

Review

Connecting microbial community assembly and function

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Microbial ecology is moving away from purely descriptive analyses to experiments that can determine the underlying mechanisms driving changes in community assembly and function. More species-rich microbial communities generally have higher functional capabilities depending on if there is positive selection of certain species or complementarity among different species. When building synthetic communities or laboratory enrichment cultures, there are specific choices that can increase the number of species able to coexist. Higher resource complexity or the addition of physical niches are two of the many factors leading to greater biodiversity and associated increases in functional capabilities. We can use principles from community ecology and knowledge of microbial physiology to generate improved microbiomes for use in medicine, agriculture, or environmental management.

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Introduction

All ecological communities are formed by the interplay of four general processes: dispersal, selection, diversification, and drift [1,2]. The relative influence of these processes depends on the abiotic and biotic context; for example, if a certain habitat has one primary food source and constant high temperatures, these factors will strongly filter the species able to colonize and thrive in the habitat, resulting in conditions where ecological selection is likely to play a larger role than dispersal or drift. Communities of microbes are affected by the four

processes just like those of animals or plants, although distinctive features of some microbes (e.g. long-term dormancy or global dispersal on air currents) can shift which processes dominate [2]. These processes drive community assembly, affecting both composition and, perhaps most importantly, function.

Community function is studied in a few different ways. One approach is through examining traits of different species and summarizing them across a community or assemblage [3]. Another approach is using the concept of ecosystem functioning — an ambiguous term that sometimes refers to the way in which energy or nutrients move through an ecosystem, while at other times, it is used in a more human-centric way to refer to ecosystem services [4]. It can be difficult to determine which mechanisms drive relevant functions for a particular community or ecosystem, even if the general goal is defined (e.g. a function-related goal might be increased biomass production or increased resistance to perturbations). Furthermore, some of the more general ‘community functions’ used for microbial communities, such as respiration, may not capture other more nuanced functions, such as hydrolytic enzyme activity, that are more likely to vary in a focal ecosystem and could affect end goals [5]. Thus, a researcher must be familiar with their particular system in order to identify the traits or ecosystem functions of interest.

Synthetic, or constructed, microbial communities (SynComs) are built by combining individual strains, while enrichment communities are sampled from natural sources and established in laboratory conditions through environmental selection. Defining key functions in SynComs is often simpler than for enrichment cultures or wild communities because the goal is usually the degradation or generation of certain compounds, often related to the health of a host. For example, in the human gut microbiome, there are four main categories of beneficial functions: cometabolism, fermentation, ecoresilience, and immune training [6]. Cometabolism is when microbes utilize compounds produced by the host, fermentation is when they assist with digestion, ecoresilience is stability in the face of pathogens or perturbations, and immune training primes the host immune system. For the most part, these categories apply to other types of host-associated microbiomes as well. For example, in an agricultural setting, managers would likely want to know how their plant-associated microbiomes act in terms of cometabolism, ecoresilience, and immune training.

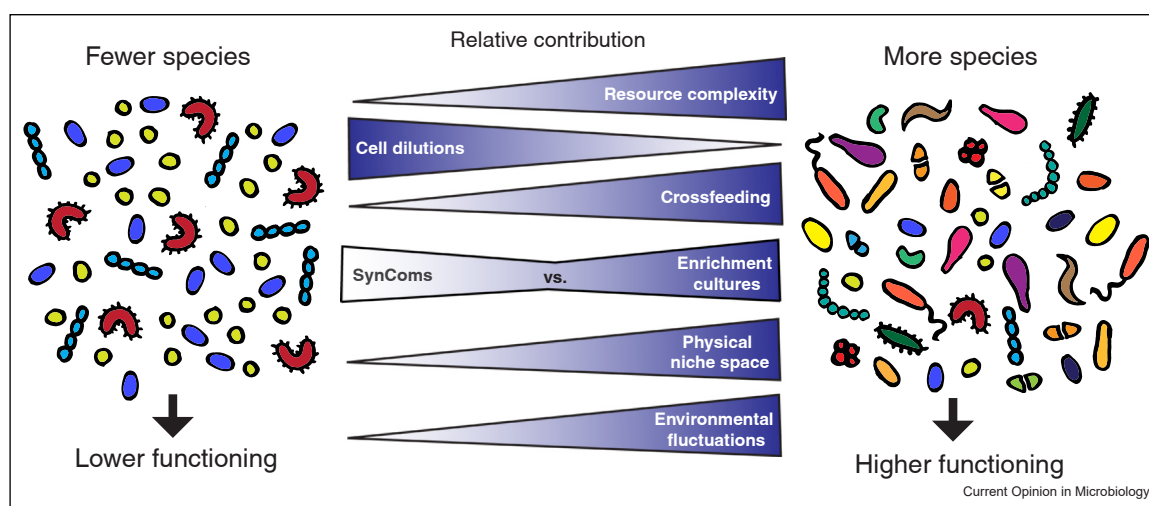
This review highlights recent literature and concepts connecting assembly and function of microbial communities. A primary link between microbial community assembly and functioning is through increased coexistence that supports higher species richness; therefore, I emphasize recent insights into the mechanisms supporting microbial biodiversity such as resource complexity, cross-feeding, and other factors that expand niche space or reduce fitness differences. The review focuses on bacterial communities, reflecting the majority of studies in this field. However, as a scientific community, microbial ecology needs to move beyond characterizing just the interactions among bacteria to also include the other players in microbial communities. For example, fungi and bacteria interact to produce unique functions such as flavor in fermented foods [7], the alleviation of amino acid and iron limitation in bacteria [8], or more effective suppression of plant disease [9]. Multitrophic interactions, such as predator–prey relationships between protozoa and bacteria [10] or viral infections that alter nutrient cycling [11,12], can also have large effects on community composition and function. Overall, even though the scientific literature (including this review) often uses the term ‘community’ for a single taxonomic grouping, communities are inherently multitrophic and contain species across different domains of life. Some recent studies reflect a broader diversity by including both prokaryotes and eukaryotes (e.g. [9,13,14]), and hopefully, many others will follow in their footsteps.

Current research is uncovering the connections between community assembly and function and the mechanisms driving observed patterns. Increased knowledge will inform how researchers build synthetic communities, with great potential for improving human health, agriculture, and ecological restoration. A variety of different factors affect how a SynCom or enrichment community will assemble and function, including resource complexity, the timing and strength of dilutions, and the source and starting point of the community, among others. I will discuss these factors after outlining the connection between community assembly and function.

The connection between microbial community assembly and function

Community assembly is intertwined with community function because both the identity and the interactions of the species in a focal community will affect its functional capabilities. In the Biodiversity Ecosystem Functioning (BEF) literature, studies generally find positive relationships where higher biodiversity (or richness) leads to higher ecosystem function (e.g. productivity) in plant or microbial communities [4,15,16] (Figure 1). A positive relationship between biodiversity and ecosystem function can be attributed to either selection or complementarity [17]. Here, selection refers to sampling effects: if there are more species, it is more likely that a high-functioning species is present in the community. Complementarity refers to how niche partitioning or facilitation can lead to a higher functional output when species are together

Figure 1



Conceptual diagram highlighting some of the factors affecting community richness (more vs fewer species) and the connection to community functioning. Organism abundance is similar on each side, with large differences in richness. The triangles in the middle represent how different factors contribute to biodiversity. Note that this diagram represents the norm, but fewer species can lead to higher functioning (e.g. when certain species are good competitors and high producers) and vice versa.

instead of alone. Despite the prevalence of positive BEF relationships, a recent study used a dynamical consumer-resource model of microbial decomposer communities to investigate the circumstances where a negative BEF relationship might emerge and found that these relationships occurred when different species inhibited the functioning of others (negative complementarity) or when a competitive hierarchy was present, and better competitors were not strong producers (negative selection) [18]. However, as also reflected in the rest of the literature, the majority of situations in this modeling study led to positive BEF relationships.

In some cases, particular species drive ecosystem or host effects, while in other cases, it appears to be more of a whole-community effect. In an example where particular species drive relevant function, a study measuring plant-pathogen defense by 130 leaf-associated, 5-species SynComs found that strain identity mattered most for pathogen reduction [19]. Further research identified how opportunistic *Xanthomonas* pathogens could trigger whole-community dysbiosis via enzyme secretion from their Type II Secretion System [20]. A whole-community effect has been observed in other studies. A 10-strain SynCom, on average, increased microbial productivity and host plant growth more than single-strain inoculations [21]. Rare species drove multifunctionality (a combination of 16 ecosystem functions related to nutrient provisioning, element cycling, pathogen control, and plant-microbe symbiosis) in a study of agricultural soils [22]. A particular bacterial community increased growth of a carnivorous pitcher plant — likely via assistance with insect prey degradation — but the effect could not be tied to particular bacteria and was likely caused by a functional rather than taxonomic shift [23]. In general, it appears that the community assembly factors driving higher species richness are also relevant for ecosystem functioning (Figure 1).

Common abiotic factors driving microbial community assembly and function

One of the most important factors affecting microbial community assembly is the complexity of resources provided (Figure 1). Microbial communities experience stronger selection when only a single carbon or nitrogen source is used. This is in part due to strong competition for the available resource (e.g. see Tilman's R^* theory [24]) and in part because only certain species may have the metabolic machinery to use a particular resource. Recent research shows that microbial use of particular carbon sources can be predictable [25–29]. For example, genome content can predict catabolic preferences [27] and metabolically similar substrates select for taxonomically similar communities [25], while community richness only increases by one species, on average, with

one additional resource [26]. Importantly, a more complex resource, such as cellulose, that can generate more metabolic products, can support many more species than a simple resource, such as citrate [26]. As researchers move forward in investigating the effects of different resources, we need to use more realistic and complex mixed-resource substrates because these will increase the richness and complexity of the microbial communities we can assemble and their resulting functional potential [5] (Figure 1).

Resource concentration also affects biodiversity, but with mixed results. Recent studies have found that bacterial richness decreases with higher resource concentrations, although the decrease is less prominent when using more complex resources [30–32]. There are probably multiple mechanisms driving this observation, including stronger competition among species at higher resource levels and also how certain fast-growing species can change their environment to make it unfavorable to other species by altering pH or producing toxic metabolites [30]. Laboratory experiments generally use far higher resource concentrations than those found in natural environments, and this may skew findings, as theoretical studies predict higher diversity at higher nutrient flux [33]. Real ecosystems likely do not obey a universal nutrient–diversity relationship [34]. In situations where the desired functional outcome is biomass production, then higher resource concentrations are necessary, even if diversity is reduced, in order to build additional biomass. Thus, higher concentrations of more complex resources would be the best way to minimize biodiversity reduction while supporting higher overall biomass.

Beyond resources, another consideration when working with microbial communities in a laboratory setting is the dilution ratio and the timing of passages. The dilution rate and frequency set a minimal average division time that each species needs to achieve to survive. A higher dilution of the cells making up the community (e.g., 1 to 1000 vs 1 to 10) will allow for more growth in the new media, which could increase a focal function of interest but will also create a bottleneck where less abundant organisms might not be transferred, thus increasing the effects of drift. Similarly, more frequent transfers or dilutions will allow for more generations but will favor fast-growing species that use up the most accessible resources first and may not give time for cross-feeding or the breakdown of more recalcitrant compounds. For example, in a bacterial community made up of glucose specialists and acetate specialists, the acetate specialists were excluded when only glucose was supplied and the transfer time was shortened from 48 to 12 hours due to a lack of time for organic acids to accumulate and support growth via cross-feeding [28].

Interactions and species-specific functional differences affect community assembly and function

Strains may not grow well alone in part because they rely on cross-feeding (Figure 1), which is widespread in microbial communities. Certain metabolic pathways often require different microbes to complete them; for example, complete anaerobic digestion of plant matter, such as in a rumen, requires three different microbial functional groups [35]. In the ocean, complete denitrifiers exist, but the vast majority of microbes only have genes for some of the steps of the NO_3^- to N_2 pathway [36]. Beyond these examples, cross-feeding can also happen even when multiple species are not required to complete a metabolic pathway. Sometimes fast growth leads to overflow metabolism, where a species capable of the whole pathway only completes the first part and excretes metabolites that are useful for other species. This was found for glucose specialists that produce acetate and support organic acid specialists [28]. Interestingly, more metabolically similar substrates select for taxonomically similar communities because the substrates lead to more similar metabolic by-products that are used by particular species [25]. To further support our knowledge of bacterial cross-feeding, an ecology-based computational method, GutCP, was recently developed to predict cross-feeding interactions in the human gut microbiome [37]. The increase in genomic information and metabolic modeling for bacteria should help to elucidate the full complexity of these relationships and which partners might be necessary for building synthetic communities that excel in particular functions.

An emerging axis for defining metabolic capability in microbial communities is if species preferentially degrade carbon compounds using glycolytic or gluconeogenic pathways. In a set of 186 heterotrophic marine microbes, their sugar to organic acid preference was highly correlated ($R^2 = 0.92$) with the first principal component of variation in growth over 17 days on 118 different substrates, as well as how quickly strains would switch between substrates [27]. There is a lag time in two-resource media with a trade-off between fast growers being slow switchers and slow growers being faster switchers [38,39]. This allows for more coexistence in multiresource environments [40]. Furthermore, spatial organization within a community can affect growth resumption after an environmental shift. For example, if one species is metabolically dependent on another, then the cells closest to partner cells will restart growth more quickly, leading to a population bottleneck [41]. Thus, coexistence and even intraspecific diversity can depend on differences in functional traits and spatial organization among different cross-feeding taxa.

It can be difficult to build microbial communities from individual strains [42]. Using enrichment cultures from

natural sources is often more effective than building SynComs from individual axenic strains. This could be due to the humpty-dumpty effect [43], which states that a community cannot be put back together by combining just the species currently present, or it could be because it is difficult to obtain all of the community members as axenic strains in culture. In the latter situation, an enrichment is effectively aiding the dispersal and arrival of species that cannot grow alone. Enrichments are generally more even and diverse and fluctuate less over time than synthetic communities [44] (Figure 1). This begs the question of what we are missing when we build communities one by one. What are the emergent properties that we could harness to increase richness, stability, and functional output in synthetic communities?

Interactions among species certainly affect biodiversity and function, but it is unclear if higher richness and stability are supported more by positive or negative interactions. Among microbial ecologists, a controversy has emerged regarding the prevalence and functional effects of positive versus negative interactions in bacterial communities [13,45,46]. Necessary and mutually beneficial interactions between two species (i.e. obligate mutualisms) are relatively rare, as they require both species to be present and interacting in a particular way and can lead to constraints on evolution or community collapse when a keystone partner is not present. However, beneficial interactions can also be one-sided (i.e. commensalism or parasitism) or opportunistic and not required for survival. We know that competition (a negative-negative interaction) is common in bacterial communities [46], but metabolic cross-feeding (most often a positive-neutral interaction) is also very common [37,45]. Laboratory studies generally use high nutrient conditions with low toxicity and stable environments that promote competitive interactions, while harsher environments may better support facilitation among taxa [47]. A global, observational, field study of multitrophic soil communities found that positive associations among both species pairs and triads governed microbial networks and supported higher biodiversity and resistance to disturbances [13]. Perhaps, the most productive approach is to recognize that both negative and positive interactions are present and that direct interactions in microbial communities are as relevant and as likely to affect richness and stability as those of animal or plant communities [48].

Connecting ecological theories of coexistence with microbial community function

Ecological theory developed in plant or animal systems can inform our understanding of coexistence in microbial communities and how it leads to increased diversity and function. Most studies exploring coexistence mechanisms,

and even our underlying mathematical theory, focus on very few species. Often only two species or strains are used [49–51], which limits our understanding of the mechanisms contributing to coexistence in complex microbial communities. Modern Coexistence Theory (MCT) [52] is a widely used approach that focuses on species' rates of invasion into a community from low abundances as a metric of coexistence. It also highlights the two main factors driving coexistence: niche differences and fitness differences. MCT has drawbacks (e.g. see Ref. [53]) and even recent advances have primarily been applied to relatively species-poor plant and animal systems [54]. But new implementations might have potential for more diverse and realistic communities, like those involving microbes. Alternatively, it may be best to move away from heavy use of the competitive exclusion principle and instead of asking why so many species are able to coexist, we should ask why species do not coexist when we see a clear prevalence of species-rich communities in natural systems [55].

Traditional methods for growing bacteria or yeasts were developed for single-species cultures and thus do not promote multispecies coexistence. One of the likely reasons why it is difficult to generate stable, diverse communities in the laboratory is that sufficient habitat and community complexity must be present in order to provide sufficient niche space for different organisms to thrive and coexist. Ecological theory highlights how spatial structure, temperature fluctuations, dispersal, and other habitat characteristics that lead to temporal asynchrony can all increase the stability of biodiverse communities [56–58]. Empirical examples with microbes support this theory and show how physical or temporal niche space can be incorporated into SynCom design.

With regard to physical niche space (Figure 1), a 2008 study of a three-species synthetic community found that microscale spatial structure was both necessary and sufficient for the stable coexistence of the species [59]. Similarly, the use of a sterile soil silt matrix allowed for a more biodiverse soil-based SynCom [60]. Fluctuations in the environment (Figure 1) lead to increases in temporal niche space and asynchronous responses of taxa. A study of a kefir microbial community found that spatio-temporal niche partitioning led to stable coexistence of diverse community members [61]. A SynCom experiment of wood decay fungi found that temperature fluctuations facilitated coexistence as well as decomposition [62]. And a mesocosm study of soil microbial communities found that higher diversity and asynchrony in activity led to increased stability in terms of ecosystem functions (here measured as biomass production, plant diversity, litter decomposition, and soil carbon assimilation). Different bacteria and fungi were active at different times, complementing each other [14]. Even the interactions among species can lead to additional niches. Two recent studies found that diversity begets diversity

in microbial community assembly, where metabolic niche construction and cross-feeding interactions lead to new niche space [63,64]. Together, the evidence suggests that increasing niches via spatial and temporal diversity in laboratory conditions will likely lead to higher overall community richness and associated increases in ecosystem function for synthetic microbial communities.

Conclusions

In recent years, our understanding of the mechanisms driving change in microbial community composition and function has grown considerably. Microbial communities in laboratory settings are likely to contain more species and greater functional capabilities when grown with increased physical niche space, no extreme dilution bottlenecks, fluctuations in their environments, and more complex resources to promote cross-feeding (Figure 1). We can harness the ecological processes of dispersal, selection, diversification, and drift in order to increase the biodiversity and resilience of synthetic communities used for industrial processes or for host and ecosystem health.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

None.

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