these two fields. This includes leveraging coexistence theory to foster rigour and conceptual synthesis to 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.

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Author Contributions

JAO, DWA, and ADL conceived the research. JAO performed the systematic review and wrote the first draft of the manuscript with input from ADL and DWA. All authors contributed to the final manuscript.

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Supplementary Material for

Coexistence theory for microbial ecology, and vice versa

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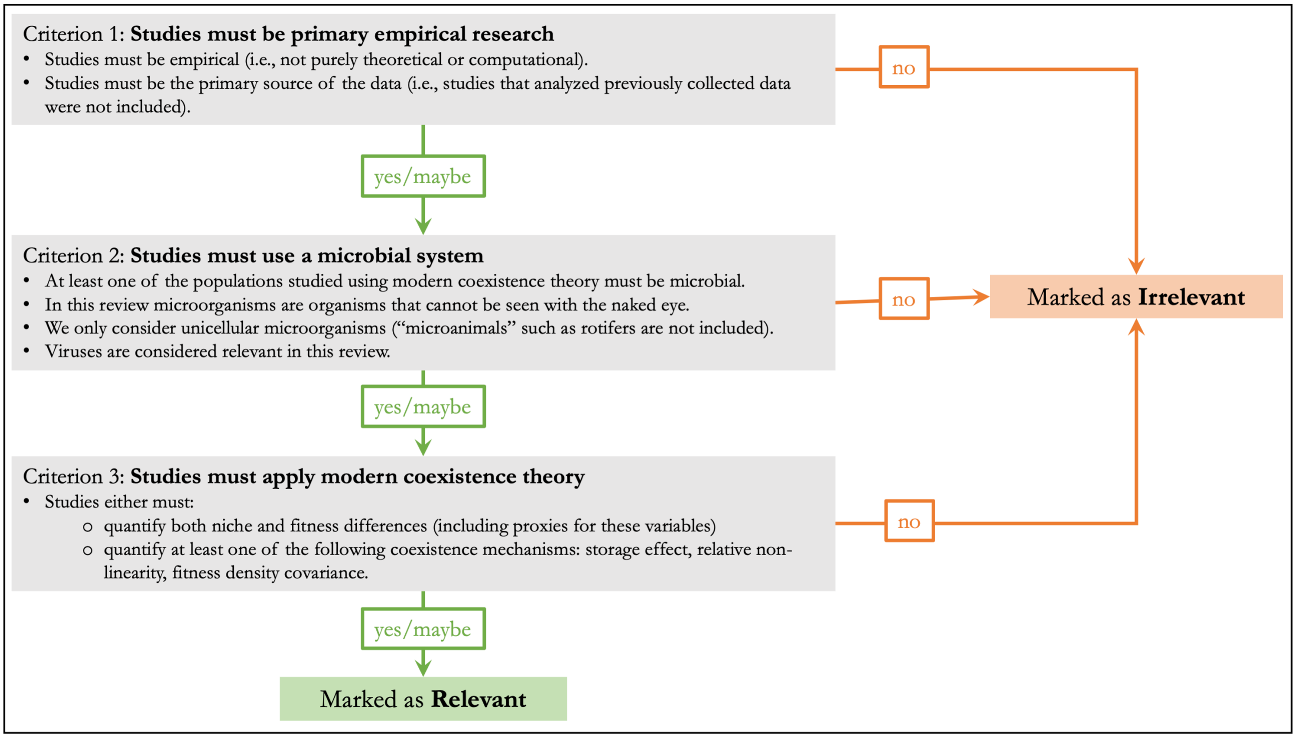
Contents

1 Overview of systematic review

We performed a systematic review to find empirical coexistence theory conducted in microbial systems. First we established three distinct inclusion criteria that a study had to meet in order to be included (Fig. S1). We considered a study relevant if it was primary empirical research and it was conducted in microbial systems and it applied (modern) coexistence theory. We identified relevant studies through previous systematic reviews and by performing our own search of the literature using a machine learning framework for abstract screening (Fig. S2).

The previous systematic reviews that we initially used to identify studies were of: (i) 29 empirical studies where both niche and fitness differences could be quantified (Buche, Spaak, Jarillo, & De Laender, 2022), (ii) 49 empirical studies that empirically applied any form of coexistence theory (Terry & Armitage, 2024), and (iii) 96 empirical studies that generally tested mechanisms of species coexistence (Hawlena, Garrido, Cohen, Halle, & Cohen, 2022). Of the 174 studies identified from previous reviews, 15 studies were potentially relevant based on how they had been labelled by the authors of those reviews (Fig. S2). Six of these studies were excluded at the full text screening stage for not meeting at least one of our inclusion criteria (Fig. S1). The remaining nine studies were included in our systematic review.

Guided by the search terms used by the previous systematic reviews, we then performed our own search of the literature and we used a novel machine learning framework for abstract screening. The methods and results of this AI-aided literature review are outlined below.



Criterion 1: **Studies must be primary empirical research**
- Studies must be empirical (i.e., not purely theoretical or computational).
- Studies must be the primary source of the data (i.e., studies that analyzed previously collected data were not included).

Criterion 2: **Studies must use a microbial system**
- At least one of the populations studied using modern coexistence theory must be microbial.
- In this review microorganisms are organisms that cannot be seen with the naked eye.
- We only consider unicellular microorganisms ("microanimals" such as rotifers are not included).
- Viruses are considered relevant in this review.

Criterion 3: **Studies must apply modern coexistence theory**
- Studies either must:
    - quantify both niche and fitness differences (including proxies for these variables)
    - quantify at least one of the following coexistence mechanisms: storage effect, relative non-linearity, fitness density covariance.

Figure S1: Decision tree with our three inclusion criteria. For a study to be included it had to meet all three criteria. This decision tree was also used during abstract screening where "yes" or "maybe" led to a record being marked as (potentially) relevant.

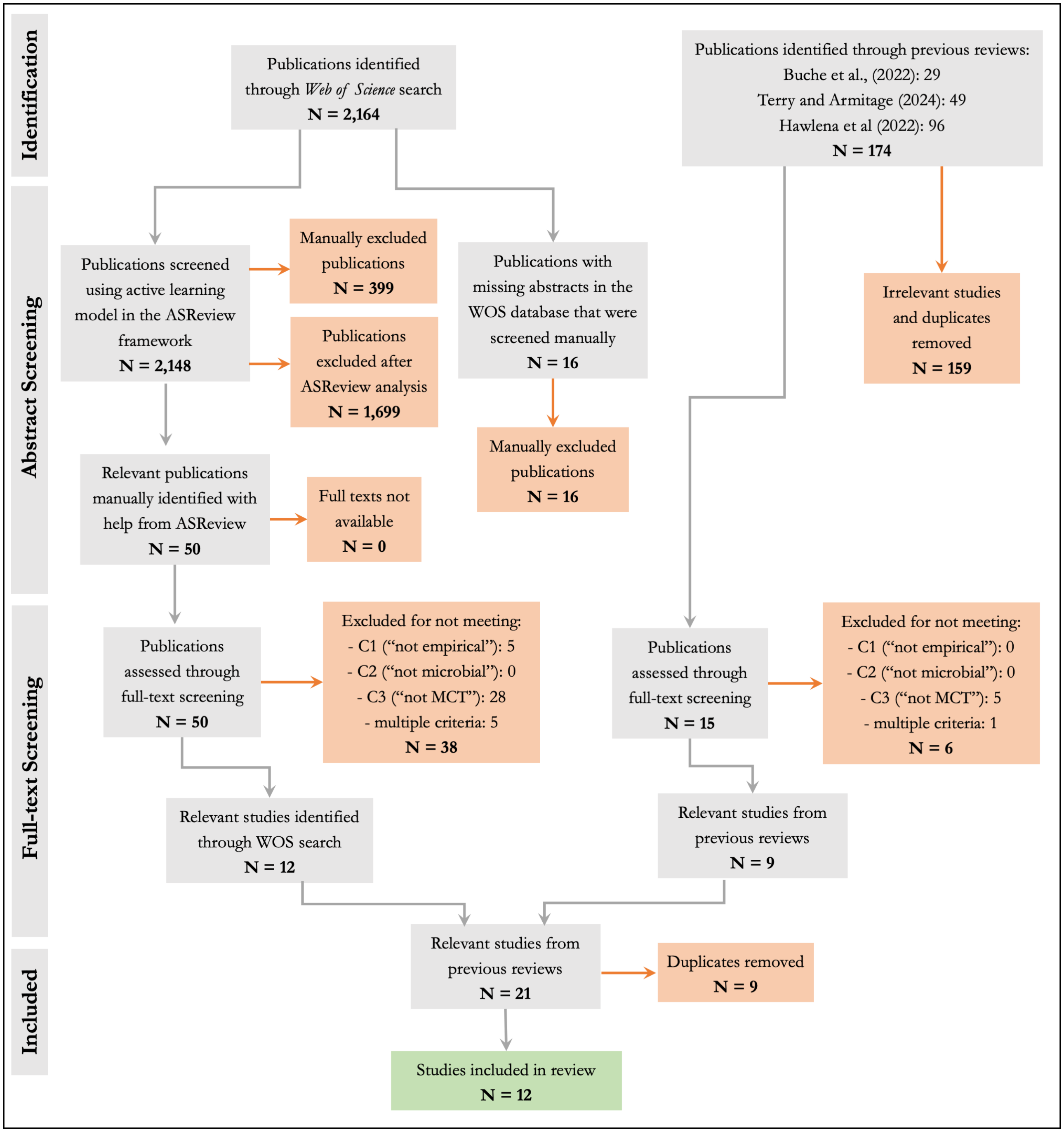


Figure S2: PRISMA-style flow chart summarizing the steps of our systematic review from study identification, to abstract screening to full-text screening, to study inclusion.

2 AI-aided literature review

A literature search was performed on *Web of Science* on the 19th of May 2024 using the following advanced search where a study needed to have a word associated with microbial systems *and* a word associated with modern coexistence theory in either its title, abstract, or keywords:

***Web of Science* search.** TS = Topic (title, abstract, or keyword).

TS = ("microb*" OR "microorganism*" OR "bacteri*" OR "fung*" OR "yeast*" OR "alga*" OR "phytoplankton" OR "diatom*" OR "cyanobacteria" OR "protozoa" OR "protist*" OR "ciliate*" OR "archaea" OR "virus*" OR "biofilm")

AND

TS = ("coexistence theory" OR "co-existence theory" OR "species coexistence" OR "species co-existence" OR "niche differ*" OR "fitness diff*" OR "niche overlap" OR "stabili$ing mechanism*" OR "equali$ing mechanism*" OR "fitness ratio*" OR "fluctuation-*dependent" OR "fluctuation *dependent" OR "relative non-linearit*" OR "relative non linearit*" OR "storage effect*" OR "lottery effect*" OR "lottery model*" OR "invas* criteri*" OR "invas* condition*")

This broad search returned 2,164 records, including all nine relevant studies that had been identified from previous systematic reviews. We then used a machine learning framework called *ASReview*, where active learning models help to accelerate abstract screening (Van De Schoot et al., 2021). In summary, a human screener will input their unlabelled dataset to the model and they will identify some "training" data (labelled abstracts that are known to be relevant or irrelevant based on prior knowledge). The active learning model then ranks the entire unlabelled dataset in order of relevancy based on the words (or sentences) that are found in the abstracts that were marked by the human as irrelevant or relevant. The paper that the active learning model marks as most relevant is shown to the human screener, who then reads that abstract and uses their inclusion criteria to determine whether to mark it as relevant or irrelevant. The active learning model is then updated based on the decision of the human screener and the order of the remaining records in the unlabelled dataset are updated based on their new relevancy scores. This screening process continues until some *a priori* stopping criterion is met. If the active learning model is working as expected, plotting the number of records screened against the number of relevant records found should return a saturating curve.

This framework requires that all records have abstracts, but there were 16 papers in our search results that did not have abstracts. The full texts of these papers were screened and all 16 were marked as irrelevant. The remaining 2,148 papers were screened using *ASReview*. The importance of the training data for these active learning models has been shown to be extremely low, given that the models are constantly being updated. Nonetheless, for the relevant training records we chose three studies from the nine relevant studies identified through previous reviews that were diverse in terms of the study organisms and approach of applying coexistence theory (Letten, Dhami, Ke, & Fukami, 2018; Li, Tan, Yang, Ma, & Jiang, 2019; Narwani, Alexandrou, Oakley, Carroll, & Cardinale, 2013), and for the irrelevant training records we randomly selected three records that were confirmed to be irrelevant. Our *a priori* stopping criterion, which was informed by a recent simulation study (Campos et al., 2024), was a combination of time driven and data driven heuristics where screening would stop when at least 20% of the records had been screened and then when the number of records marked as irrelevant in a row equalled at least 5% of the total number of records marked as irrelevant. We used the default active learning model settings (i.e., "Naive Bayes" as the classifier and "Term Frequency-Inverse Document

Frequency" for feature extraction). This combination works well for fields were terminology if well defined (e.g., "niche differentiation" or "temporal storage effect" have very specific meanings) and has been shown to be very efficient and accurate in simulation studies (Van De Schoot et al., 2021; Campos et al., 2024).

By the time our stopping criterion was met, we had marked 50 records as relevant and 399 records as irrelevant. When we stopped the abstract screening process, 65 records had been marked as irrelevant in a row. All nine relevant records from previous studies had been identified during the abstract screening process and the recall curve was clearly saturating (Fig. S3). 1,699 records were automatically excluded as they were not suggested to be relevant by the active learning model before the stopping criterion was met. The 50 records manually marked as relevant progressed to full text screening and all of their full texts were available. Following full-text screening, 38 of these records were excluded (five studies were not empirical, 28 studies did not use coexistence theory, and five studies did not meet multiple inclusion criteria). The 12 studies that passed through full-text screening included the nine relevant studies identified from previous reviews as well as three additional relevant studies (Fig. S2).

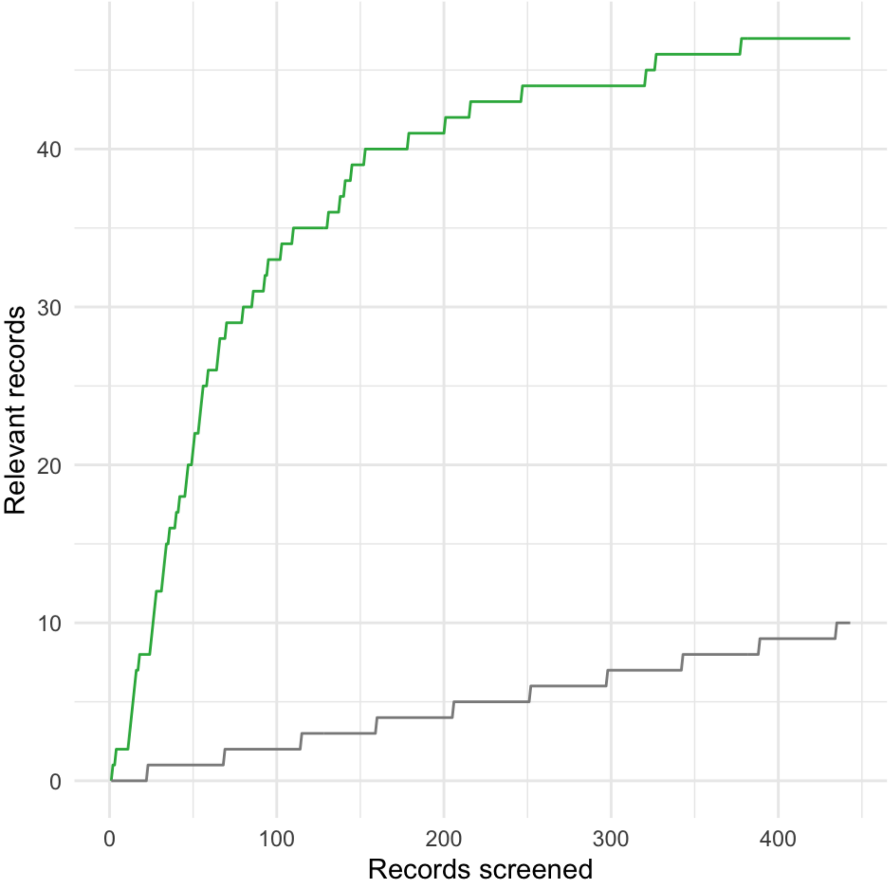


Figure S3: Recall curve showing the number of relevant records identified plotted against the number of records screened (green line). The grey line shows the number of relevant records that would have been expected by random sampling of the unlabelled dataset (i.e., traditional abstract screening). The gap between the green and the grey line shows the efficiency of the machine learning abstract screening framework.

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