Are microbes colimited by multiple resources?

Noelle A. Held*
Department of Biological Sciences, Marine and Environmental Biology Division,
University of Southern California, Los Angeles, CA, USA

Michael Manhart†
Center for Advanced Biotechnology and Medicine and Department of Biochemistry and Molecular Biology,
Robert Wood Johnson Medical School, Rutgers University, Piscataway, NJ, USA
(Dated: March 1, 2024)

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.

Keywords: colimitation; resource limitation; microbial growth; microbial ecology; marine microbes; biogeochemistry

WHAT IS COLIMITATION?

The growth of microbes, like all living things, depends on the availability of resources in the environment, along with various non-resource factors such as predators, toxins, or inhibitors [1]. These resources include macronutrients (e.g., sources of carbon and nitrogen), micronutrients (e.g., metals), complex nutrients (e.g., vitamins, amino acids, or prey), as well as non-chemical resources such as light and space. Some of these resources are essential — their consumption is required for growth — while others are non-essential, such as an amino acid that a cell can synthesize but will also consume if present in the environment. 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]. However, the Law of the Minimum is violated if the availabilities of multiple resources simultaneously affect growth, a scenario known as resource *colimitation*. While this phenomenon has long been considered in the context of microbes and other organisms, 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. 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 particular, Fig. 1 shows a map of key concepts (green) related to colimitation, along with its potential causes (blue) and consequences (red) as discussed in this article's following sections.

The first key aspect of resource colimitation is the choice of biological scale (single cell, clonal population, species, multispecies community, or whole ecosystem) at which we are considering growth in response to resources [7]. Colimitation can differ across these scales, for example, when the limitation of a whole population does not match the limitation of each constituent cell [8]. The second key aspect of colimitation is the growth trait which is affected by the resources. 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 may limit rate and yield differently; analogously, a car's speed (cf. growth rate) is typically limited by air intake or

---

* To whom correspondence should be addressed. Email: nheld@usc.edu

† To whom correspondence should be addressed. Email: mmanhart@rutgers.edu

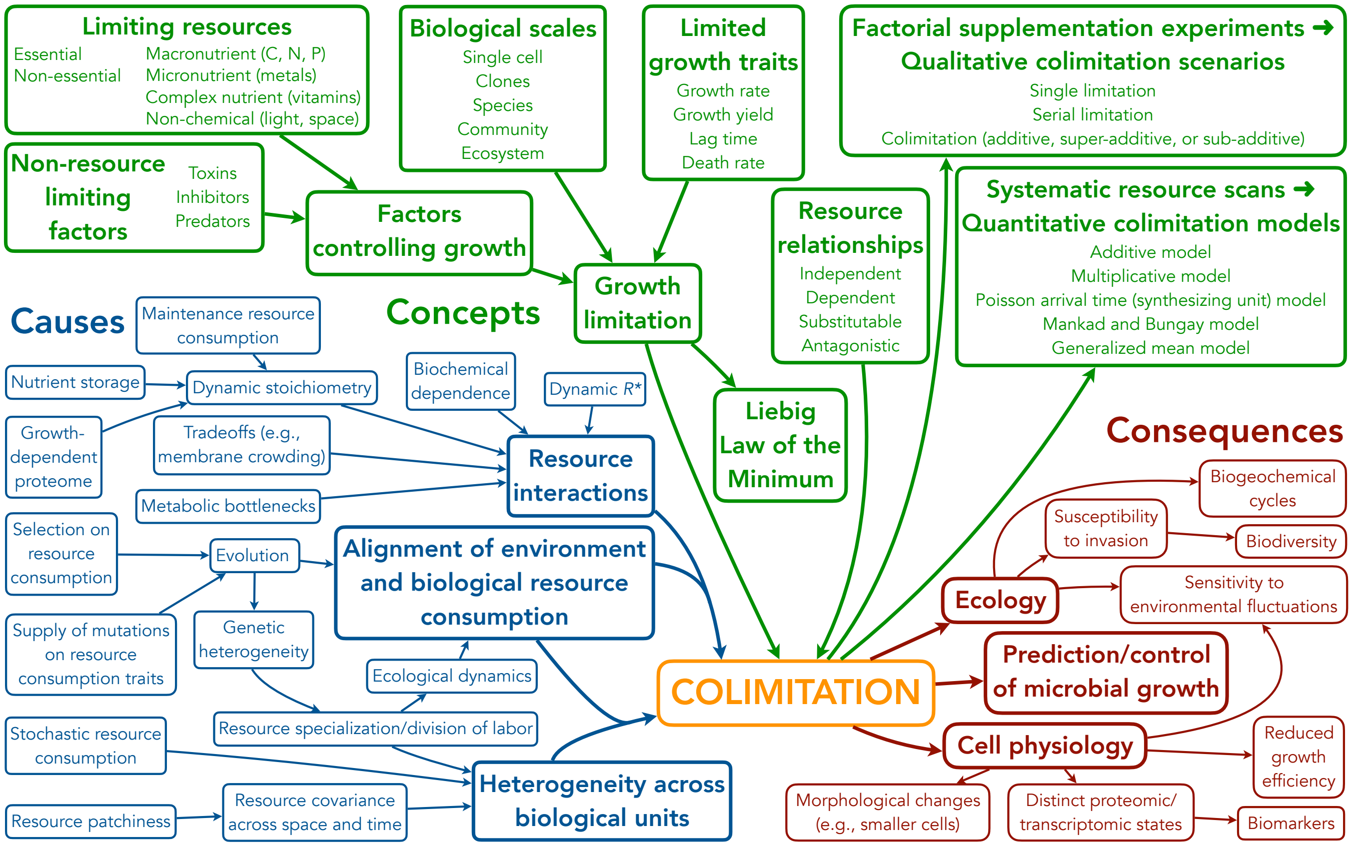

FIG. 1. **Map of relationships between concepts, causes, and consequences of resource colimitation, as discussed in this article.**

drag, while its range (cf. growth yield) is limited by fuel or battery. While yield limitation is often emphasized over rate limitation [9], especially in field studies owing to the ease of measuring total biomass over biomass rate of change [10], these concepts are important in different contexts. For example, rate limitation is likely more important to evolution since growth rate is generally under positive selection, whereas growth yield is only selected when there is spatial structure or another mechanism to privatize resources [11]. On the other hand, yield limitation may be more relevant to biogeochemical cycles, when tracking the overall stocks and flows of elements on Earth.

The third key aspect of resource colimitation is the relationship between the resources, which can be independently consumed (variously referred to in the literature as type I, heterologous, non-interactive, 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 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.

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 (Fig. 2a–d) [9, 12]. 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, 13]. Moreover, the limited quantitative data resulting from these experiments means their outcomes are usually interpreted qualitatively according to some discrete, pre-defined categories (Fig. 2a–d, e.g., single limitation, serial limitation, additive colimitation, super-additive colimitation, etc.) [7, 9]. An alternative approach is to systematically scan the concentration of each resource over some range and quantitatively measure the growth response (Fig. 2e,f) [6, 7, 13–21]. 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

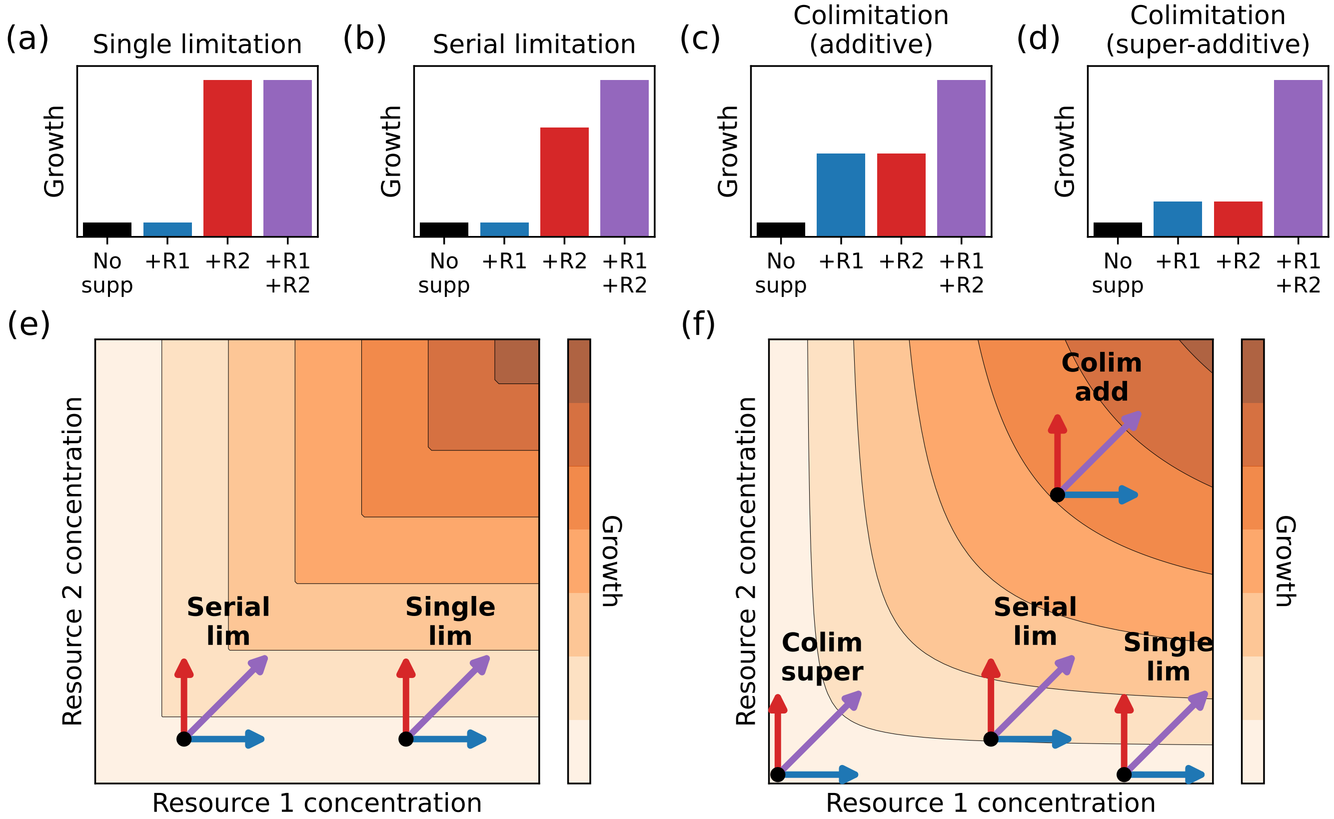

FIG. 2. **Approaches to measuring colimitation.** Schematics of factorial supplementation experiments for measuring colimitation, where two resources are added separately (+R1, +R2) and together (+R1+R2) to test their effects on a growth trait (e.g., growth rate or growth yield): (a) single limitation (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) super-additive colimitation (the growth response of supplementing both resources is greater than the sum of responses for each resource supplemented alone). Other scenarios such as sub-additive colimitation and antagonistic resources are possible [7] but are not realized by most common models (Box 1). Factorial supplementation experiments that test only single concentrations of each resource on a single background condition can be difficult to interpret. Instead, one must measure the growth response across a systematic scan of resource concentrations: for example, (e) a resource scan where growth obeys the Law of the Minimum (Box 1, Eq. 2) and (f) a resource scan where growth can be colimited (Box 1, Eq. 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).

of the Minimum hypothesis and resolve true colimitation compared to serial limitation. 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 [13, 22].

ARE MICROBES COLIMITED IN NATURAL ENVIRONMENTS?

While there has been evidence of colimitation of microbial growth in laboratory environments for many years now [13, 15, 18, 20, 27], there is also growing evidence that microbes experience resource colimitation across many natural habitats as well. 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). We summarize these results in Fig. 3, breaking them down by habitat (Fig. 3a), growth trait (rate or yield; Fig. 3a, inset), and resource (Fig. 3b). Altogether 55% of these tests claim to find evidence for colimitation; considering that a meta-analysis performed in 2011 found evidence for colimitation in only 28% of its studies [9], this suggests that colimitation is becoming more commonly observed as the evidence grows. Futhermore, 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 [13], as well as the possibility of environmental patchiness in space or

Box 1: Mathematical models of growth dependence on multiple resources. 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 growth rate g depends on a resource concentration R is the Monod model:

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

where $g_{\max}$ is the maximum growth rate when the resource is unlimited and K is the half-saturation concentration [2, 23]. Other models of this dependence (e.g., Droop [24], Blackman [25], Bertalanffy [16], and Hill models [26]) 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 [15, 18].

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 usually implemented mathematically by assuming the realized growth rate is the minimum of potential (Monod-dependent) growth rates for each resource (Fig. 2e) [6, 7, 15, 16, 18]:

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

In contrast, alternative models such as the "additive model" [15] allow the growth rate to depend on multiple resources simultaneously (and hence describe colimitation; see Fig. 2f):

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

Other models of rate colimitation include the multiplicative Monod model [6, 7, 16], the Poisson arrival time model [15, 16] (also known as the synthesizing-unit model [18]), and the Mankad and Bungay model [26, 27]. These models all aim to describe independent essential resources; other models exist for substitutable, chemically-dependent, or antagonistic resources [6, 7]. There is little empirical data to test this dependence in microbes, primarily due to the need to measure growth rate at low cell densities [23], but that which exists generally refutes the Law of the Minimum model (Eq. 2) in favor of colimitation [13, 15, 16, 18, 20]. 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. While the precise extent of colimitation varies across models (for example, the multiplicative model predicts significant colimitation over a wider range of resource concentrations than does the Poisson arrival time model [13]), the population dynamics resulting from these models appear to be qualitatively similar (but see Poggiale et al. [24] for a counterexample). In fact, recent work has suggested that these models are simply different mathematical approximations of the same underlying process [18].

It is also possible to model the dependence of growth yield on resource concentrations. Note that 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) is proportional to the supplied resource concentration R [2, 21]:

$$y(R) = sR, \tag{4}$$

where s is the stoichiometry of biomass to resource. Thus, the Law of the Minimum for yield would dictate that the total yield depends on whichever resource has the minimum potential yield [13]:

$$y(R_1, R_2) = \min\left(s_1 R_1, s_2 R_2\right). \tag{5}$$

As with Eq. 2 for growth rate, the Law of the Minimum for growth yield (Eq. 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 [9], these analyses can categorize yield dependence into qualitative classes (e.g., single limitation, serial limitation, additive colimitation, super-additive colimitation, etc.; see Fig. 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 [21]). 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 [13]. Recent work has instead proposed a phenomenological model for this dependence based on the power mean of each resource's potential yield [13]:

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

where $q < 0$ is a parameter that tunes the degree of colimitation ($q \to -\infty$ recovers the Eq. 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.

time [28].

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 ecosystem 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 [13, 15, 16, 18, 20], but they will require careful design to give any insights into natural environments. Both rate and yield colimitation have been tested in marine systems (Fig. 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), 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. Furthermore, all of this evidence relies on factorial supplementation experiments (cf. Fig. 2a–d) rather than systematic scans of resource concentrations (cf. Fig. 2e,f), which constrains their ability to definitively resolve different colimitation scenarios [7, 13].

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 could differ between particular molecular forms of that element (e.g., ammonium versus 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 freshwater), perhaps due to the relative ease of performing resource supplementation experiments in liquid ecosystems compared to terrestrial or animal microbiomes (Fig. 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 (Fig. 3b); nitrogen and phosphorus are the most tested and identified colimiting resource pair, followed by nitrogen and iron (Fig. 3b, inset). Among non-chemical resources, light has also been found to colimit phytoplankton growth [29]. Colimitation in marine non-primary 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 [30]. There are also few tests of resource colimitation on ocean coasts, though there are some examples at the poles, where experiments have been conducted on microbial communities at the sea ice edge [31].

In freshwater systems such as lakes, resource colimitation has primarily been tested for nitrogen and phosphorus (although iron and light are also popular considerations [32]). 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 Phosphorous Limitation Paradigm [33]. In these systems there are consistent super-additive effects of supplementing multiple resources compared to single resources; this effect commonly occurs across the spectrum of nitrogen-to-phosphorus availability, suggesting an intrinsic biological as opposed to extrinsic chemical mechanism [34]. Like in the oceans, the focus in freshwater habitats is on primary producers, though there are examples in freshwater bacteria (e.g., carbon versus phosphorus colimitation [35]). 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 (Fig. 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 [34].

Similar to freshwater microbes, terrestrial (soil) microbes exhibit diverse and complex responses to the addition of multiple resources. Resource supplementation experiments in soils require considerable effort, limiting the examples in the literature. Among the available examples, heterotophic soil bacteria have repeatedly been claimed to have colimitation for nitrogen and phosphorus [36, 37] as well as potentially carbon [38]. Interestingly, resource colimitation has been associated with reduced biodiversity of soil bacteria communities [38].

At this time, there is limited information about colimitation in other microbial ecosystems, although there are isolated examples in animal microbiomes [39], streams [32, 40], groundwater bacteria [41], wastewater sludge [42], and specialized habitats such as hypersaline lakes [43]. 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.

Lastly, when considering natural communities, it should not be forgotten that other factors besides resource availability can limit growth. Examples of non-resource, "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 na-

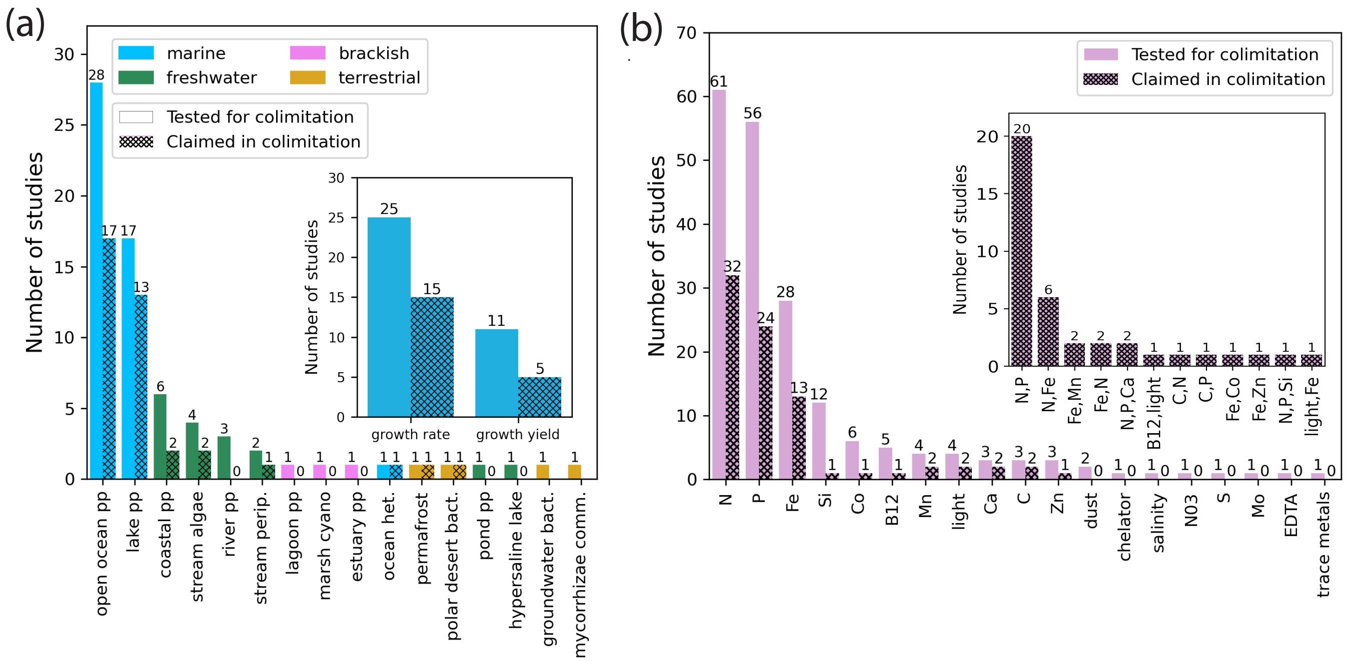

FIG. 3. **Evidence in the literature for resource colimitation of microbes in nature.** (a) Number of studies that tested for colimitation (clear bars) and claimed evidence of colimitation (hashed bars), broken down by habitat. PP = primary production, perip = periphyton, het = heterotrophs, bact = bacteria, comm = community. The inset shows the number of marine systems measured for growth rate limitation versus those measured for growth yield limitation. (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.

ture, these controls will be layered on the "bottom up" control of resource limitation, leading to the possibility of resource-predator colimitation [44].

WHAT ARE THE CAUSES OF COLIMITATION?

In general, colimitation occurs when biological resource consumption aligns with environmental resource availability (Fig. 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 [45] — which is sometimes linked to the ability to form storage compounds [5, 46] — and in other cases growth-dependent proteome allocation [47–49], situations in which growth stops at nonzero resource concentrations that depend on each other (i.e., when R^* is dynamically set) [50], and changes in maintenance resource consumption [51].

Colimitation of growth rate is likely to involve different types of interactions. Metabolic bottlenecks have been the main interaction underlying models of growth rate colimitation [18], but physiological tradeoffs are also an important possibility. These tradeoffs imply a constraint on resource uptake and utilization causing resource interactions. This could take the form of limited space for membrane transporters [25, 52–54], the need to devote energy or resources to resource uptake and utilization [51], prioritization of growth over productivity/carbon fixation [45], and noncompetitive inhibition of growth under one resource by another resource [49]. 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 have been generated through modeling insights as opposed to direct experimentation, and that literature tends to be focused on autotrophic organisms [9].

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 [8, 55, 56]. 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 [57]. 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 [58] 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 the large scale due to linked biogeochemical cycles, such as the difference between a rainy or dry season [59], episodic events as in an animal microbiome after a meal [60], or at the microscale, such as a gradient of organic matter around a marine snow particle [61]. Relatedly, 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].

Lastly, the relationship between microbes and resource availability is driven by evolution, especially over long time scales. Since selection will be strongest on traits for the most rate-limiting resource (by definition), the evolutionary steady state of this process would be for all resources to have similar levels of limitation [62], which is a state of rate colimitation. 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 always expected to be under selection. Indeed, this may explain why we observe somewhat fewer instances of yield colimitation than we do for rate colimitation (Fig. 3a, inset). These processes are related to the coevolution of life and the environment [63, 64], 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?

Both rate and yield colimitation can have a variety of consequences across biological scales (Fig. 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) [13, 16]. Colimitation may change cell morphology; for example, smaller cells with increased surface area-to-volume ratios have been a hypothesized response to colimitation [45, 65]. Finally, colimitation may also engender distinct molecular phenotypes, such as the transcriptome or proteome, although this remains to demonstrated. On one hand, the growth law hypothesis posits that cell physiology is largely dictated by growth rate, regardless of the specific (co)limitation condition [47]. However, other studies have shown that different limitation conditions can indeed elicit resource-specific responses to cell physiology [48, 66, 67], although physiology may eventually converge across conditions after genetic adaptation [68]. If the cellular 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 [10]. This would also suggest that laboratory experiments, which usually involve artificial conditions of single limitation, may give us an unrealistic pictures of cell physiology in nature, if in fact microbes are often colimited in nature.

At the scale of a microbial population, knowing whether it is colimited is important for predicting and controlling its growth. 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 [13], 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 [55, 69].

At the scale of the global ecosystem, colimitation presents a potential challenge to predicting microbial contributions to biogeochemical cycles [18], 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 [65]. It remains to be seen whether accounting for microbial colimitation in biogeochemical models changes the elemental composition of environments [12]. For example, 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 anthropogenic influences.

WHAT ARE THE PRIORITIES FOR FUTURE COLIMITATION RESEARCH?

While recent years have generated progress in understanding the concept of colimitation [7, 13, 18] (Figs. 1 and 2) and its empirical basis [9, 12, 13, 16, 19, 20] (Fig. 3), several major challenges remain to establish its role in microbial physiology and ecology. First, we need to test possible causes of colimitation at the molecular and cellular scale (Fig. 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 are not possible, then our priority should be to collect more data on the growth response of natural samples over systematic scans of resource concentrations (Fig. 2e,f), rather than traditional factorial supplementation (Fig. 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 (Figs. 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, we must test the effect of microbial colimitation on microbe-mediated processes, such as biogeochemical cycles for global nutrient cycling [18] and the functions of animal microbiomes. 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 Fig. 3.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We thank Aswin Krishna, Donat Crippa, and Justus Fink for contributing to our conceptual understanding of colimitation.

[1] M. Schaechter, J. L. Ingraham, and F. C. Neidhardt. *Microbe.* ASM Press, Washington, DC, 2006.

[2] J. Monod. The growth of bacterial cultures. *Annu Rev Microbiol*, 3:371–394, 1949.

[3] J. von Liebig. *Organic chemistry in its application to agriculture and physiology.* Taylor and Walton, London, 1840.

[4] D. Tilman. *Resource competition and community structure.* Princeton University Press, Princeton, NJ, 1982.

[5] M. Zinn, B. Witholt, and T. Egli. Dual nutrient limited growth: models, experimental observations, and applications. *J Biotechnol*, 113:263–279, 2004.

[6] M. A. Saito, T. J. Goepfert, and J. T. Ritt. Some thoughts on the concept of colimitation: Three definitions and the importance of bioavailability. *Limnol Oceanogr*, 53:276–290, 2008.

[7] E. Sperfeld, D. Raubenheimer, and A. Wacker. Bridging factorial and gradient concepts of resource co-limitation: towards a general framework applied to consumers. *Ecol Lett*, 19:201–215, 2016.
**** The authors present an outstanding synthesis of a wide range of concepts in colimitation. Besides summarizing and connecting disparate terminology, they synthesize the approach of factorial supplementation experiments with systematic resource scans, showing that the former is an imprecise estimate of the latter. This means that factorial supplementation experiments cannot be reliably used to distinguish between different colimitation scenarios.**

[8] M. Danger, T. Daufresne, F. Lucas, S. Pissard, and G. Lacroix. Does Liebig's law of the minimum scale up from species to communities? *Oikos*, 117:1741–1751,

2008.
**** This work develops a theoretical and experimental understanding that community-level stoichiometry adjusts to resource availability. In particular, it shows how even if the Law of the Minimum holds at the scale of individual species, it will not hold at the scale of a whole community, meaning that colimitation should be possible in microbial communities.**

[9] W. S. Harpole, J. T. Ngai, E. E. Cleland, E. W. Seabloom, E. T. Borer, M. E.S. Bracken, J. J. Elser, D. S. Gruner, H. Hillebrand, J. B. Shurin, and J. E. Smith. Nutrient co-limitation of primary producer communities. *Ecol Lett*, 14:852–862, 2011.
**** This work provides a comprehensive analysis of resource colimitation across terrestrial, marine, and freshwater ecosystems, with a focus on plants and phytoplankton. The authors distinguish between serial limitation and strict colimitation. The data demonstrates that colimitation, particularly for nitrogen and phosphorus, is an important feature of primary producer ecosystems.**

[10] J. W. Fink and M. Manhart. How do microbes grow in nature? The role of population dynamics in microbial ecology and evolution. *Curr Opin Syst Biol*, 36:100470, 2023.

[11] H. Bachmann, M. Fischlechner, R. N. Barfa, F. Branco dos Santos, D. Molenaar, and B. Teusink. Availability of public goods shapes the evolution of competing metabolic strategies. *Proc Natl Acad Sci USA*, 110:14302–14307, 2013.

[12] T. J. Browning and C. M. Moore. Global analysis of ocean phytoplankton nutrient limitation reveals high prevalence of co-limitation. *Nat Commun*, 14:5014, 2023.
**** This paper provides a comprehensive profile of literature biogeochemical data and shipboard incubation experiments that test for resource limitation and colimitation of open ocean phytoplankton communities. The authors conclude that surface ocean phytoplankton are commonly colimited.**

[13] N. A. Held, A. Krishna, D. Crippa, A. Dragan, and M. Manhart. Quantifying nutrient colimitation of microbial growth. *bioRxiv preprint*, doi:10.1101/2023.09.27.559472, 2023.

[14] D. Tilman. Resources: A graphical-mechanistic approach to competition and predation. *Am Nat*, 116:362–393, 1980.

[15] R. V. O'Neill, D. L. DeAngelis, J. J. Pastor, B. J. Jackson, and W. M. Post. Multiple nutrient limitations in ecological models. *Ecol Modelling*, 46:147–163, 1989.

[16] E. Sperfeld, D. Martin-Creuzburg, and A. Wacker. Multiple resource limitation theory applied to herbivorous consumers: Liebig's minimum rule vs. interactive colimitation. *Ecol Lett*, 15:142–150, 2012.

[17] H. M. Halvorson, E. Sperfeld, and M. A. Evans-White. Quantity and quality limit detritivore growth: Mechanisms revealed by ecological stoichiometry and colimitation theory. *Ecology*, 98:2995–3002, 2017.

[18] J. Tang and W. J. Riley. Finding Liebig's law of the minimum. *Ecol Appl*, 31:e02458, 2021.
*** The authors analyze a kinetic model of resource uptake and metabolism to show how different models of growth rate colimitation can be derived as different approximations of the same underlying process. This work establishes a theoretical connection between widely-used models of growth rate colimitation that are otherwise treated phenomenologically.**

[19] A. Redoglio, K. Radtke, and E. Sperfeld. How nitrogen and phosphorus supply to nutrient-limited autotroph communities affects herbivore growth: Testing stoichiometric and co-limitation theory across trophic levels. *Oikos*, 2022:e09052, 2022.
*** This work combines a systematic resource scan with traditional factorial supplementation experiments to determine colimitation. It is also notable for mapping resource limitation and colimitation across trophic levels (phytoplankton and a herbivore that consumes them).**

[20] F. Liu, L. Gaul, A. Giometto, and M. Wu. Colimitation of light and nitrogen on algal growth revealed by an array microhabitat platform. *arXiv preprint*, doi:10.48550/arXiv.2307.02646, 2023.

[21] O. Golan, O. Gampp, L. Eckert, and U. Sauer. Overall biomass yield on multiple nutrient sources. *bioRxiv preprint*, doi:10.1101/2023.02.16.528813, 2023.

[22] M. Rutgers, P. A. Balk, and K. van Dam. Quantification of multiple-substrate controlled growth-simultaneous ammonium and glucose limitation in chemostat cultures of Klebsiella pneumoniae. *Arch Microbiol*, 153:478–484, 1990.

[23] J. W. Fink, N. A. Held, and M. Manhart. Microbial population dynamics decouple growth response from environmental nutrient concentration. *Proc Natl Acad Sci USA*, 120:e2207295120, 2023.

[24] J.-C. Poggiale, M. Baklouti, B. Queguiner, and S. a. L. M. Kooijman. How far details are important in ecosystem modelling: the case of multi-limiting nutrients in phytoplankton–zooplankton interactions. *Phil Trans R Soc B*, 365:3495–3507, 2010.

[25] J. R. Casey and M. J. Follows. A steady-state model of microbial acclimation to substrate limitation. *PLoS Comput Biol*, 16:1–17, 2020.

[26] L. Xie, A. E. Yuan, and W. Shou. Simulations reveal challenges to artificial community selection and possible strategies for success. *PLoS Biol*, 17:e3000295, 2019.

[27] T. Mankad and H. R. Bungay. Model for microbial growth with more than one limiting nutrient. *J Biotechnol*, 7:161–166, 1988.

[28] M. S. Hale, W. K. W. Li, and R. B. Rivkin. Meridional patterns of inorganic nutrient limitation and colimitation of bacterial growth in the Atlantic Ocean. *Prog Oceanogr*, 158:90–98, 2017.

[29] M. T. Maldonado, P. W. Boyd, P. J. Harrison, and N. M. Price. Co-limitation of phytoplankton growth by light and Fe during winter in the NE subarctic Pacific Ocean. *Deep Sea Res Part II Top Stud Oceanogr*, 46:2475–2485, 1999.

[30] M. M. Mills, C. M. Moore, R. Langlois, A. Milne, E. Achterberg, K. Nachtigall, K. Lochte, R. J. Geider, and J. La Roche. Nitrogen and phosphorus co-limitation of bacterial productivity and growth in the oligotrophic subtropical North Atlantic. *Limnol Oceanogr*, 53:824–834, 2008.

[31] E. M. Bertrand, J. P. McCrow, A. Moustafa, H. Zheng, J. B. McQuaid, T. O. Delmont, A. F. Post, R. E. Sipler, J. L. Spackeen, K. Xu, D. A. Bronk, D. A. Hutchins, and

A. E. Allen. Phytoplankton–bacterial interactions mediate micronutrient colimitation at the coastal antarctic sea ice edge. *Proc Natl Acad Sci USA*, 112:9938–9943, 2015.

[32] D. R. Warren, S. M. Collins, E. M. Purvis, M. J. Kaylor, and H. A. Bechtold. Spatial Variability in Light Yields Colimitation of Primary Production by Both Light and Nutrients in a Forested Stream Ecosystem. *Ecosystems*, 20:198–210, 2017.
*** This paper provides an example of light and nutrient colimitation of stream periphyton communities. It indicates that patchiness in environmental resource availability can lead to the emergent property of resource colimitation.**

[33] R. W. Sterner. On the Phosphorus Limitation Paradigm for Lakes. *Int Rev Hydrobiol*, 93:433–445, 2008.

[34] A. R. Bratt, J. C. Finlay, J. R. Welter, B. A. Vculek, and R. E. Van Allen. Co-limitation by n and p characterizes phytoplankton communities across nutrient availability and land use. *Ecosystems*, 23:1121–1137, 2020.

[35] I. Dorado-García, J. M. Medina-Sánchez, G. Herrera, M. J. Cabrerizo, and P. Carrillo. Quantification of carbon and phosphorus co-limitation in bacterioplankton: New insights on an old topic. *PLoS ONE*, 9:e99288, 2014.

[36] D. Zhang, L. Wang, S. Qin, D. Kou, S. Wang, Z. Zheng, J. Peñuelas, and Y. Yang. Microbial nitrogen and phosphorus co-limitation across permafrost region. *Glob Change Biol*, 29:3910–3923, 2023.

[37] M. Kaspari and J. S. Powers. Biogeochemistry and Geographical Ecology: Embracing All Twenty-Five Elements Required to Build Organisms. *Am Nat*, 188:S62–S73, 2016.

[38] Z. T. Aanderud, S. Saurey, B. A. Ball, D. H. Wall, J. E. Barrett, M. E. Muscarella, N. A. Griffin, R. A. Virginia, A. Barberán, and B. J. Adams. Stoichiometric shifts in soil c:n:p promote bacterial taxa dominance, maintain biodiversity, and deconstruct community assemblages. *Front Microbiol*, 9:1401, 2018.

[39] A. T. Reese, F. C. Pereira, A. Schintlmeister, D. Berry, M. Wagner, L. P. Hale, A. Wu, S. Jiang, H. K. Durand, X. Zhou, R. T. Premont, A. M. Diehl, T. M. O'Connell, S. C. Alberts, T. R. Kartzinel, R. M. Pringle, R. R. Dunn, J. P. Wright, and L. A. David. Microbial nitrogen limitation in the mammalian large intestine. *Nat Microbiol*, 3:1441–1450, 2018.

[40] R. L. Lowe, S. W. Golladay, and J. R. Webster. Periphyton response to nutrient manipulation in streams draining clearcut and forested watersheds. *J North Am Benthol Soc*, 5:221–229, 1986.

[41] R. Hofmann and C. Griebler. DOM and bacterial growth efficiency in oligotrophic groundwater: Absence of priming and co-limitation by organic carbon and phosphorus. *Aquatic Microb Ecol*, 81:55–71, 2018.

[42] E. Lee and Q. Zhang. Integrated co-limitation kinetic model for microalgae growth in anaerobically digested municipal sludge centrate. *Algal Res*, 18:15–24, 2016.

[43] R. Jellison and J. M. Melack. Nitrogen limitation and particulate elemental ratios of seston in hypersaline Mono Lake, California, U.S.A. *Hydrobiologia*, 466(1-3):1–12, 2001.

[44] B. B. Cael. The good, the bad, and the tiny: A simple, mechanistic-probabilistic model of virus-nutrient colimitation in microbes. *PLoS ONE*, 10:e0143299, 2015.

[45] N. Yang, Y.-A. Lin, C. A. Merkel, M. A. DeMers, P.-P. Qu, E. A. Webb, F.-X. Fu, and D. A. Hutchins. Molecular mechanisms underlying iron and phosphorus co-limitation responses in the nitrogen-fixing cyanobacterium Crocosphaera. *ISMEJ*, 16:2702–2711, 2022.

[46] T. Egli. On multiple-nutrient-limited growth of microorganisms, with special reference to dual limitation by carbon and nitrogen substrates. *Antonie van Leeuwenhoek*, 60:225–234, 1991.

[47] M. Scott, C. W. Gunderson, E. M. Mateescu, Z. Zhang, and T. Hwa. Interdependence of cell growth and gene expression: Origins and consequences. *Science*, 330:1099–1102, 2010.

[48] S. H.-J. Li, Z. Li, J. O. Park, C. G. King, J. D. Rabinowitz, N. S. Wingreen, and Z. Gitai. Escherichia coli translation strategies differ across carbon, nitrogen and phosphorus limitation conditions. *Nat Microbiol*, 3:939–947, 2018.
*** The authors measure RNA to protein (R/P) ratio as a proxy for proteome investment in translation. Contrary to the growth law hypothesis that this ratio depends only on the growth rate, they find that *E. coli* has different R/P at the same growth rates between limitation conditions — carbon and nitrogen limitation are very similar, but phosphorus limitation achieves the same growth rate for much lower R/P. Altogether they find that all three limitation conditions entail different translation strategies.**

[49] S. Sharma and R. Steuer. Modelling microbial communities using biochemical resource allocation analysis. *J R Soc Interface*, 16:20190474, 2019.
*** The authors develop a resource allocation model of phytoplankton growth and find that resource colimitation is an emergent property. The colimited growth is consistent with the additive model of growth rate colimitation. Their model also provides a tool to simulate proteome composition under different limitation conditions.**

[50] K. R. Arrigo. Marine microorganisms and global nutrient cycles. *Nature*, 437:349–356, 2005.

[51] T. Egli. How to live at very low substrate concentration. *Water Res*, 44:4826–4837, 2010.

[52] R. J. Hudson and F. M. M. Morel. Trace metal transport by marine microorganisms: Implications of metal coordination kinetics. *Deep Sea Res Part I Oceanogr Res Pap*, 40:129–150, 1992.

[53] N. Norris, N. M. Levine, V. I. Fernandez, and R. Stocker. Mechanistic model of nutrient uptake explains dichotomy between marine oligotrophic and copiotrophic bacteria. *PLoS Comput Biol*, 17:e1009023, 2021.

[54] N. A. Held, E. A. Webb, M. M. McIlvin, D. A. Hutchins, N. R. Cohen, D. M. Moran, K. Kunde, M. C. Lohan, C. M. Mahaffey, E. M. S. Woodward, and M. A. Saito. Co-occurrence of Fe and P stress in natural populations of the marine diazotroph Trichodesmium. *Biogeosciences*, 17:2537–2551, 2020.

[55] D. Tilman. The resource-ratio hypothesis of plant succession. *Am Nat*, 125:827–852, 1985.

[56] M. Sebastián and J. M. Gasol. Heterogeneity in the nutrient limitation of different bacterioplankton groups in the Eastern Mediterranean Sea. *ISMEJ*, 7:1665–1668, 2013.

[57] C. Bannon, I. Rapp, and E. M. Bertrand. Community interaction co-limitation: Nutrient limitation in a

marine microbial community context. *Front Microbiol*, 13:846890, 2022.

[58] J. A. Schwartzman, A. Ebrahimi, G. Chadwick, Y. Sato, B. R. K. Roller, V. J. Orphan, and O. X. Cordero. Bacterial growth in multicellular aggregates leads to the emergence of complex life cycles. *Curr Biol*, 32:3059–3069.e7, 2022.

[59] G. V. Lacerda-Júnior, M. F. Noronha, L. Cabral, T. P. Delforno, S. T. P. de Sousa, P. I. Fernandes-Júnior, I. S. Melo, and V. M. Oliveira. Land use and seasonal effects on the soil microbiome of a Brazilian dry forest. *Front Microbiol*, 10:648, 2019.

[60] A. Benítez-Páez, P. Belda-Ferre, A. Simón-Soro, and A. Mira. Microbiota diversity and gene expression dynamics in human oral biofilms. *BMC Genomics*, 15:311, 2014.

[61] R. Stocker. Marine microbes see a sea of gradients. *Science*, 338:628–633, 2012.

[62] N. Shoresh, M. Hegreness, and R. Kishony. Evolution exacerbates the paradox of the plankton. *Proc Natl Acad Sci USA*, 105:12365–12369, 2008.

[63] T. M. Lenton, S. J. Daines, J. G. Dyke, A. E. Nicholson, D. M. Wilkinson, and H. T. P. Williams. Selection for Gaia across Multiple Scales. *Trends Ecol Evol*, 33:633–645, 2018.

[64] F. M. M. Morel. The co-evolution of phytoplankton and trace element cycles in the oceans. *Geobiology*, 6:318–324, 2008.

[65] N. G. Walworth, F.-X. Fu, E. A. Webb, M. A. Saito, D. Moran, M. R. Mcllvin, M. D. Lee, and D. A. Hutchins. Mechanisms of increased Trichodesmium fitness under iron and phosphorus co-limitation in the present and future ocean. *Nat Commun*, 7:1–11, 2016.
*** In this work, the authors subject a marine cyanobacterium to iron/phosphorus colimitation for a period of 7 years and demonstrate a complex and irreversible change in physiology. It suggests that organisms evolution in resource colimitation leads to different outcomes than single resource limitations.**

[66] J.-J. Park, H. Wang, M. Gargouri, R. R. Deshpande, J. N. Skepper, F. O. Holguin, M. T. Juergens, Y. Shachar-Hill, L. M. Hicks, and D. R. Gang. The response of Chlamydomonas reinhardtii to nitrogen deprivation: a systems biology analysis. *Plant J*, 81:611–624, 2015.

[67] A. Bren, J. O. Park, B. D. Towbin, E. Dekel, J. D. Rabinowitz, and U. Alon. Glucose becomes one of the worst carbon sources for E. coli on poor nitrogen sources due to suboptimal levels of cAMP. *Sci Rep*, 6:24834, 2016.

[68] M. Tamminen, A. Betz, A. L. Pereira, M. Thali, B. Matthews, M. J.-F. Suter, and A. Narwani. Proteome evolution under non-substitutable resource limitation. *Nat Commun*, 9:4650, 2018.
*** The authors evolve an algae under different resource limitation conditions and measure the changes in proteome composition. They find that the proteome changes significantly, but not in terms of up-regulation of transporters specific to the limiting resource. Rather, most changes are to core metabolic proteins and are similar across limitation conditions.**

[69] S. A. Levin. Community equilibria and stability, and an extension of the competitive exclusion principle. *Am Nat*, 104:413–423, 1970.