

Review

Are microbes colimited by multiple resources?

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Resource colimitation — the dependence of growth on multiple resources simultaneously — has become an important topic in microbiology due both to the development of systems approaches to cell physiology and ecology and to the relevance of colimitation to environmental science, biotechnology, and human health. Empirical tests of colimitation in microbes suggest that it may be common in nature. However, recent theoretical and empirical work has demonstrated the need for systematic measurements across resource conditions, in contrast to the factorial supplementation experiments used in most previous studies. The mechanistic causes of colimitation remain unclear in most cases and are an important challenge for future work, but we identify the alignment of resource consumption with the environment, interactions between resources, and biological and environmental heterogeneity as major factors. On the other hand, the consequences of colimitation are widespread for microbial physiology and ecology, especially the prediction and control of microbial growth, motivating continued consideration of this state in microbiology.

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What is colimitation?

The growth of microbes, like all living things, depends on the availability of resources in the environment, along with various nonresource factors such as predators, toxins, or inhibitors [1]. Resources include macronutrients (e.g. sources of carbon, nitrogen, and phosphorus), micronutrients (e.g. trace metals), complex nutrients (e.g. vitamins, amino acids, or prey), as well as nonchemical resources such as light and space. Some of these resources are essential — their consumption is required for growth — while others are nonessential,

such as an amino acid that a cell will consume if present in the environment but that it can also synthesize. The dependence of growth on resource availability is known as *resource limitation* [2] and is a fundamental concept across biological systems, owing to its conceptual and practical consequences (addressed later in this article).

Although microbes require multiple resources to grow, many studies have assumed the dependence of growth on resources follows a principle called the Law of the Minimum (attributed to Justus von Liebig in the context of agricultural yields [3]), which states that only a single resource at a time can limit growth. (The ‘minimum’ in the law is due to the corollary that the realized growth is the minimum potential growth allowed by any one resource; see Box 1 for mathematical models of this assumption.) The Law of the Minimum is explicitly or implicitly assumed in a large body of classical ecological models, including resource-ratio theory [4].

Box 1: Quantitative studies of colimitation require mathematical models of how growth traits (usually growth rate or growth yield) depend on the concentrations of resources in the environment. The most well-known model of how per-capita growth rate g (e.g. per hour) depends on a resource concentration R (e.g. in mM or g/ml) is the Monod model:

$$g(R) = g_{\max} \frac{R}{R + K}, \quad (1)$$

where g_{\max} is the maximum growth rate when the resource is unlimited and K is the half-saturation concentration [2,25]. The maximum growth rate g_{\max} typically depends on the identity of the focal resource (e.g. growth will be faster for a saturating amount of glucose than for a saturating amount of mannose [26,27]), and both g_{\max} and K for the focal resource depend on all other media conditions as well. Other models of this dependence (e.g. Droop [28], Blackman [29], Bertalanffy [19], and Hill models [30]) have been studied in some systems but are less common. Although the Monod model is generally taken to be a phenomenological description of growth, it can be rationalized in terms of Michaelis-Menten kinetics of resource uptake and metabolism [9,21].

Studying colimitation requires generalizing the Monod model to multiple resources. The Law of the Minimum, in which only one resource at a time can limit growth, is implemented mathematically by assuming the realized growth rate is the minimum of potential (Monod-dependent) growth rates for each resource (Figure 2e) [9,6,19,7,21]:

$$g(R_1, R_2) = g_{\max} \min\left(\frac{R_1}{R_1 + K_1}, \frac{R_2}{R_2 + K_2}\right). \quad (2)$$

In contrast, alternative models such as the ‘additive model’ [9] (not to be confused with the colimitation scenario in which resources have additive effects on a growth trait, as shown in Figure 2c) allow the growth rate to depend on multiple resources simultaneously (and hence describe colimitation; see Figure 2f):

$$g(R_1, R_2) = g_{\max} \frac{1}{1 + K_1/R_1 + K_2/R_2}. \quad (3)$$

Many of these models, including Equations (2) and (3) as well as other common models such as the multiplicative Monod model [6,19,7,31] and the Poisson arrival time model [9,19] (also known as

the synthesizing-unit model [21,32]), can be derived at least heuristically from some underlying mechanism of resource uptake and metabolism. Other models, such as the Mankad and Bungay model [8,30] and the Hill model [17], serve only as phenomenological descriptions of data. These models all aim to describe independent essential resources; other models exist for substitutable, chemically dependent, or antagonistic resources [6,7].

Fitting these models to empirical data requires growth rate measurements across a wide range of resource concentrations, ideally starting below the half-saturation concentrations K_i and extending into the regime where growth rate saturates. Unfortunately, there are few such data sets, primarily due to the need to measure growth rate at low cell densities [25], but those which exist generally refute the Law of the Minimum model (Equation 2) in favor of colimitation [9,19,21,23,17]. However, it has so far been difficult to distinguish among the different colimitation models using empirical data, given the mathematical similarity of the models and large uncertainties in the measurements. In any case, it is unclear whether these colimitation models differ in any meaningful biological sense. For example, the population dynamics resulting from these models appear to be qualitatively similar (but see Poggiale et al. [28] for a counterexample), and recent work has suggested that these models are simply different mathematical approximations of the same underlying process [21,31].

It is also possible to model the dependence of growth yield on resource concentrations. Unlike growth rate, growth yield depends on the total amount of a resource supplied to the biomass, rather than the concentration of the resource at a single instant in time. The simplest model of this dependence is to assume a fixed stoichiometry of biomass to the resource, such that the total yield y (biomass concentration, e.g. in units of optical density or cells/ml) is proportional to the supplied resource concentration R [2,24]:

$$y(R) = sR, \quad (4)$$

where s is the stoichiometry of biomass to resource (biomass concentration per unit resource). Thus, the Law of the Minimum for yield would dictate that the total yield depends on whichever resource (among independent essential resources) has the minimum potential yield [17]:

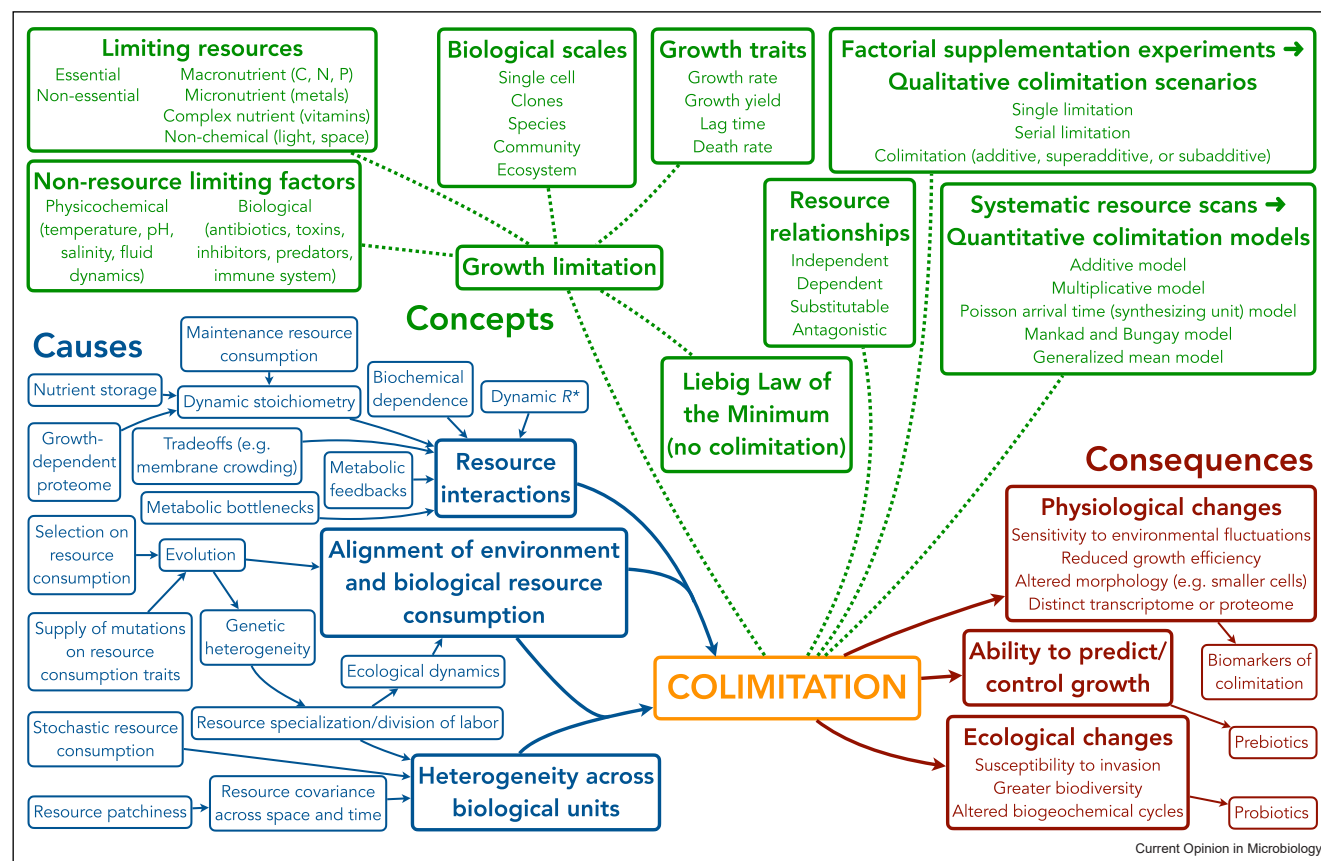
$$y(R_1, R_2) = \min(s_1 R_1, s_2 R_2). \quad (5)$$

As with Equation (2) for growth rate, the Law of the Minimum for growth yield (Equation 5) has no colimitation by construction. Since most experiments testing yield colimitation only test a single concentration of each resource rather than scanning a range of concentrations [12], these analyses can categorize yield dependence into qualitative classes (e.g. single limitation, serial limitation, additive colimitation, super-additive colimitation, etc.; see Figure 2a–d) but cannot test quantitative models [7]. As a result, there are no widely used quantitative models for yield that describe colimitation (but see a recent model for substitutable resources [24]). It is possible to derive models based on specific mechanisms (e.g. dynamic stoichiometry), but these contain too many parameters to reliably fit to data [17]. Recent work has instead proposed a phenomenological model for this dependence based on the power mean of each resource’s potential yield [17,33]:

$$y(R_1, R_2) = ((s_1 R_1)^q + (s_2 R_2)^q)^{1/q}, \quad (6)$$

where $q < 0$ is a dimensionless parameter that tunes the degree of colimitation ($q \rightarrow -\infty$ recovers the Equation (5) Law of the Minimum with no colimitation). However, future work will need to establish the connection (if any) between such low-dimensional phenomenological models and specific mechanisms.

Figure 1



Map of relationships between concepts, causes, and consequences of resource colimitation, as discussed in this article. Solid lines with arrows indicate causal relationships (e.g. resource interactions cause colimitation), and dashed lines without arrows represent conceptual relationships (e.g. growth traits are a conceptual aspect of growth limitation).

However, the Law of the Minimum is violated if the availabilities of multiple resources simultaneously affect growth, a scenario known as resource *colimitation* [5–7]. While this phenomenon has long been considered in the context of microbes and other organisms [8–10], the renaissance of microbial physiology and ecology over the last decade has brought new relevance to the topic. In particular, the development of these fields has raised new questions about whether resource colimitation actually occurs in microbes, and if so, what are its causes and consequences. Understanding the consequences of colimitation is important for evaluating whether the Law of the Minimum is a useful, if not exactly true, model of microbial growth, or if there are important aspects of microbes that fundamentally depend on the existence of colimitation. Since the conceptual foundation of colimitation, especially the plethora of associated terminology, has often been a source of confusion in previous literature, we summarize its most important elements here (see also previous syntheses [5–7]). In contrast to previous reviews, this article aims to provide a unified map (depicted schematically in Figure 1) of key

concepts (green) related to colimitation, along with its potential causes (blue) and consequences (red) as discussed in this article's following sections. We also assemble an updated survey of evidence for colimitation across habitats in the next section.

The first key aspect of resource colimitation is the choice of biological scale (single cell, clonal population, species, multispecies community, or whole ecosystem; Figure 1) at which we are considering growth in response to resources [7]. Colimitation can differ across these scales, for example, when the limitation state of a whole population does not match the limitation state of each constituent cell [11]. The second key aspect of colimitation is the growth trait affected by the resources (Figure 1). The two most common growth traits for studying limitation are the growth rate and the growth yield (sometimes known as kinetic limitation and stoichiometric limitation [5]). Resources can limit rate and yield differently; analogously, a car's speed (cf. growth rate) is typically limited by air intake or drag, while its range (cf. growth yield) is limited by fuel or battery.

While yield limitation is often emphasized over rate limitation [12], especially in field studies owing to the feasibility of measuring total biomass compared with measuring biomass rate of change [13], these concepts are distinct and important in different contexts. For example, rate limitation is likely more important to evolution in well-mixed environments since fast growth rate is generally selected in these systems, whereas efficient growth yield is selected when there is spatial structure or another mechanism to privatize resources [14]. Similarly, growth rate tends to be more closely linked to aspects of microbial activity, particularly at very low-resource concentrations, whereas growth yield is more relevant to stocks of resources and depends more strongly on resource stoichiometry.

The third key aspect of resource colimitation is the relationship between the resources (Figure 1), which can be independently consumed (variously referred to in the literature as type I, heterologous, noninteractive, complementary, or essential resources [5–7]; e.g. a source of carbon and a source of nitrogen); substitutable, meaning the resources are consumed interchangeably (also known as type II, homologous, or mixed resources [5,6]; e.g. two sources of carbon such as glucose and galactose); biochemically-dependent, where the uptake or usage of one resource depends on the other (also known as type III resources [6]; e.g. a source of phosphorus and a source of zinc); or antagonistic, where one resource inhibits uptake or usage of the other [7]. These different relationships lead to distinct possibilities for colimitation between the resources. For example, two substitutable resources may not be colimiting if a cell only activates a pathway for one at a time, as occurs under diauxic growth [15].

Given a choice of biological scale, growth trait, and a set of focal resources, how do we quantify colimitation? Empirically, colimitation is usually measured by factorial supplementation experiments, where each resource is supplemented into the medium by itself and in combination with another resource (Figure 2a–d) [12,16]. The outcome of these experiments can be difficult to interpret when the supplementations only test a single concentration of each resource on a single set of background concentrations [7,17]. Moreover, the limited quantitative data resulting from these experiments means their outcomes are usually interpreted qualitatively according to some predefined categories (Figure 2a–d, e.g. single limitation, serial limitation, additive colimitation, super-additive colimitation, etc.) [7,12]. An alternative approach is to systematically scan the concentration of each resource over some range and quantitatively measure the growth response (Figure 2e,f) [18,9,6,19,7,20–23,24,17]. This allows one to test quantitative hypotheses in the form of mathematical models (see Box 1); in particular,

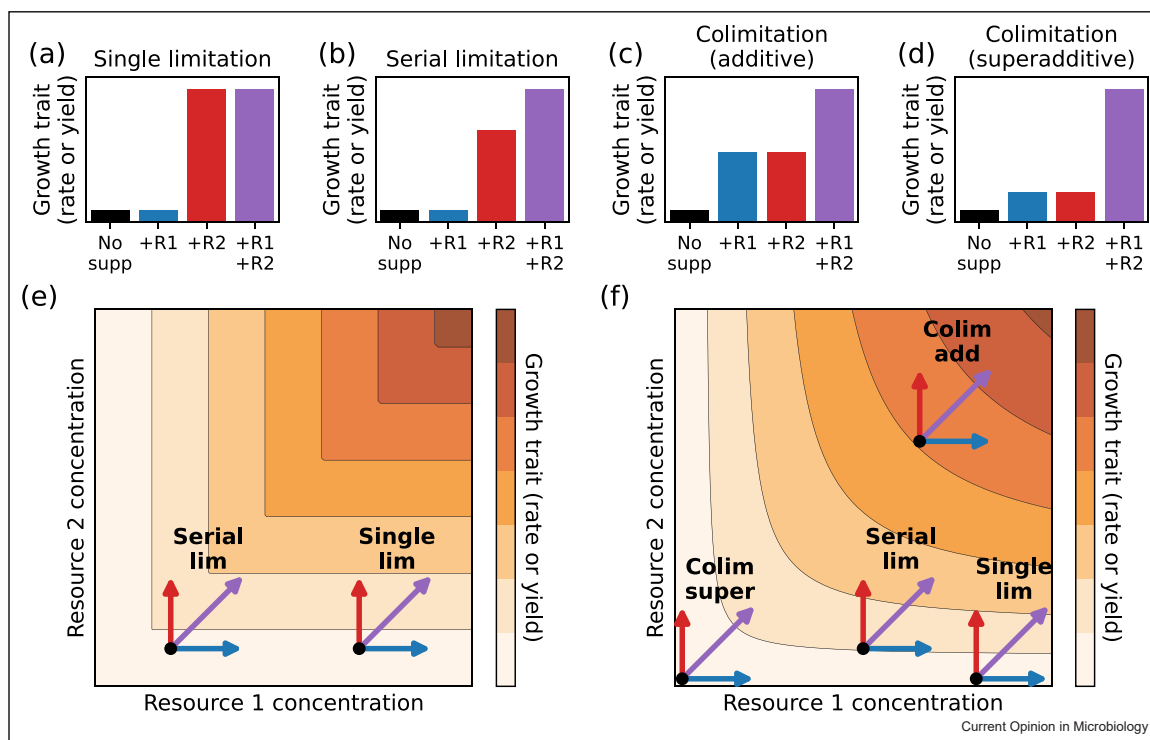
this approach is necessary to rigorously reject the Law of the Minimum hypothesis and resolve true colimitation compared with serial limitation. This is because, as shown in Figure 2e,f, many different outcomes of factorial supplementation experiments are possible even in the same underlying model depending on the starting conditions and supplemented concentrations [7]. Furthermore, these systematic resource scans motivate thinking of resource (co)limitation as a quantitative, rather than binary (e.g. limiting or not), property of resources in a biological system [10,17].

Are microbes colimited in natural environments?

While there has been evidence of microbial growth colimitation in laboratory environments for many years now [8,9,21,23,17], an increasing number of recent studies have been reporting colimitation of microbes in their natural habitats. We have compiled results from 71 previously published tests for colimitation in a variety of ecosystems spanning marine, freshwater, brackish, and terrestrial habitats (Table S1, drawn primarily from surveys by Harpole et al. [12] and Browning and Moore [16]). We summarize these results in Figure 3, breaking them down by habitat (Figure 3a), growth trait (rate or yield; Figure 3a, inset), and resource (Figure 3b). Altogether 46% of these tests claim to find evidence for colimitation, compared with the 28% of studies in a 2011 meta-analysis that found evidence for colimitation [12]. This increase is presumably not because colimitation is becoming more common in nature but because a greater appreciation and understanding of the phenomenon has led more researchers to test for it. Furthermore, there is reason to believe that the actual proportion of colimited systems is even higher, given that colimitation is tricky to access experimentally because of the need to sample the correct elemental ratios and absolute concentrations [17], as well as the possibility of environmental patchiness in space or time [34].

The existing literature has generated a good appreciation for colimitation in nature, but there are gaps in the current evidence. These studies invariably study colimitation at the microbial community scale since they test growth of natural samples (e.g. seawater). Thus, there is little to no information about colimitation at smaller biological scales (single cells, clones, species) in natural environments; laboratory experiments may be able to fill this gap [9,19,21,23,17], but they will require careful design to give any insights into natural environments. Both rate and yield colimitation have been tested in marine systems (Figure 3a, inset), with rate colimitation having a somewhat higher frequency of detection than yield colimitation has. However, measurements of yield colimitation dominate other habitats (Table S1),

Figure 2



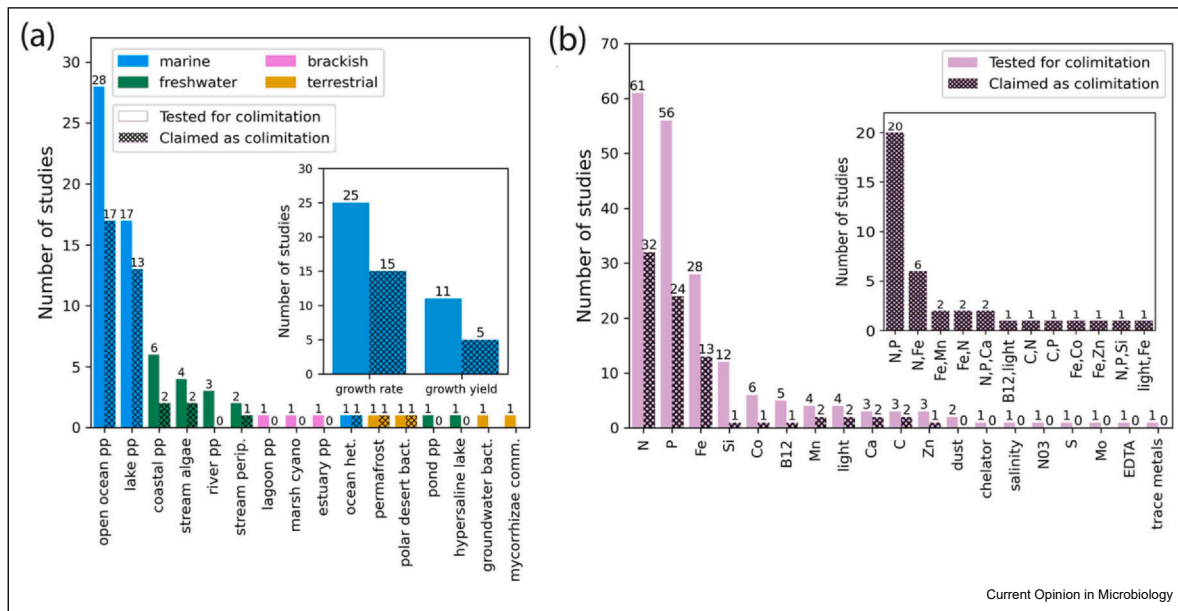
Two approaches to measuring colimitation. The traditional approach is factorial supplementation experiments, in which two resources are added separately (blue bar for +R1, red bar for +R2) and together (purple bar for +R1+R2) to test their effects on a growth trait (e.g. growth rate or growth yield) relative to no supplementation (black bar). We show four qualitative scenarios for these experiments: **(a)** single limitation (for resource 2 only), **(b)** serial limitation (initial limitation is for resource 2 only, but upon adding resource 2, the limitation switches to resource 1), **(c)** additive colimitation (the growth response of supplementing both resources is the sum of responses for each resource supplemented alone), **(d)** superadditive colimitation (the growth response of supplementing both resources is greater than the sum of responses for each resource supplemented alone). Other scenarios such as subadditive colimitation and antagonistic resources are possible [7] but are not realized by most common models (Box 1). However, factorial supplementation experiments that test only a single added concentration of each resource on a single background condition can be difficult to interpret. An alternative, more systematic approach is to measure the growth response across a scan of resource concentrations. For example, we show **(e)** a resource scan where growth obeys the Law of the Minimum (Box 1, Equation 2) and **(f)** a resource scan where growth can be colimited (Box 1, Equation 3). The arrows in panels **(e,f)** represent factorial supplementation experiments starting on different background resource concentrations and thus correspond to the different qualitative scenarios in **(a-d)**. The example scans in **(e,f)** are for independent resources (such as a carbon source and nitrogen source), but similar scenarios exist for other resource relationships.

leaving it unknown what level of rate colimitation exists in those environments. Additionally, most experiments looking at rate colimitation measure growth rate only from an initial and final time point after incubation with the supplemented resources, so the actual growth dynamics are usually unknown and the measured rates are likely lower bounds. That is, if the biomass grows rapidly right after resources are added, but the final time point is measured after growth has stopped, then the growth rate inferred from that data will be lower than the initial growth rate since that inferred rate will be an average over the stationary phase as well. Furthermore, all of this evidence relies on factorial supplementation experiments (cf. Figure 2a–d) rather than systematic scans of resource concentrations (cf. Figure 2e,f), which constrains our ability to definitively resolve different colimitation scenarios [7,17].

In terms of resources, most tests of colimitation in natural environments focus on independent or biochemically dependent resources. We note that while it is common in these studies to speak of (co)limitation for individual elements such as nitrogen, limitation likely differs between molecular forms of that element (e.g. ammonium vs. nitrate), so it is more precise to speak of limitation for those specific forms of the element rather than an element alone. In the case of autotrophs, the molecular form of most elemental resources is usually assumed, but this point is critical in heterotrophs, which often consume complex molecules containing multiple elemental resources (e.g. amino acids containing both carbon and nitrogen).

The largest body of experiments testing for resource limitation is from the aquatic sciences (marine and

Figure 3



Evidence in the literature for resource colimitation of microbes in nature. **(a)** Number of studies that tested for colimitation (clear bars) and claimed evidence of colimitation (hashed bars), broken down by habitat. Abbreviations: pp = primary production, perip. = periphyton, het. = heterotrophs, bact. = bacteria, comm. = community. The inset shows the number of marine systems measured for growth rate limitation versus those measured for growth yield limitation (all other habitats have only yield data). **(b)** Same as **(a)** but broken down by tested resource instead of habitat. The inset shows specific combinations of resources that have been claimed to be colimiting.

freshwater), perhaps due to the relative ease of performing resource supplementation experiments in liquid ecosystems compared with terrestrial or animal microbiomes (Figure 3a). Of all systems, surface ocean phytoplankton communities are the most well represented in the literature. Nitrogen, iron, phosphorus, cobalt/vitamin B12, and manganese have been claimed to be colimited for these communities (Figure 3b); nitrogen and phosphorus are the most tested and identified colimiting resource pair, followed by nitrogen and iron (Figure 3b, inset). Among nonchemical resources, light has also been found to colimit phytoplankton growth, alongside iron or vitamin B12 [35]. Colimitation in marine nonprimary producers, such as heterotrophic bacteria or zooplankton, is not well characterized yet, though a few examples exist, such as nitrogen and phosphorus colimitation of bacterial respiration [36]. There are also few tests of resource colimitation on ocean coasts, although there are some examples at the poles, where experiments have been conducted on microbial communities at the sea ice edge [37].

In freshwater systems such as lakes, resource colimitation has primarily been tested for nitrogen and phosphorus (although iron and light are also popular considerations [38]). One hypothesis is that nitrogen fixation alleviates nitrogen limitation, driving systems to phosphorus limitation, but that organisms can

‘overshoot’ phosphorus limitation by growth and become limited again by nitrogen or another resource; this is an extension of the so-called Phosphorus Limitation Paradigm [39]. In these systems, there are consistent super-additive effects of supplementing multiple resources compared with single resources; this effect commonly occurs across the spectrum of nitrogen-to-phosphorus availability, suggesting an intrinsic biological as opposed to extrinsic chemical mechanism [40]. Like in the oceans, the focus in freshwater habitats is on primary producers, though there are examples in freshwater bacteria (e.g. carbon vs. phosphorus colimitation [41]). In the freshwater literature, the focus on elemental ratios makes it sometimes difficult to resolve serial limitation versus simultaneous colimitation because the absolute concentrations of the resource additions also play a role in the experimental outcomes (Figure 2). In spite of this, it is common to see complex responses to the addition of multiple resources that cannot be explained through serial limitations alone [40].

Similar to freshwater microbes, terrestrial (soil) microbes exhibit diverse and complex responses to the supplementation of multiple resources, but resource supplementation experiments in soils require considerable effort, limiting the examples in the literature. Among the available examples, heterotrophic soil bacteria have repeatedly been claimed to be colimited for nitrogen and

phosphorus [42,43] as well as potentially carbon [44]. Interestingly, resource colimitation has been associated with reduced biodiversity of soil bacteria communities [44].

At this time, there is limited information about colimitation in other microbial ecosystems, although there are isolated tests in animal microbiomes [45], streams [38,46], groundwater bacteria [47], wastewater sludge [48], and specialized habitats, such as hypersaline lakes [49]. This may be because of the difficulty in experimentally assessing even a single resource limitation in these systems. However, the commonality of colimitation responses in experiments indicates that wherever scientists start to look for colimitation, they are likely to find it. Lack of experimentation across many habitats and systems makes it difficult to assess the full extent of this phenomenon and adding to this evidence will be an important step forward for comparing across natural habitats.

Finally, when considering natural communities, it should not be forgotten that other factors besides resource availability can limit growth (Figure 1). Examples of nonresource, ‘top down’ controls include the presence of predators, grazers, and phages; the role of the immune system for animal microbiome communities; and toxins produced by members of the microbial community. In nature, these controls will be layered on the ‘bottom up’ control of resource limitation, leading to the possibility of resource-predator colimitation [50].

What are the causes of colimitation?

In general, colimitation occurs when biological resource consumption aligns with environmental resource availability (Figure 1), but the important question is what specific mechanisms create such an alignment. At the cellular scale, interactions between resources are one of the principal ways that biological need and environmental supply can be aligned. For growth yield, mechanisms leading to resource interactions include dynamic stoichiometry in response to external resource availability [51,52], which may be caused by forming storage compounds [5,53], growth-dependent proteome allocation [54–57,58], and changes in maintenance resource consumption [59]. Resource interactions also emerge when growth stops at nonzero resource concentrations that depend on each other (i.e. when R^* is dynamically set) [60].

Colimitation of growth rate is likely to involve different types of interactions. Colimitation in typical growth rate models (Box 1) arises from a metabolic bottleneck where both resources must be jointly assimilated to form biomass [21]. Regulatory feedbacks between metabolic processes can also lead to resource interactions by

allowing coordination of nutrient availability with acquisition and use [61], such as coordination of carbon and nitrogen metabolism through pools of α -ketoglutarate in the metabolome of *E. coli* [62].

On a broader level, physiological tradeoffs may also lead to growth rate colimitation. These tradeoffs imply a constraint on resource uptake and utilization causing resource interactions. This could take the form of limited membrane space for transporters [63–65,29], the need to devote energy or resources to resource uptake and utilization [59], prioritization of growth over productivity/carbon fixation [52], and noncompetitive inhibition of growth under one resource by another resource [57]. There is evidence for all of these processes, but whether we should understand them as true tradeoffs leading to colimitation is not yet clear, because in general mechanisms of cell growth and biomass production are not well characterized. Indeed, most knowledge about mechanisms of colimitation has been generated through modeling insights as opposed to direct experimentation and that literature tends to be focused on autotrophic organisms [12], limiting what is known about nutrient colimitation of microbes in general.

At the scale of populations or ecosystems, heterogeneity across biological units may cause alignment of environmental supply and biological need, leading to colimitation for either growth rate or growth yield. In mixed microbial communities, colimitation is predicted by foundational ecological theory, such as the resource competition model, wherein different organisms have different resource needs and responses, allowing them to specialize and draw down multiple resources simultaneously [66,11,67]. More direct linkages are also possible, such as a division of labor when a resource must be processed by an organism that is limited by another independent resource [68]. Even clonal populations of microbes can be heterogeneous, leading to the possibility that individual cells have different elemental stoichiometries or resource preferences, such as when a subpopulation can produce storage molecules [69] or when there is stochastic resource consumption. At this time, most mechanistic studies of resource colimitation are focused on mixed microbial communities, and there is a need to study this phenomenon in clonal populations and in individual cells. In reality, the mechanisms of population and community colimitation are most likely layered on the biochemical/physiological mechanisms within individual cells.

Biological heterogeneity can be caused or exacerbated by patchiness in resource availability in the environment, such as when resources are available in high concentrations in localized areas. This can occur on a large scale due to linked biogeochemical cycles, such as

the difference between a rainy or dry season [70] and episodic events as in an animal microbiome after a meal [71], or at the microscale, such as a gradient of organic matter around a marine snow particle [72]. Conditional bioavailability of resources has also been proposed, such as when trace metal bioavailability is altered by metal-ligand interactions, which are produced biologically, leading to patchiness and interdependencies among resource and organism distributions [6].

Finally, the relationship between microbes and resource availability is driven by evolution, especially over long time scales. Since selection will usually be strongest on traits for the most rate-limiting resource, the evolutionary steady state of this process would be for all resources to have similar levels of limitation [73], which is a state of rate colimitation. For example, metabolic feedbacks, such as α -ketoglutarate that coordinates carbon and nitrogen consumption, may have evolved in this manner. Selection for different limitation traits in different spatial niches may also be a cause of genetic heterogeneity and division of labor. However, the effects of mutation supply (e.g. whether spontaneous mutations tend to reduce limitation for multiple resources simultaneously or induce tradeoffs) and other population genetic forces (such as horizontal gene transfer) remain less clear. These arguments hold mainly for growth rate colimitation, since limitation for growth rate, unlike growth yield, is more often expected to be under selection. Indeed, this may explain why we observe somewhat fewer instances of yield colimitation than we do for rate colimitation (Figure 3a, inset). These processes are related to the coevolution of life and the environment [74,75], likely underpin observed relationships between resource ratios in the environment and in biomass (such as the marine Redfield ratio), and form the basis of the fields of biogeochemistry and ecological stoichiometry.

What are the consequences of colimitation?

Even if the Law of the Minimum does not hold exactly for microbial populations [9,19,21,23,17] (Box 1), its usefulness as an approximation depends on whether the alternative scenarios of colimitation lead to significantly different consequences (Figure 1). For individual cells, colimitation (where multiple resources also have high absolute levels of limitation) entails greater sensitivity to environmental fluctuations since the cellular growth rate or yield depends on multiple resources rather than just one. Growth is also less efficient under colimitation conditions, in the sense that growth rate or yield is lower than would be expected from extrapolating single limitation conditions (Box 1) [17,19]. Colimitation may change cell morphology; for example, smaller cells with increased surface area-to-volume ratios have been a hypothesized response to colimitation [52,76]. Finally, both

rate and yield colimitation may also engender distinct molecular phenotypes, such as the transcriptome or proteome. For example, the proteome under carbon–nitrogen colimitation may be distinct from the proteome under nitrogen–phosphorus colimitation or under single limitation for any of these resources. Indeed, studies have shown that different limitation conditions can indeed elicit resource-specific responses to cell physiology [77,27,55], although physiology may eventually converge across conditions after genetic adaptation [78]. If the molecular phenotypes of colimitation are in fact distinct from single limitation phenotypes, we could use those differences to define biomarkers of colimitation that we can test for in natural samples without performing (often difficult) explicit growth measurements [13]. This would also suggest that laboratory experiments, which usually involve artificial conditions of single limitation, may give us an unrealistic picture of cell physiology in nature, if in fact microbes are often colimited in situ.

At the scale of a microbial population, knowing whether it is colimited is important for predicting and controlling its growth (Figure 1). These consequences of colimitation are especially important in the human gut microbiome, where promoting commensal growth using prebiotic compounds (such as fiber) or colonizing by probiotic species has potential health benefits [79]. For example, if we want to promote the growth of a commensal species, colimitation would mean that we must supplement all of the colimiting resources simultaneously. On the other hand, colimitation of a pathogen or invasive species could simplify growth inhibition since we must only remove one of the colimiting resources. Colimitation at the scale of populations and communities also can affect their susceptibility to invasions: a population should be more susceptible to invasion if it is colimited for growth rate, as that creates two niches that can be exploited by an invader (e.g. the invader can grow faster by being less limiting for either resource) rather than just one. This may enable rate-colimited populations to evolve more rapidly and generate greater biodiversity. Colimitation among substitutable resources implies balanced resource concentrations [17], which also promotes biodiversity since the concentration of each substitutable resource supports a commensurate abundance of a separate species according to the competitive exclusion principle [66,80].

At the scale of the global ecosystem, rate and yield colimitation present a potential challenge to predicting microbial contributions to biogeochemical cycles [21], both in terms of standing stocks (e.g. concentration of microbial biomass and how much carbon is bound up in microbial biomass) and fluxes (contributions of microbial enzymes to processes such as organic matter degradation, nitrogen fixation, and remineralization). The picture is further complicated by the knowledge that resource colimitation can alter the adaptive trajectories

of common marine cyanobacteria [76]. It remains to be seen whether accounting for microbial colimitation in biogeochemical models changes the elemental composition of environments [16]. For example, are there quantitative differences in element cycling and microbial community dynamics when we use growth rate models with colimitation rather than the Law of the Minimum (Box 1)? Does colimitation of nitrogen and phosphorus in phytoplankton lead to significant differences in net primary production compared to single limitation for nitrogen? Will colimitation alter the evolutionary trajectories of microbes and the biogeochemical processes they mediate? Determining the role of colimitation in biogeochemical cycles is especially critical, given the importance of predicting how those cycles will change with the climate and other human influenced processes.

What are the priorities for future colimitation research?

While recent years have generated progress in understanding the concept of colimitation [7,21,17] (Figures 1 and 2) and its empirical basis [12,19,22,16,23,17] (Figure 3), several major challenges remain to establish its role in microbial physiology and ecology. We believe the first priority should be to test possible causes of colimitation at the molecular and cellular scale (Figure 1) for a range of microbes and resources to establish whether these causes are generic or idiosyncratic to specific systems. If a mechanism is in fact common to many systems, then we can use that mechanism to identify biomarkers for measuring colimitation in natural samples, without performing explicit but laborious growth measurements.

However, if such biomarkers do not exist, then our next priority should be to collect more data on the growth response of natural samples over systematic scans of resource concentrations (Figure 2e,f), rather than traditional factorial supplementation (Figure 2a–d). In practice, we may need to prioritize testing more conditions over replicates if we hope to evaluate quantitative (Box 1) rather than qualitative models (Figures 2a–d and 3). These studies may require simulating natural environments in the laboratory rather than relying solely on natural samples, which would also have the advantage of enabling us to probe colimitation at the scale of single cells, clones, and species, rather than just whole communities.

Finally, a key priority is to test the effect of microbial colimitation on microbe-mediated processes, such as biogeochemical cycles for global nutrient cycling [21] and the functions of animal microbiomes. We hope to see greater consideration of colimitation across fields of research, from molecular and systems biology to ecology and evolution. These results would

determine whether colimitation is simply a fascinating aspect of microbiology or a critical driver of microbial activity in environmental science, biotechnology, and human health.

Data Availability

Table S1 contains information on literature data for resource colimitation studies used in Figure 3.

Declaration of Competing Interest

None.

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Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mib.2024.102509.

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