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Review

Trophic Interactions and the Drivers of Microbial Community Assembly

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SUMMARY

Despite numerous surveys of gene and species content in heterotrophic microbial communities, such as those found in animal guts, oceans, or soils, it is still unclear whether there are generalizable biological or ecological processes that control their dynamics and function. Here, we review experimental and theoretical advances to argue that networks of trophic interactions, in which the metabolic excretions of one species are the primary resource for another, constitute the central drivers of microbial community assembly. Trophic interactions emerge from the deconstruction of complex forms of organic matter into a wealth of smaller metabolic intermediates, some of which are released to the environment and serve as a nutritional buffet for the community. The structure of the emergent trophic network and the rate at which primary resources are supplied control many features of microbial community assembly, including the relative contributions of competition and cooperation and the emergence of alternative community states. Viewing microbial community assembly through the lens of trophic interactions also has important implications for the spatial dynamics of communities as well as the functional redundancy of taxonomic groups. Given the ubiquity of trophic interactions across environments, they impart a common logic that can enable the development of a more quantitative and predictive microbial community ecology.

Introduction

Through the integration of different metabolic functions distributed among multiple microbial species, microbial communities govern the cycling of the elements and the health and productivity of the planet. Microbial communities are composed of multiple species interconnected by the exchange of metabolites, many of which act as primary energy substrates — the waste of one species being the substrate of another. Despite their importance and ubiquity, we lack principles that help us to understand how these systems self-assemble (that is, what determines their composition and diversity), how they function (how they metabolize resources collectively and with what efficiency), and how they respond to the perturbations that they regularly face in the environment (such as changes in resources, or environmental fluctuations). The lack of guiding principles in microbial community ecology makes it hard to infer processes from patterns and to distinguish what is idiosyncratic to an environment and what is universal among different microbial communities [1].

Despite major advances driven by molecular technologies over the last two decades, little has emerged to date in the way of theoretical frameworks and principles for microbial communities. The study of microbial communities relies heavily on a few key parameters: catalogs of fine-grained features like 16S rRNA types or gene fragments from metagenomes present in a large-scale assemblage, such as a human gut; physiological studies of single species in isolation from their ecological context; or bulk measurements of O_2, CO_2, NO_3, CH_4, etc., which are the main inputs and outputs of central energy-transducing reactions. Each approach has its own shortcomings: bulk chemical measurements elucidate little about the inner workings of a community, large-scale measurements of 16S types or gene content end up as statistical descriptors that are hard to map to function [2,3], and single species descriptions are difficult to relate to their function in a community context, which typically comprises a large number of species. Thus, although these approaches provide valuable information about the microbial world, it remains difficult to link physiology (for example, metabolism) to ecological patterns (such as diversity) to achieve a comprehensive picture of the processes that structure microbial communities.

In this review, we address this conceptual gap in the field by expanding and building on the perspective that microbial communities are fundamentally decentralized and distributed metabolic systems, and as such, are driven by the interactions between cells and resources [4–6]. From this perspective, to understand the principles that drive microbial community assembly we need to see beyond catalogs of species, genes, and metabolites, and instead focus on the flows of energy and biomass precursors that drive population growth and turnover. We argue that in order to build an understanding of the interplay between metabolism and community ecology (the collection of biotic factors that control species abundance profiles), we need to understand the trophic interactions that take place between microorganisms in the environment. Although we focus

mainly on heterotrophic, carbon-limited communities, the principles we discuss in the following also apply to other types of communities such as those where the primary substrate is an inorganic substance (for example, algae–bacteria consortia).

The Role of Microbial Interactions and Niche Modification during Community Assembly

In many environments, periodic perturbations create opportunities for microorganisms to invade an environment and for communities to re-assemble. Examples of this process are the colonization of animal guts during the first days after birth [7,8], the colonization of patches of particulate organic matter in the ocean, for example, a fragment of a dead copepod or diatom [9,10], or the colonization of plant roots [11], to name a few. The process of colonization is intrinsically stochastic, with microbes arriving in a newly opened environment in varying numbers and at different times. After arrival, the success of the different colonizers is determined by a combination of abiotic factors, such as the availability of nutrients, and biotic factors such as the interactions between newcomers and resident microbes, which may change as the community assembly proceeds. Thus, the growth of an individual microbial species in a community is often transient, resulting in successions whereby shifting conditions during the assembly create waves of bloom and demise for individual microbial species.

To illustrate, consider the assembly of microbial communities in one of the best studied host–microbe symbiosis systems, the cow rumen [12]. After a calf is born, the rumen is colonized by microbes transmitted from the mother and from the environment [7]. Most of the microbes that initially enter the rumen are unable to grow in the conditions they find there. However, a few have the right metabolic capacity to grow and are therefore 'ecologically selected' by this new environment. These initial colonizers exploit the conditions of high carbon and oxygen availability, inducing a dramatic decrease in the oxygen content of the rumen [8]. This shift in the environment created by the biological activity of early colonizers opens new niches for anaerobic microbes, which in turn drive the growth of other populations by providing new metabolites, and so on. These microbial interactions, mediated through pH and oxygen-content modification, metabolite production, and other mechanisms, create complex successional assembly patterns [7,13]. Although this story may seem specific to anaerobic digesters, the same process of random colonization, environmental selection and recurrent niche modification has been found to drive the assembly of communities in very different environments such as marine particles [9], in vitro pitcher-plant microbiomes [14], cheese rinds [15], kefir [16], and even in controlled assembly experiments in the lab [17,18]. It is this commonality across systems that suggest to us that sufficiently general principles can be derived in order to make — hopefully quantitative — predictions of how communities should assemble in new environments.

To derive such general principles of microbial community assembly and microbial ecology in general, we argue that a conceptual shift from pairwise interactions to community-wide trophic interactions is needed and requires a synthesis of several approaches. For decades, microbiologists have attempted to discover microbial interactions by examining pairwise interactions in the laboratory, from plate assays designed to measure antibiosis or growth facilitation [19–22], to co-cultures in liquid media or as biofilms [23–25]. This focus on pairwise interactions is arguably due to a variety of factors: besides the relative difficulty of performing experiments and interpreting data with tens of species or more, classical ecological models are often based on pairwise interactions, and even in surveys of complex microbial communities with many species, current analysis tools are often designed to describe a community in terms of pairwise interactions [26–28].

Nevertheless, when studying complex communities containing many species, pairwise interaction networks may not be the simplest way to conceptualize the processes governing the community. Pairwise assays produce dense networks of potential interactions, many of which may not be realized in the community [22,24] and could be specific to the type of growth medium used in the experiment. Moreover, higher-order interactions — whereby a pairwise interaction is altered by the presence of one or more other species — and the dynamics of metabolite exchange can prohibit the prediction of even moderately complex community outcomes through pairwise interactions alone [29–32]. These observations may lead one to conclude that microbial communities are too complex and disordered to make sense of how they are organized and to predict how they assemble. Yet although fully quantitative predictions of the dynamics of individual species in the community are still (and may remain) an elusive goal, there are emerging patterns (described in this paper) that point towards universal mechanisms guiding community assembly. This is akin to a similar situation in the physics of gases: although the dynamics of individual gas molecules cannot be predicted, there is no doubt that macro-scale observables, such as pressure and volume, describe a container of gas in a meaningful way. Whether such coarse-graining of the process of microbial community assembly can be achieved, and what the microbial equivalents of 'pressure' and 'volume' may be, remains to be seen. We argue that focusing on trophic interactions may be a step towards such a coarse-grained description of microbial community assembly.

How Do Trophic Interactions Arise?

In contrast to the widespread view of microbiomes as complex, intractable systems, community assembly experiments performed in recent years with synthetic communities or controlled enrichment cultures have revealed that, despite the multitude of possible microbial interactions, the assembly of microbial communities can sometimes be highly reproducible [9,15,17,33–36]. Community assembly experiments are different from classical pairwise interaction assays in that they involve a diverse set of (potentially uncultured) species, which are allowed to colonize, grow, and self-organize in an environment. In contrast to pairwise interaction assays, the focus of these experiments is the dynamic behavior of the collective. As we discuss in detail below, the collective dynamics that emerge from these experiments reveal that metabolic excretions associated with the partial metabolism of substrates are common and create conditions that allow the coexistence of many different species on a single, externally supplied carbon source. This perspective is consistent with the classical view of anaerobic microbial ecosystems, as described in most environmental microbiology

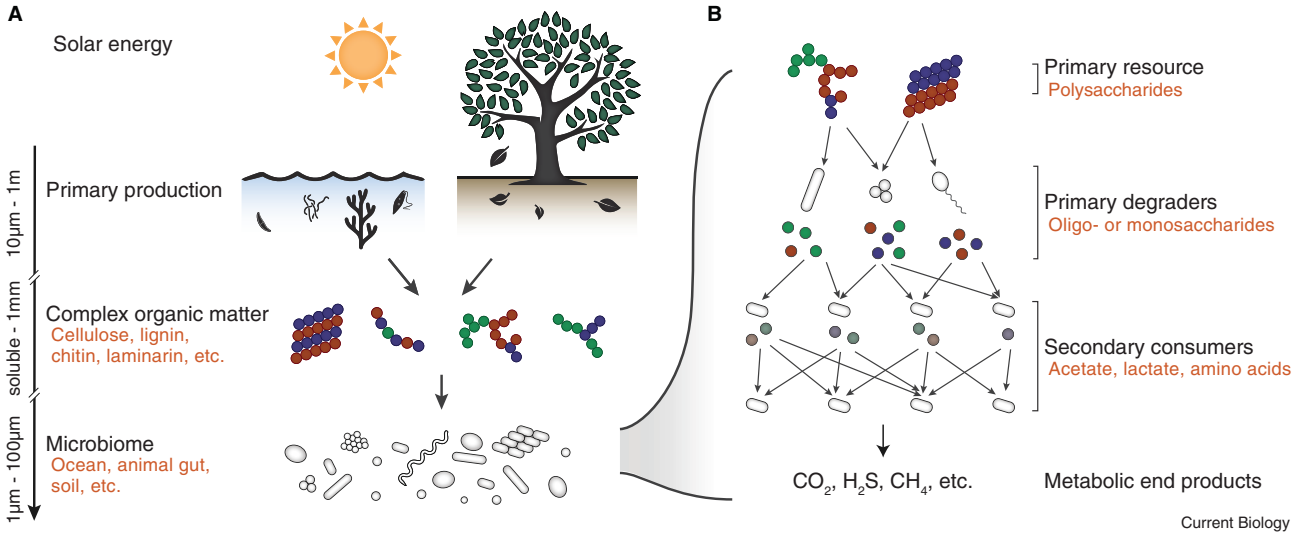

Figure 1. Microbial communities in the context of global ecosystems.
(A) Heterotrophic microbial communities degrade complex forms of organic matter synthesized by primary producers such as algae and plants. These heterotrophic microbes can be found in all environments, from animal guts, where they mediate digestion, to soils and oceans, where they mediate the global carbon cycle. (B) The internal structure of the heterotrophic microbiome is a trophic cascade whereby primary degraders first break down complex organic matter, such as proteins or polysaccharides. Secondary consumers then process the byproducts of hydrolysis themselves or the metabolic byproducts of primary degraders; the latter of which can act as primary sources of carbon and nitrogen for the consumers. Cascades of primary metabolite flux occur both in aerobic and anaerobic conditions, although for different reasons.

textbooks: complex forms of organic matter such as hemicellulose or chitin, found in plant or animal detritus, are enzymatically broken down by specialized organisms (Figure 1). The byproducts of this first step are utilized and transformed through fermentation cascades, whereby labor is divided across multiple species. These cascades end in organisms that convert oxidized substrates to end products like methane or CO_2 [37,38].

The division of labor displayed in anaerobic metabolic cascades appears to be hard-wired in the biology of the participating microorganisms: for example, no known microbe can degrade complex biopolymers anaerobically and at the same time perform methanogenesis [39]. However, recent experiments with synthetic communities or marine particles show that this trophic organization is not limited to anaerobic systems: it can also take place in oxygen-rich environments, like the surface ocean, where microbes degrade organic matter through a series of partial reactions performed by different organisms [9]. These metabolic cascades occur despite the fact that many primary degraders — those organisms that break down the complex substrate — are capable of performing all reactions required to oxidize the carbon source all the way down to CO_2. However, in practice, imbalances between anabolic and catabolic reactions lead to the leakage or excretion of energy-rich metabolic intermediates, which sustain the growth of many other species that cannot degrade the primary substrate [9,39,40] (Figure 2).

Incomplete metabolism of the primary substrate and the excretion of metabolites has been observed widely in microbes, including proteobacteria, firmicutes, actinobacteria, and yeasts, from a variety of environments [41], making it a likely widespread feature of bacterial (and fungal) metabolism. Such leakage may be interpreted as metabolic inefficiency, because energy in the form of metabolites is lost to the environment that could have been used for anabolic reactions (that is, biomass production). However, there are various mechanisms that can explain the excretion of metabolic intermediates as an optimal strategy in at least some ecologically relevant scenarios. One such mechanism identified in *Escherichia coli* [42] is referred to as overflow metabolism. Overflow occurs during exponential growth, when intracellular metabolite and protein concentrations are at steady state. In these conditions, if one set of proteins increases in concentration in a cell, others must decrease. Therefore, in order to maximize its growth rate, an organism can regulate its gene expression to shut down the production of the respiratory protein repertoire (which would yield a higher metabolic efficiency at a higher protein budget cost) in order to allocate resources towards replication requirements (for example, ribosome production). This strategy maximizes growth rate when resource supply is high, and it allows the organism to turn over resources rapidly and maintain a high flux of ATP [42]. This logic is consistent with very general models of metabolic control developed in the 1980s by Heinrich and Schuster [43–45]. These models show that the optimal strategy to allocate enzymes along the linear pathway depends on the external supply rate of the primary substrate. If the objective of the regulation apparatus is to maximize the flux of ATP, the organism should run the full linear pathway only when the resource supply is low. Only in such conditions will the allocation of enzymes to downstream reactions not limit the flux of resources (and ATP) through the system.

However, fast growth under high resource supply is arguably an artificial scenario for many microbes — highly suitable for quantitative biological studies in the lab, but a poor reflection of the ecological realities of many environmental populations. In scenarios in which nutrients are scarce, maximizing growth rate may not be the primary determinant of microbial fitness,

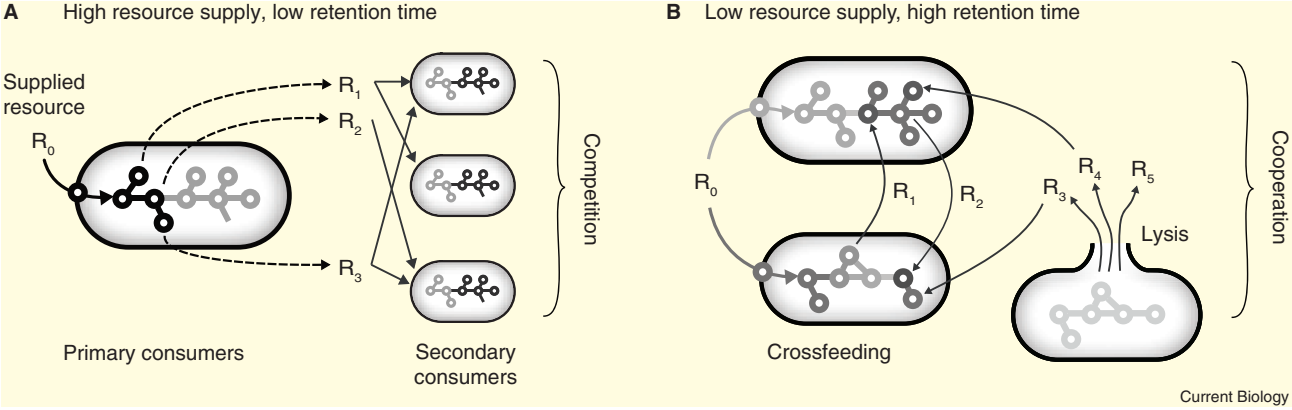

Figure 2. Competition and cooperation as emergent properties controlled by energy supply and residence time.
(A) High resource supply is often coupled with high dilution rates (that is, low residence times) in continuous or semi-continuous culture conditions, to prevent the accumulation of biomass waste products. Under such conditions, the primary consumers of externally supplied resources will excrete a large array of primary metabolites, which in turn feed a diverse set of secondary consumers. The persistence of secondary consumers in the system depends on the extent to which they compete with each other for metabolites. (B) In conditions of low resource supply, the rates of dilution must also be low (that is, high residence times) if slow-growing organisms are to persist. In such conditions, all species are limited by resources and those groups of organisms that can best complement each other via their metabolic excretions are more likely to persist. Metabolites can be released through different processes described in the main text, including phage-induced lysis.

and there are other mechanisms that can explain why it may be optimal for an organism to excrete carbon- or nitrogen-rich molecules instead of using them for growth. One such process of high ecological relevance is the release of metabolites during nutrient limitation and subsequent starvation. As external resources are exhausted, *E. coli* cells begin to consume their internal pool of metabolites [46], but *E. coli* does not have the metabolic machinery to degrade and recycle all of its biomass components. For instance, *E. coli* cannot degrade aromatic amino acids such as phenylalanine, which are therefore excreted during stationary phase [47]. Similarly, in yeast, metabolic dysregulation in response to limitation for a required amino acid was shown to induce the release of organosulfur compounds, which can be then used by other community members [48]. Secondly, phage-induced lysis may be another ecologically relevant mechanism that leads to the release and subsequent consumption of the contents of lysed cells. Indeed, this process has been shown to lead to a successional community assembly in the terrestrial deep biosphere [49]. Thirdly, instead of being imported into the cells directly, some primary resources must be hydrolyzed outside the cell, releasing oligosaccharides that can be consumed by other community members [50,51]. Finally, cells may excrete carbon- or nitrogen-rich metabolites when either of these elements is in excess in the environment relative to the stoichiometric demands of the organism. Such excretions may come at no cost in terms of growth rate or yield [52]. Taken together, there is a plethora of mechanisms, with others potentially still undiscovered, that can explain the release of metabolites from microbial cells in various aerobic and anaerobic contexts. The nature of these mechanisms suggests a compelling corollary, namely that the emergence of communities with a trophic structure could be a consequence of the design principles of cellular metabolism.

The metabolic intermediates excreted during fast microbial growth are highly diverse [41,53]: in addition to key metabolites like acetate, microbes leak a variety of carbon and nitrogen-rich metabolites. For instance, marine bacteria grown on alginate and NH_4 as a nitrogen source excreted a large array of amino acids, organic acids, osmolytes, etc. [34]. Thus, although externally supplied resources can be of low diversity but complex in structure (for example, a biopolymer), microbial activity results in a diversification of the substrate into many simpler molecules. We can therefore postulate that the increase in the chemical diversity of the resource pool should subsequently lead to an increase in species diversity as additional niches open up [33,54]. Indeed, this hypothesis is consistent with experimental data of community assembly on marine particles, where secondary consumers — the organisms whose primary resource are the metabolic byproducts of degraders — are more diverse than primary degraders [9,34]. A similar rule may hold in rumen or human gut microbiomes [37].

Cooperation and Competition as Community-Level Properties

Even when conceptualizing microbial interactions as the exchange of metabolites, it is tempting to maintain a framework of fixed pairwise interactions that are independent of the biotic and abiotic environment. In such a framework, if two species consume the same metabolites, such that growth of one species is hindered by the presence of the other species, we might call their interaction competitive; conversely, if two species can mutually benefit from the metabolites excreted by the other, their interaction would be called cooperative; and so on [55]. However, multiple sources of evidence, from models to experiments, suggest that whether the interactions between organisms are cooperative or competitive depends strongly on environmental conditions, such as the rate of resource supply [18]. This means that the same pair of species could cooperate or compete, depending on how much energy and nutrients they are provided with. Hoek and coworkers studied this problem using two cross-feeding yeast strains that supplied each other essential amino acids [56]. The authors found that depending on the

amount of externally supplied amino acids, the pair of yeast strains can exhibit seven qualitatively different types of interactions, from mutualism at low resource supply rates, to parasitism, to competitive exclusion at high resource supply rates. This finding highlights the difficulty of inferring relevant interactions with laboratory assays, typically performed in a pairwise manner and in rich media.

In contrast to a static view of interactions as hard-wired links between species, an environment-dependent and thus dynamic notion of microbial interactions arises naturally when we consider trophic interactions as the driving force behind microbial community assembly. Illustrating this point, Marsland *et al.* devised a consumer-resource model with explicit metabolite exchange to study the impact of nutrient supply rates on community-scale patterns, in terms of both community composition and metabolite flux [54]. They found that a high external resource supply rate implied also a high metabolite flux between community members, as expected, and the majority of biomass was predicted to stem not from the primary resource but from the exchanged metabolites. In such conditions, community assembly was found to be niche-driven [57]; that is, what determines whether a species persists is whether their pattern of metabolite consumption is sufficiently different from others so that interspecific competition can be minimized. By contrast, when resource supply is low, then so is the flux of metabolic excretions and species are limited by the total amount of resources available. In this scenario, those species that coexist are the ones that engage in exchange of mutually beneficial, essential metabolites to fulfill their resource quota [58]. Most community assembly studies in the lab are performed in conditions of high resource supply (for example, mM concentration of carbon and nitrogen) and low residence time (high dilution rate) (Figure 2), and metabolic interdependencies are typically only observed in engineered contexts (such as selected pairs with defined auxotrophies in minimal media). By contrast, studies of metabolic interaction in anaerobic environments limited by energy supply and with high residence times of microbes and metabolites show that metabolic interdependencies can be widespread among organisms [59]. For instance, metagenomic analyses of aerobic and anaerobic hydrocarbon-degrading consortia maintained for several years showed that none of the main species in the system were able to synthesize all amino acids, but instead their amino acid production patterns were complementary [60,61]. Bringing these observations together, we may postulate that the incidence of competition and cooperation depend, in a predictable manner, on resource supply and are in general emergent properties of the assemblage and the environment rather than intrinsic properties of the species pair.

This discussion sheds a different light on a topic that has been the focus of interest in the last few years: the question of the relative contribution of competition versus cooperation in microbial communities [62–68]. Although the concepts of cooperation and competition are intuitive and part of our everyday language, the above examples illustrate that these interactions can be highly sensitive to the environment and therefore difficult to measure. It is conceivable, for example, that two organisms are metabolically co-dependent in a community, but that in a plate assay on rich medium and separated from the other community members they display strong competition. This does not mean that a community cannot be decomposed in terms of pairwise interactions [29], but doing so in a realistic community may require us to reproduce the environment experienced by each organism — a generally impractical proposition. The challenge is thus developing analytical tools that allow us to infer from the dynamics of the full community the extent to which the system is governed by metabolic interdependencies or by niche partitioning and competition [65]. Models that go beyond pairwise interactions and instead model resource dynamics, such as that of Marsland *et al.*, could be useful tools in this endeavor [54].

The Effect of Secondary Metabolites

We have focused so far on the interplay between central carbon metabolism and community assembly. However, microorganisms can also synthesize a large number of specialized metabolites that, while not continuously essential for survival, are important modulators of growth and behavior. These secondary metabolites can be vitamins or other co-factors, signaling molecules that regulate behavior, or antimicrobial compounds that can alter gene expression, inhibit growth, or induce lysis of other community members, to name a few [69–73]. These interactions can have dramatic effects on population dynamics, but it is useful to separate their impact from the effects of the underlying trophic structure that we have argued drives the successional assembly of a microbial community. As we will discuss below, although trophic interactions alone lead to highly stable communities, interactions mediated by secondary metabolites can 'steer' communities through different assembly paths.

Despite the potential importance of secondary-metabolite interactions, relatively few general principles can be postulated based on current data to relate the incidence of these interactions to environmental conditions or biological properties of the organisms. Although interactions mediated by vitamins, antimicrobial peptides, etc., have been shown to occur across distantly related taxa — in fact some of the most ecologically relevant microbial interactions are inter-kingdom mutualisms [74,75] — one potential 'rule' is the apparent dependency between secondary metabolite interactions and phylogenetic distance. For instance, some forms of antimicrobial activity [76–78] or microbial communication mediated by quorum sensing signals [70,79–81] act almost exclusively on closely related organisms. The narrow phylogenetic range of these compounds is easily interpretable: antimicrobials that act on close relatives are thought to serve as 'weapons' to fend off competitors with a high niche overlap. Similarly, quorum sensing signals allow microorganisms to coordinate their behavior, which is most advantageous among members of a clonal group or natural population.

Importantly, however, these fine levels of phylogenetic resolution may not be detectable in surveys of community composition based on 16S rRNA [82]. Many of the different natural populations of bacteria where these interactions take place have 16S rRNA sequences that are either identical or are too close to be reliably distinguished by short amplicon libraries [22,81]. Moreover, when 16S rRNA data are analyzed, the 16S rRNA sequences are often clustered at the level of genera, in order to reduce the complexity and 'noise' in the data. It is therefore likely that the impact of some of the secondary metabolite interactions, such as those that mediate antibiosis, is masked from

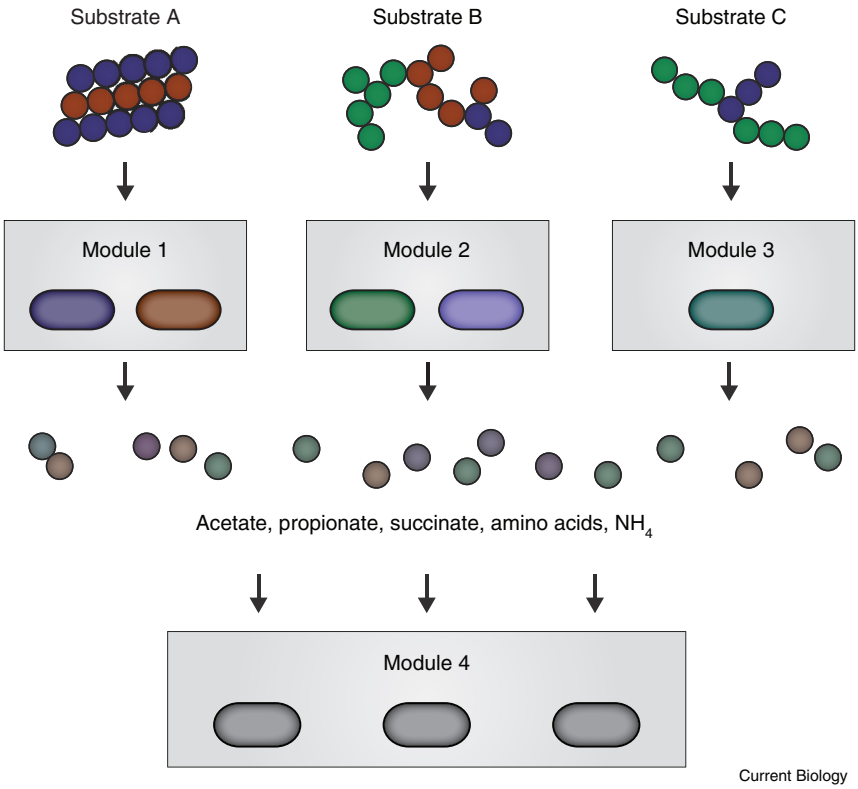

Figure 3. Modular community assembly.
The composition of communities that assemble on multiple resources can be approximated as a weighted sum of the composition of communities on the individual resources. In the context of polysaccharide degradation, primary degraders can be highly specialized. However, their metabolic byproducts are generic, such as simple intermediates of glycolysis. This means that communities assembled on different resources only change by 'replacing' the primary degrader module, without this change necessarily percolating through the rest of the community.

community composition data that do not resolve strain diversity [22,83]. Discovering the principles that govern the distribution of secondary-metabolite interactions requires adopting top-down modes of experimentation to complement the traditional bottom-up approach based on pairwise assays [55]. For example, studies that measure the impact of biosynthetic-cluster knockouts in the assembly dynamics and function of synthetic consortia will be highly informative [84].

Assembly Dynamics of Trophic Systems

We now turn our attention to the impact of primary (trophic) and secondary metabolite interactions on the dynamics of microbial community assembly. The goal is to define general principles to the extent that current information allows it, not to describe any particular system. To this end, it is useful to focus first on what we can learn from simple mathematical models before discussing similarities and differences with empirical data. Specifically, we will focus on results that can be derived from recent consumer-resource models of crossfeeding [54,85], which are useful tools to develop null expectations about the behavior of microbial communities dominated by interspecies metabolite flux.

Consider first a purely trophic system (ignoring secondary metabolites) with a strict hierarchy, such that species in the upper levels feed species in the lower levels but there is no feedback, i.e. there are no cycles introduced by interactions within levels or directed interactions from a 'lower' level to a 'higher' one (Figure 1). A typical, naïve example might be a polysaccharide-degrading community where degraders (the top trophic level) of the polysaccharides degrade the primary resource and produce metabolites that are then consumed by lower trophic levels [86] (Figure 3). A strong trophic hierarchy implies a simple mapping from primary resources to composition: the composition of a community on multiple resources can be broken down as a simple sum of the communities assembled on individual resources. This type of additive, or 'modular,' community assembly predicts that subcommunities corresponding to a particular resource are present or absent depending on whether that resource is present. Such a pattern was observed in a recent study of community assembly on model marine particles of varying composition [34], and it can also be readily reproduced with consumer-resource models [87]. Similarly, the assembly of phytoplankton-associated bacterial communities in synthetic media with mixtures of ecologically relevant carbon source was predictable from individual species' abundances in individual carbon sources, again suggesting at least some degree of modularity [35].

Another prediction of models with purely trophic hierarchies is that the final state of community assembly is independent of the composition of the founding community; that is, provided that the species are present, the system converges to a stable state regardless of their initial abundance, a state in which the surviving set of species partition the energy input optimally [33,54,88,89]. In practice, however, laboratory systems [17,29,90,91], industrial digesters [92] and natural systems [7] have been shown to display alternative assembly trajectories. The emergence of multiple states can have important practical implications. For instance, in the rumen microbiome alternative states can have different rates of methane or short chain fatty acid production [93,94]. There are many plausible biological mechanisms one could add to a purely trophic community model that could explain the existence of alternative states, also known as 'multistability'. These mechanisms could involve, for instance, phenotypic plasticity [95], simultaneous competition for multiple resources [96], metabolic trade-offs [89], or higher-order interactions [97]. Here, we choose to highlight two simple mechanisms that we think are likely to be ubiquitous across microbiomes: mutual antagonism and positive feedback loops (Figure 4).

Mutually antagonistic pairwise interactions are the textbook recipe to create a bistable ecological system. When interspecific competition is much stronger than intraspecific competition, the

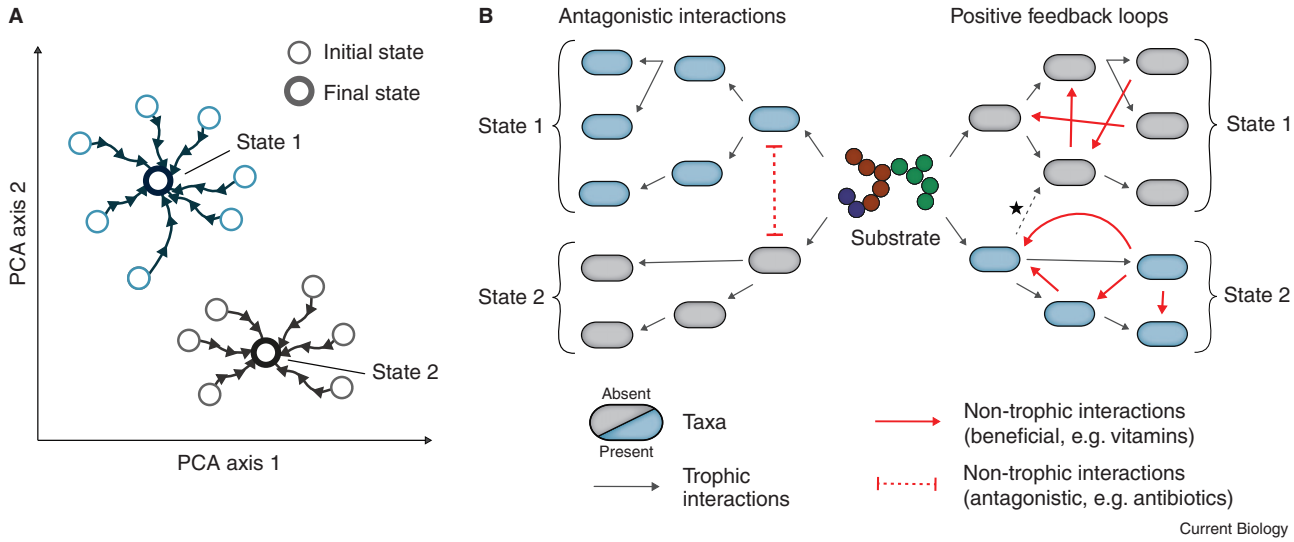

Figure 4. Multi-stability in community assembly.
(A) In an ordination plot showing the dynamics of community composition over time, different alternative states appear as regions towards which different biological replicates converge based on initial condition. Each alternative state has a basin of attraction separated by an unstable equilibrium point. Initial conditions close to this unstable point can converge to either alternative state based on miniscule differences that get amplified over time. (B) Two mechanisms generating multi-stability in hierarchical trophic networks. Left: antagonistic interactions between different trophic chains. When interspecific competition is stronger than intraspecific competition the outcome of community assembly depends on the initial species abundances, such that the more abundant species at initial stages of assembly wins. This phenomenon can propagate through the trophic cascade, creating large changes in community composition. Right: feedback loops can create dense networks of mutual facilitation between subsets of species at different levels of the trophic cascade. To the extent that these networks are exclusive to subsets of species, alternative community states can emerge whereby whole trophic chains outcompete each other depending on the initial population abundances. Even when trophic interactions are in principle possible between species exclusive to alternative stable states (dashed line indicated by star), beneficial interactions create positive feedback loops that favor retaining trophic interactions within states.

outcome depends on the initial frequency of the two species, such that the species that colonizes and grows first has a higher chance of 'winning' the contest. In a community with a strong trophic structure, this type of instability can propagate throughout the network, leading to the assembly of alternative trophic cascades. This is exactly what is believed to happen in the above example of the rumen, where the transition from methane to short chain fatty acid-producing communities is mediated by the inhibition of one trophic chain by another. In particular, the initial abundance of *Megasphaera elsdenii* controls the abundance of lactate, which acidifies the environment and inhibits methanogens [93,94]. The inhibition of methanogens then results in the redirection of the electron flow towards short chain fatty acid production, which is beneficial for both the host and the environment. In another recent example based on a controlled laboratory system, it was shown that antagonistic interactions can drive initially identical, complex communities into different stable states [17]. Interestingly, in this example, it was a negative interaction between two species on a secondary trophic level that impacted global community composition, suggesting that there was significant feedback between trophic levels allowing perturbations to travel up the trophic chain.

Positive feedback loops occur when a species downstream in the trophic chain facilitates the growth of the species upstream. These interactions can become *de facto* mutualistic, and they can be mediated through a variety of mechanisms, such as the exchange of secondary metabolites or amino acids [59], regulation of pH to mutually acceptable levels [98,99], or the consumption of inhibitory byproducts of metabolism, like hydrogen in anaerobic communities [100]. Although in principle positive feedback loops can occur in any type of community, they can become particularly relevant in anaerobic communities performing reactions near the limit at which they are thermodynamically favorable [101]. Because the forward reactions generate little Gibbs free energy, metabolic reactions can be inhibited by the accumulation of end products. Thus, the removal of these products by species downstream in the metabolic cascade itself allows the continued production of those metabolites, closing the positive feedback loop. In this way, positive feedback loops may establish inter-species crossfeeding between groups of species, and there could be multiple such groups in a community. Those groups that collectively manage to exploit the incoming resources more efficiently (by harvesting more energy through the metabolic cascade) will be able to drive competing groups to extinction, and this process may depend on the initial abundances of the individual species in each group. However, in general, mechanistic studies of multistability in anaerobic reactors are not available at this time, and more work, both from the perspective of theory and experiments, is required to understand the mechanisms of competition between groups of positively interacting species.

The Spatial Dimension of Community Assembly

Although we have focused thus far on how primary and secondary metabolites shape microbial community assembly, microorganisms are more than just metabolic factories; they are also capable of displaying complex behaviors that impact how their communities assemble and disassemble. If the system in

question is a well-mixed reactor, behaviors such as motility, chemotaxis, or aggregation may not play a big part in the macro-scale observables. However, most natural environments are far from being well-mixed reactors. Soil is naturally heterogeneous, with the topographic complexity of the medium creating strong microscale gradients [102]. The gut is also characterized by gradients, for example in nutrients, oxygen, and pH from the epithelium to the lumen [103,104]. Even aquatic environments such as the ocean, frequently thought of in the past as a mixed environment, are in fact punctuated by micro-scale patches of nutrients, originating from the death of other microorganisms such as algae or the coagulation of extracellular polysaccharides [105,106]. Here, we use these marine nutrient patches as an example for how the spatial arrangement of organisms and the gradients they sense and create can impact their assembly and ecosystem function in many, if not all, environments.

Nutrient patches in the ocean are hotspots of microbial activity and their role for carbon cycling has catalyzed extensive research on the interaction between particles and microbes [107,108]. These particles of organic matter, sometimes referred to as marine snow, can transport vast amounts of carbon to the ocean floor as they sink. How much carbon is ultimately transported from the surface oceans to the deep depends on the rate of degradation performed by the microbial communities that assemble on these particles. Many of the processes discussed thus far, such as trophic interactions and the role of stochastic arrival times, are believed to play out on particle surfaces as marine bacteria encounter these rich resource patches. However, before bacteria can successfully colonize a particle, they must first be able to find it. Modeling shows that encounter rates of bacteria with particles are enhanced by a factor of 100 to 1,000 if bacteria are motile [109], because motile bacteria explore space much more rapidly than non-motile bacteria. Encounters are further enhanced by chemotaxis, which allows bacteria to sense a nearby particle and swim towards it [110,111].

Consistent with the expectation that motility promotes particle colonization, a study of community assembly on model particles found a remarkable correlation between trophic level and motility. Early colonizers (arriving to particles within the first 48 hours of exposure to sea water) were not only primary degraders of polymers but also motile and capable of chemotaxing towards particles. By contrast, late colonizers, which relied on metabolites from primary degraders for growth, were non-motile [9]. This shows that the spatial behaviors can prime the system to assemble in a logical order, with the first trophic level arriving early and opening niches for the lower levels. Moreover, the spatial organization of primary degraders on particle surfaces is also critical for the initiation of the trophic cascade. A recent study found that, in order to degrade complex biopolymers such as chitin, marine bacteria needed to aggregate into cell clusters of 20 μm in diameter [112]. Within these clusters, cells share hydrolysis products and can overcome the large diffusive losses associated with extracellular hydrolysis. The metabolic activity of microbes within these clusters and other types of dense cell aggregates can create oxygen gradients that enable respiration with different electron acceptors, such as nitrate, to take place [113,114]. The ensuing conversion of nitrate to N_2 within aggregate cores is believed to be a process of prime importance for the cycling of nitrogen in soils and oceans [115,116]. Many other examples exist of microbial activities that depend on local, physical associations between microbes [117]. However, the integration of this physical dimension into models of microbial community assembly remains difficult, and more work is needed, particularly to learn how to scale up the observed microscale patterns to predict the global impact of these interactions.

The Challenge Ahead: Functional Groups

There are many important open challenges in microbial ecology, for example, leveraging genomic data to infer function and interactions from species catalogs, or integrating insights gained from small-scale communities into global models of carbon and nitrogen cycles, to name a few. Dealing with many of these challenges requires us first to translate species catalogs into functionally meaningful units, representing key metabolic or ecological roles. Traditionally, ecologists have focused on species as units of diversity, and microbiologists have followed suit in using species content as a descriptor of a community. This is the approach widely used in microbiome science, enabled by the advances in next generation amplicon sequencing. However, in most conceivable cases, we study a particular microbiome in order to understand, predict, and potentially control its functioning, with no particular regard for species content. Multiple studies published in the last decade have shown that the abundance of metabolic pathways in a microbiome and the abundance of the different taxa are weakly coupled, at best [3,118,119]. The main reason for this is that functionally redundant taxa can replace each other, while maintaining the stability of the main metabolic process they mediate. Therefore, unless genetic diversity is the ultimate focus of study, current methods describing communities in terms of ambiguous variables (for example 'species' defined as 16S rRNA sequence clusters) leave it up to the researchers to translate those variables into interpretable functions. In the context of communities controlled by trophic interactions, functionally redundant taxa can be conceived as species with similar patterns of metabolite consumption and excretion [12,86]. In large-scale surveys, the replacement of one species by a metabolically redundant one gives rise to anticorrelations between their abundances, which makes it difficult to interpret the abundance of any individual species relative to biochemical measurements such as temperature or nitrate concentrations (Figure 5A). By contrast, appropriately selecting sets of functionally redundant species and adding their abundances can help identify environmental drivers of microbiome composition [120]. However, we still lack systematic strategies — experimental or computational — to identify those functional groups without requiring exhaustive phenotyping of culture collections.

In general, functional annotations are decoupled from taxa simply by virtue of the fact that the same pathway can be distributed across many taxa. However, the degree of coupling may depend on the type of metabolic function and the speed at which it evolves. Indeed, for many large-scale functional annotations, such as aerobic heterotrophy or nitrogen fixation, there exists no taxonomic scale at which that function is always present or absent [3] (Figure 5B). However, on finer functional and taxonomic scales, we do expect some degree of coupling between function and taxonomy, depending on whether the function is coded by pathways that lie at the periphery or in the core of

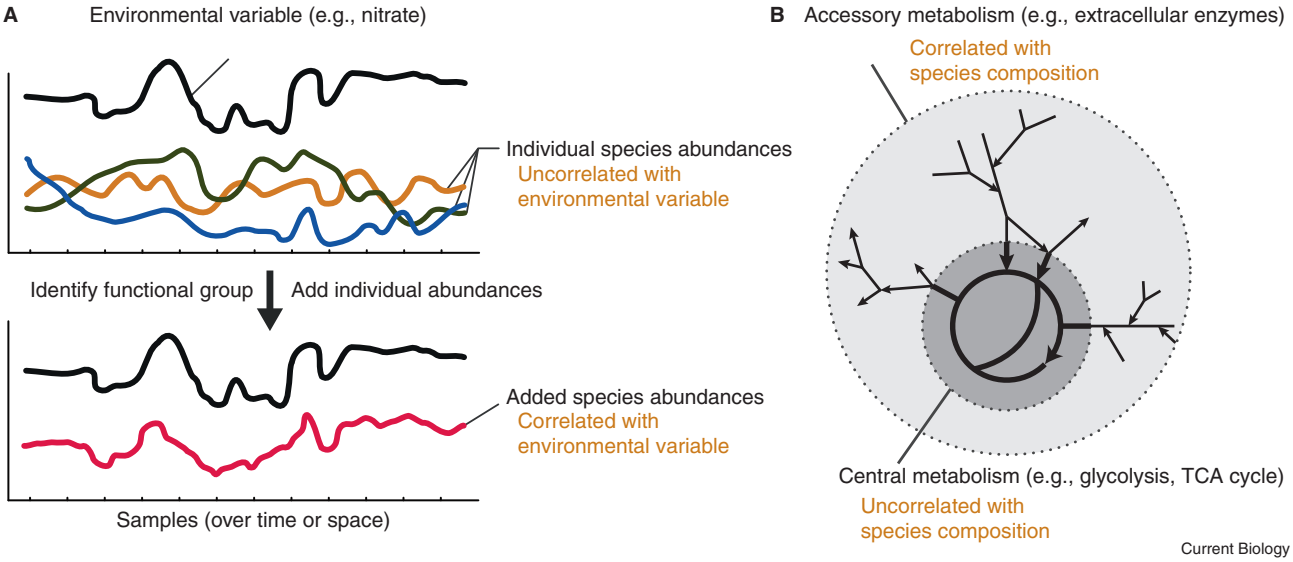

Figure 5. Functional groups in microbial ecology.
(A) Functional groups can be considered as groups of species with the similar metabolic functions. If the community has reached equilibrium, metabolically redundant species are expected to be anticorrelated in their abundance, either over time, space or across replicates. The sum of the abundances of the functionally redundant species is more stable and reflects the availability of resources for the species within the functional group. (B) Not all functions are equally decoupled from species abundances. In general, we should expect core metabolic functions, such as glycolysis or the TCA cycle (central circle), to be conserved and partly redundant across different species. However, peripheral pathways, such as those that determine the uptake of substrates or genes that code for extracellular enzymes, evolve much faster and are less likely to be conserved across different species. Therefore, function and taxonomy can remain coupled when the function of interest is the profile of resources that are metabolized by the community.

the metabolic network. For instance, a highly conserved component of the metabolic network for harvesting energy from organic matter and producing biomass precursors is the tricarboxylic acid cycle. Because this cycle is shared among most heterotrophs, its measured abundance is bound to remain 'stable' across metagenomic samples despite variation in taxonomic composition. On the other extreme, at the periphery of the network, there are a large number of feeding pathways and transport mechanisms to take up specific carbohydrates, proteins, and lipids from the environment. These pathways evolve by frequent horizontal gene transfer and gene loss and they are therefore highly variable across taxa [121]. We thus expect peripheral pathways, such as those that determine substrate specificity, to vary across taxa. Building on this logic, a decoupling between taxa and function should be expected in particular for core metabolic functions, whereas peripheral functions are more likely to be correlated with the taxonomic composition of the community. This exact pattern was observed in a recent study by Bittleston *et al.*, where 10 different communities at different stages of assembly were profiled for their ability to metabolize 31 simple substrates [14]. The patterns of substrate utilization and taxonomic composition as measured by 16S rRNA sequencing were strongly correlated, revealing the expected coupling between taxa and function. By contrast, the different communities were indistinguishable in terms of their CO_2 output, as expected from the fact that most heterotrophic microbes are capable of performing aerobic respiration.

Quo Vadis Microbial Ecology?

Trophic interactions are ubiquitous across communities, forming the backbone onto which species assemble. We have shown how this backbone constrains assembly dynamics, the emergence of cooperative or competitive interactions, as well as the potential for alternative assembly trajectories. Given this perspective, we would like to point out a few areas of future work that could accelerate research into microbial communities.

First, we need new methods to infer properties of the interaction network, such as the degree of hierarchy or interdependence between species, that do not require pairwise assays. The main obstacle at the moment is the difficulty to perturb specific community members, which would allow us to study how perturbations propagate to other species. An underused opportunity in this regard is the *in vitro* assembly of complex synthetic communities consisting of a large number of environmental isolates. This allows the creation of (in principle) complete phenotype and genotype libraries, which can then be used to develop computational methods to understand and predict community assembly. Perhaps more importantly, synthetic communities also enable the rational assembly of communities using, for instance, 'leave-one-out' protocols [122], in which the community is assembled with or without a focal strain, or using known functional relationships, such as when a secondary community of crossfeeders is combined with a set of degrader strains. Such experimental designs can shine a light on the degree to which individual strains can impact community assembly dynamics and functional output.

A second important item in this 'wishlist' is learning more about the conditions that lead to inefficient metabolism and metabolite secretion in nature. Ideally, this question should be addressed in non-model organisms and in the context of different environmental limitations, for example, carbon,

nitrogen, or phosphorus. Different environmental limitations will also impact which metabolites will be excreted, whether or not microbes tend to invest in storage compounds, and which genomically predicted capabilities are actually realized in a given community context.

Finally, we would like to emphasize the importance of developing techniques — both experimental and computational — to convert 'omics' data into functionally meaningful descriptions of microbial communities that go beyond 16S rRNA profiles. Many efforts are underway that will aid in this endeavor, be it the assembly of large isolate libraries [123], the creation of community metabolic models from available metagenomic and 16S rRNA data sets [65], or the development of techniques for high-throughput screening of microbial communities [124] or *in situ* single-cell physiological measurements [125]. What is needed now is to combine these efforts to validate computational models with experimental data and develop a predictive understanding of microbial interactions within communities. We believe that addressing these three issues is critical to advance microbial community ecology in this new decade.

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