Towards synthetic ecology: strategies for the optimization of microbial community functions

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Microbial communities are able to carry out myriad functions of biotechnological
interest, ranging from the degradation of industrial waste to the synthesis of
valuable chemical products. Over the past years, several strategies have emerged
for the design of microbial communities and the optimization of their functions.
Here we provide an accessible overview of these strategies. We highlight how
principles of synthetic biology, originally devised for the engineering of individual
organisms and sub-organismal units (e.g., enzymes), have influenced the
development of the field of synthetic microbial ecology. With this, we aim to
encourage readers to critically evaluate how insights from synthetic biology
should guide our approach to community-level engineering.

1 Introduction

Fulfilling the promise of a circular economy, where waste byproducts can be reutilized
in closed-loop processes, relies on the development of sustainable alternatives to non-
renewable resources. In recent years, it has become increasingly evident that addressing this
challenge requires harboring the immense potential of microorganisms (Aswani et al.,
2024). Microbes carry out a variety of functions that could be harnessed for biotechnological
purposes. For example, some microbes are capable of degrading plastics (Taghavi et al.,
2021), transforming complex compounds like lignocellulose (often present in municipal
waste) into biofuels (Cragg et al., 2015; Ling et al., 2014; Prasad et al., 2019; Senne De
Oliveira Lino et al., 2021), producing biomaterials (Laurent et al., 2024), or synthesizing
high-value molecules including drugs (Zhang et al., 2022), plant natural products (Cravens
et al., 2019; Walls et al., 2023), or vitamins (Fang et al., 2017). Microbes are now even
emerging as a potential sustainable source of food (Graham and Ledesma-Amaro, 2023).

Unlocking the full biotechnological potential of microorganisms requires us to be able
to optimize the relevant functions they provide. Humans have been harvesting microbial
functions for millenia, with evidence that early agricultural societies already produced
fermented beverages around 7,000 years ago (Wang et al., 2021). Yet, it was only in recent
decades that the engineering of microbial functions began to be performed in a rational and
methodical manner, driven by technological advances in microorganism identification,
characterization, and manipulation. The development of genetic engineering tools (gene
cloning, recombinant DNA technology, or more recently CRISPR-Cas9) fueled the

establishment and expansion of the field of synthetic biology. Synthetic biology has aimed to systematize the engineering of microbial systems with a dual aim: understanding the principles by which living organisms process information, and constructing synthetic biosystems with enhanced (or even novel) traits for concrete biotechnological applications (Khalil and Collins, 2010; Leonard et al., 2008). While progress has been made over recent years, the latter objective has remained elusive (Hanson and Lorenzo, 2023; Zakeri and Carr, 2015).

Synthetic biologists have been mostly concerned with the engineering of individual strains or particular subcellular units, such as proteins or metabolic pathways. Some of the earliest examples include the genetic manipulation of yeast (Hansen and Kielland-Brandt, 1996; Romano et al., 1985) and bacteria (McKay and Baldwin, 1990) for food and beverage fermentation, the development of enzymes with high substrate specificity or catalytic activity through directed evolution (Schmidt-Dannert and Arnold, 1999; Yano et al., 1998; You and Arnold, 1996), or the optimization of intracellular biochemical reactions for increased enantioselectivity (Reetz et al., 1997). More recently, engineered microorganisms have been developed for modern applications such as carbon sequestration (Hu et al., 2019) or cancer immunotherapy (Chowdhury et al., 2019).

Engineered microorganisms, however, present various important limitations. The expression of synthetic genetic circuits is often inevitably noisy and subject to crosstalk with the native machinery of the host cells — a problem that is accentuated as these circuits become more complex (Kwok, 2010; Slusarczyk et al., 2012). In addition, heterologous expression often comes at a fitness cost, making engineered microbial functions sensitive to purging by evolution or to exclusion from better-adapted competitor species. Even when fitness costs are negligible, mere genetic drift may disrupt the expression of synthetic functions if they do not confer a benefit to the host. Synthetic biologists have proposed formal rules for the design of biosystems (Slusarczyk et al., 2012) which attempt to mitigate the effects of noise (Perrino et al., 2021), crosstalk (Müller et al., 2019), or evolution (Bull and Barrick, 2017); however challenges remain to this day.

The prospect of engineering microbial communities (as opposed to single organisms) has emerged as a promising alternative (Großkopf and Soyer, 2014; McCarty and Ledesma-Amaro, 2019; Shong et al., 2012). Communities present several advantages with respect to individual strains. They enable the compartmentalization of functional components (i.e., division of labor), alleviating fitness costs on individual strains (Beck et al., 2022; Wang M. et al., 2022; Roell et al., 2019). Functions that emerge at the community level may also be more robust (or at least change in predictable ways) in the face of evolution of constituent species (McEnany and Good, 2024; Venkataram and Kryazhimskiy, 2023). Communities may often be able to resist invasions from external species (Wagner, 2022; Mickalide and Kuehn, 2019) or groups of species (Diaz-Colunga et al., 2022; Lechón-Alonso et al., 2021). Furthermore, the composition and function of microbial communities may be modulated without the need to genetically engineer any member species, minimizing concerns on the environmental or health hazards that genetically modified organisms may pose (EFSA et al., 2020).
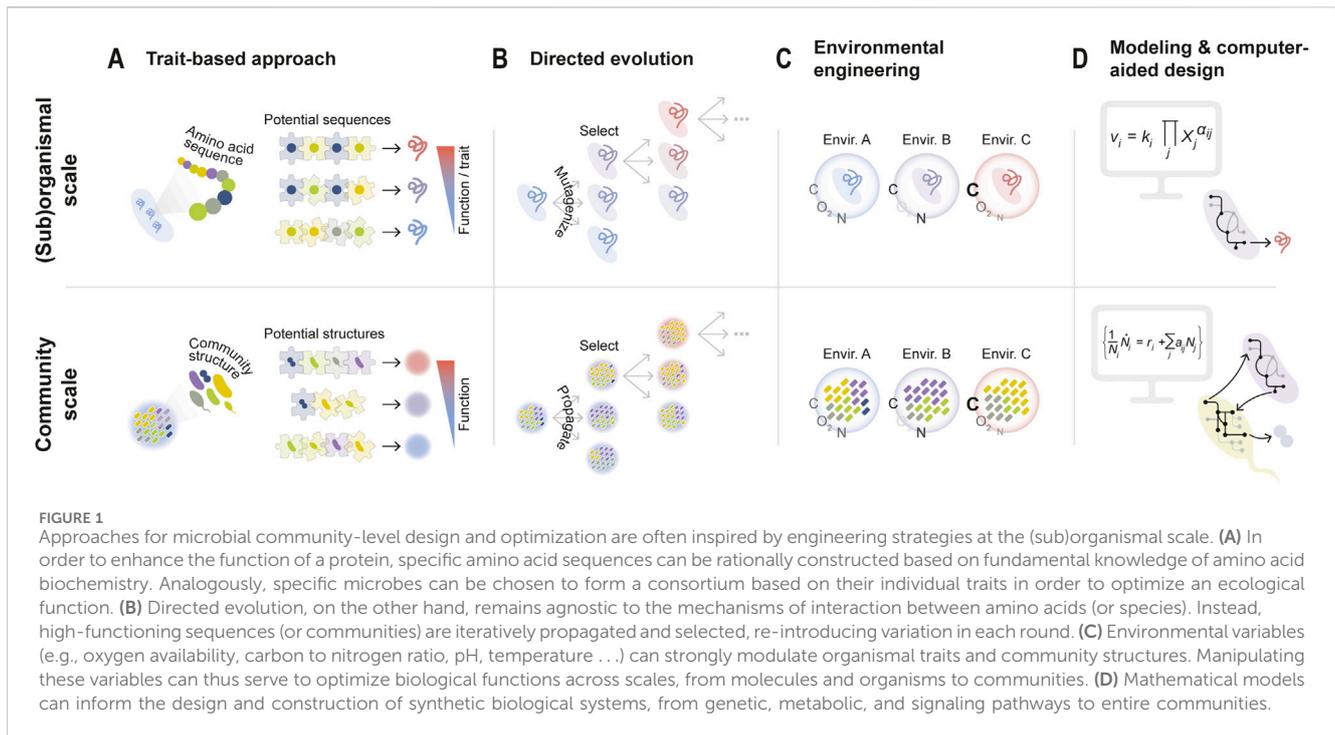
Here we review a variety of strategies which have been proposed for optimizing the functions of microbial communities, many of them inspired by synthetic biology at the level of organisms and subcellular units. These strategies range from bottom-up approaches, where defined sets of (typically few) species are combined into consortia with the aim of maximizing a function, to top-down approaches, where a community (which can be of high complexity and even of undefined composition) is manipulated through rational interventions (San León and Nogales, 2022). We also review methods based on mathematical modeling, including mechanistic models (most notably metabolic models) as well as more recent data-driven models. With this, we seek to provide an accessible overview of the rapidly-expanding field of synthetic ecology. We aim to prompt readers to carefully consider the extent to which the lessons learned from synthetic biology should guide our path towards community-level engineering.

2 Trait-based approaches

Perhaps the most commonly used strategy for community-level engineering has relied on the rational, bottom-up assembly of synthetic consortia. In short, based on some known traits of a set of microbial species/strains, a consortium is constructed with the aim of maximizing a target function, as well as (potentially) its ecological and evolutionary stability (Krause et al., 2014; Lajoie and Kembel, 2019; Li et al., 2024).

This trait-based approach is reminiscent of how early efforts at protein design were based on searching for optimal amino acid sequences starting from the basic principles of amino acid biochemistry (Song et al., 2023; Bryson et al., 1995) — i.e., the biochemical "traits" of each amino acid. Using biochemical reasoning to predict protein function from sequence can be seen as solving a "puzzle" by carefully examining each of the pieces, with each piece being a specific amino acid (Figure 1A, top panel). In the case of synthetic organisms, the pieces could be genes within an artificial plasmid, while for communities they would represent the different member species (Figure 1A, bottom panel).

The strategy of "solving the puzzle" has sometimes yielded good results. In some cases, consortia have been assembled by leveraging the natural capabilities of wild-type microbial species for performing specific tasks. For example, Park et al. used a two-species bacterial co-culture for the production of bioethanol by leveraging the natural ability of C. phytofermentans to hydrolyze cellulose and the potential of E. coli to ferment cellobiose catabolism byproducts into ethanol, respectively (Park et al., 2020). Alternatively, researchers have relied on the genetic manipulation of different member species/strains within a consortium. Examples include the construction of two-species/strain systems for the generation of photovoltaic energy (Zhu et al., 2019) or the production of resveratrol, a plant natural product (Camacho-Zaragoza et al., 2016). In this last example, two E. coli strains were engineered to express a complementary part of the resveratrol biosynthesis pathway. Genetic engineering has sometimes also served to enhance the stability of the community and facilitate the coexistence of its members, for instance through the imposition of obligate mutualisms (Pignon et al., 2024; Sgobba et al., 2018).

FIGURE 1
Approaches for microbial community-level design and optimization are often inspired by engineering strategies at the (sub)organismal scale. **(A)** In order to enhance the function of a protein, specific amino acid sequences can be rationally constructed based on fundamental knowledge of amino acid biochemistry. Analogously, specific microbes can be chosen to form a consortium based on their individual traits in order to optimize an ecological function. **(B)** Directed evolution, on the other hand, remains agnostic to the mechanisms of interaction between amino acids (or species). Instead, high-functioning sequences (or communities) are iteratively propagated and selected, re-introducing variation in each round. **(C)** Environmental variables (e.g., oxygen availability, carbon to nitrogen ratio, pH, temperature …) can strongly modulate organismal traits and community structures. Manipulating these variables can thus serve to optimize biological functions across scales, from molecules and organisms to communities. **(D)** Mathematical models can inform the design and construction of synthetic biological systems, from genetic, metabolic, and signaling pathways to entire communities.

Beyond these and other biotechnological applications, it is worth noting that synthetic communities have also been used to address more fundamental questions in microbial ecology, such as how microbial interactions determine the structure and dynamics of communities (Hu et al., 2022; Van Vliet et al., 2022; Cordero and Datta, 2016). Researchers have used synthetic consortia as laboratory model systems to mimic natural communities in the soil (Coker et al., 2022) or associated with animal and human hosts (Bonillo-Lopez et al., 2024; Clark et al., 2021), among other contexts. This is again reminiscent of how synthetic organisms have also served purposes beyond biotechnology, such as providing insights on cellular information processing and signal transduction (Gao et al., 2023; Hanson and Lorenzo, 2023).

Yet, for biotechnology, the trait-based construction of synthetic communities presents important challenges. First, the traits expressed by an organism often depend on its ecological context, that is, which other species (and potentially at which abundances) may be present (Diaz-Colunga et al., 2024a; Yang, 2021). In addition, even if the contribution of each individual microbial cell to a community function was constant, species' population sizes often vary differently across ecological contexts (Baichman-Kass et al., 2023; Sanchez et al., 2023), so the total functional contribution of a species may change depending on the presence/absence or abundance of other community members. Interactions between species can often present higher-order components, e.g., a third species may affect how two members of the community interact (Morin et al., 2022; Sanchez-Gorostiaga et al., 2019; Mickalide and Kuehn, 2019; Guo and Boedicker, 2016). In the "puzzle" analogy, it would be as if the shape of each piece changed every time we included a new one. Perhaps for this reason, the construction of synthetic consortia for biotechnology through trait-based approaches has been mostly limited to low-complexity communities, typically of two or three species/strains

(Park et al., 2024; Park et al., 2020; Zhu et al., 2019). This also owes to the fact that the rational assembly of microbial consortia in high-throughput remains an experimentally tedious process. The development of new experimental methodologies (Diaz-Colunga et al., 2024a), including based on microfluidic devices (Kehe et al., 2019; Kehe et al., 2021), promises to facilitate this process and potentially expand the bottom-up approach to more complex communities.

Much like in the case of synthetic organisms, communities engineered through this trait-based approach may be disrupted by evolution, environmental fluctuations, or the influx of invader species (Amor et al., 2020; Shibasaki and Mitri, 2020). Several strategies have been proposed for enhancing stability. For example, it has been shown that synthetic communities engaging in division of labor exhibit increased stability when not only the fitness costs, but also the benefits of expressing a function are allocated evenly across member species (Wang M. et al., 2022). Stability may also be achieved by rationally modulating intercellular interactions (Deter and Lu, 2022; Karkaria et al., 2021; Kong et al., 2018; Wu et al., 2024), e.g., interspecies metabolic cross-feeding (Li et al., 2022; Park et al., 2024; Peng et al., 2024; Ziesack et al., 2019), or by imposing a defined spatial structure to physically separate different subpopulations (Wang L. et al., 2022).

It is notable how these challenges (eco-evolutionary stability, scalability beyond low-complexity constructs, etc.) are similarly faced when engineering single organisms. This is a direct consequence of the trait-based design of microbial communities being very explicitly based on engineering principles from synthetic biology (Johns et al., 2016; San León and Nogales, 2022) — which becomes particularly evident in the case of consortia made up by genetically engineered strains (Park et al., 2024; Sgobba et al., 2018; Camacho-Zaragoza et al., 2016). In this case, the reasoning is straightforward: the target function to express is encoded in a

synthetic genetic circuit, but the burden of its expression is distributed across different strains (i.e., multiple "chassis") instead of assigned to a single one. While the basic idea is appealing, we must consider its practical limitations if the end goal is to develop microbial communities for biotechnological purposes which can be deployed on a large scale. Over the past decades, synthetic biologists have devoted much effort to addressing the challenges of engineering single organisms (e.g., Perrino et al., 2021; Müller et al., 2019; Slusarczyk et al., 2012), with only limited practical success (Hanson and Lorenzo, 2023; Zakeri and Carr, 2015). As the field of synthetic ecology develops, we must carefully assess the risk of falling into a similar stage of stagnation.

3 Community-level directed evolution

Artificial selection, most notably directed evolution, has been used for decades to improve the traits of individual organisms or the properties of subcellular units such as enzymes (e.g., Schmidt-Dannert and Arnold, 1999; You and Arnold, 1996) (Figure 1B, top panel). Directed evolution mitigates many of the limitations of rational design approaches, as it bypasses the need for a detailed understanding of the underlying mechanisms that may determine the function/trait of a molecule/organism. The basic strategy can be conceptualized as an iterative exploration of a genotype space in search of optimal phenotypes, e.g., an exploration of a sequence-function landscape in the context of protein engineering.

Artificial selection at the microbiome level can be similarly framed as a guided exploration of a community structure space (defined as the set of all possible community compositions in terms of species presence/absence or abundance) in search of states that optimize a target community-level function. Such mappings between community compositions and functions have received the name of *ecological landscapes* or *community-function landscapes* (George and Korolev, 2023; Sanchez et al., 2023; Skwara et al., 2023), by analogy with the concept of genetic fitness landscapes.

In its most general form, an exploration of a community-function landscape through directed evolution would involve (i) ranking a set of microbial communities based on a target community-level function, (ii) selecting the best-performing ones, and (iii) propagating them into a new set of "offspring" communities, which can be ranked and propagated again in subsequent rounds of selection (Figure 1B, bottom panel). Despite its conceptual simplicity, this premise was not directly tested until the early 2000s, when Swenson et al. attempted to select for microbial ecosystems which efficiently performed functions such as the degradation of 3-chloroaniline (Swenson et al., 2000a) or the promotion of plant growth (Swenson et al., 2000b). More recent efforts have aimed to select microbiomes with the ability to induce early or late flowering in plant hosts (Panke-Buisse et al., 2015), or to degrade environmental pollutants (Arias-Sánchez et al., 2024). Community-level artificial selection has been typically implemented as a top-down strategy, where communities are selected and propagated without necessarily dissecting their species-level composition (Chang et al., 2021; Chang et al., 2020; Swenson et al., 2000a; Swenson et al., 2000b; Xie et al., 2019; Panke-Buisse et al., 2015). More recently, bottom-up variations have also

been proposed, where the species-level composition of a community is well defined and experimentally manipulated in each round of selection (Arias-Sánchez et al., 2024; George and Korolev, 2023). While there exist promising studies of *in silico* community-level artificial selection (Chang et al., 2021; Lalejini et al., 2022; Xie et al., 2019), experiments have generally yielded only modest functional improvements, in some cases barely exceeding typical day-to-day fluctuations.

Selection (whether artificial or natural, at the level of organisms or groups) requires that there exists variation in the target trait/function, and that this variation can be passed from parents to offspring — i.e., that the trait is *heritable* to some extent (Lewontin, 1970). When organisms reproduce, they pass their genetic information to the next-generation, such that those phenotypes that are (at least partially) determined by the organism's genotype can exhibit some degree of heritability. The very same process can also reintroduce trait variation through mutation or recombination, upon which selection can further act.

Directed evolution of communities, however, entails important nuances with respect to that of single organisms (Arias-Sánchez et al., 2019; Blouin et al., 2015; Sánchez et al., 2021; Xie et al., 2019). Communities, unlike organisms, cannot naturally self-replicate, and thus generating an "offspring" community from a "parental" one requires the intervention of the experimenter. The first attempts at microbiome breeding addressed the issue of community-level reproduction by taking inspiration from earlier experiments of group-level selection in small animal populations [e.g., of chickens (Muir, 1996) or beetles (Wade, 1976; Wade, 1977)]. In short, offspring populations were initialized from a random sample of individuals from the highest-functioning parental ones.

Yet, this approach was less successful when applied to microbial communities (Swenson et al., 2000a; Swenson et al., 2000b). This may be explained, at least partially, by an effect of population size (Sánchez et al., 2021): If the number of individuals sampled from a parent population is very large, all offspring populations may end up being compositionally very similar to one another, as the effect of stochastic sampling becomes negligible (Blouin et al., 2015). This issue is typically more prominent in microbial communities than in animal populations, as the former often have very large population sizes (e.g., a single colony can contain millions of microbial cells). The loss of structural (and therefore functional) variation across communities can naturally obstruct further selection (Blouin et al., 2015; Chang et al., 2020). Other mechanisms could also lead to the exhaustion of variation or even to functional collapse: for instance, evolution of member species within a community could have such effects under certain conditions (Shibasaki and Mitri, 2020; Venkataram and Kryazhimskiy, 2023; Xie et al., 2019).

Several strategies have been proposed to tackle this issue. Structural variation can be externally re-introduced into the offspring communities, for instance through the co-inoculation of invader species or groups of species, through the application of harsh population bottlenecks (Chang et al., 2021; Sánchez et al., 2021), or through the propagation of not only the highest-function community but also of sub-optimal ones (Xie et al., 2019). It has also been suggested that selection schemes inspired by evolutionary computing could aid in maintaining functional variation across communities, leading to better selection outcomes (Arias-Sánchez et al., 2024; Lalejini et al., 2022).

Community-level artificial selection, in principle, could substantially boost our ability to engineer microbiomes, the same way it revolutionized our ability to engineer enzymes (Schmidt-Dannert and Arnold, 1999). There is extensive theoretical and empirical evidence showing that artificial selection can act above the organismal level (Doulcier et al., 2020; Goodnight, 1990a; Goodnight, 1990b; Lewontin, 1970; Wade, 1976; Wade, 1977), and thus it is not immediately obvious why experiments of microbiome-level breeding have found only modest success in general. It is possible that current strategies for community-level selection may be limited by methodological aspects, such as the choice of community-level reproduction method or the maintenance of functional variation across communities. It is also possible that there exist more fundamental factors which may intrinsically limit community-level artificial selection. These could include stochastic fluctuations in species abundances, or the rapid emergence of "cheater" strains, especially when the expression of the target function is costly (Blouin et al., 2015; Shibasaki and Mitri, 2020; Smith and Schuster, 2019).

It is important to note that targets of selection, artificial or natural, do often have fundamental limits at the organismal scale. These limits are evidenced, for instance, by the common observation of *diminishing returns* at the level of organismal fitness — i.e., beneficial mutations having smaller positive fitness effects in genetic backgrounds which are already well-adapted (Chou et al., 2011; Schoustra et al., 2016; Wünsche et al., 2017). Diminishing returns have also been recently observed at the level of community function (Diaz-Colunga et al., 2024b), suggesting that when a community function is high, most interventions will tend to disrupt it rather than improve it. This could pose an obstacle towards community-level artificial selection. Such limitations may become more pronounced when the individual members of a community benefit from the expression of the target ecological function — for instance, when the function is the clearance of a toxin (Arias-Sánchez et al., 2024). When such organismal-level pressures exist, the function may strongly dictate the composition of the community in a very deterministic manner, leading to reduced across-community functional variation. This would then constrain further optimization through selection.

As promising as community-level directed evolution may be, further work is necessary before it can become a viable option for biotechnological applications. Are there intrinsic limits to selection at the level of microbial communities? If not, why has directed evolution not yet been successfully applied to microbiomes? If the reasons are purely methodological, there are perhaps reasons for optimism, as *in silico* simulations have proven useful to inform the design of selection protocols (Chang et al., 2021; Lalejini et al., 2022; Xie and Shou, 2021). These methods, however, remain to be tested empirically.
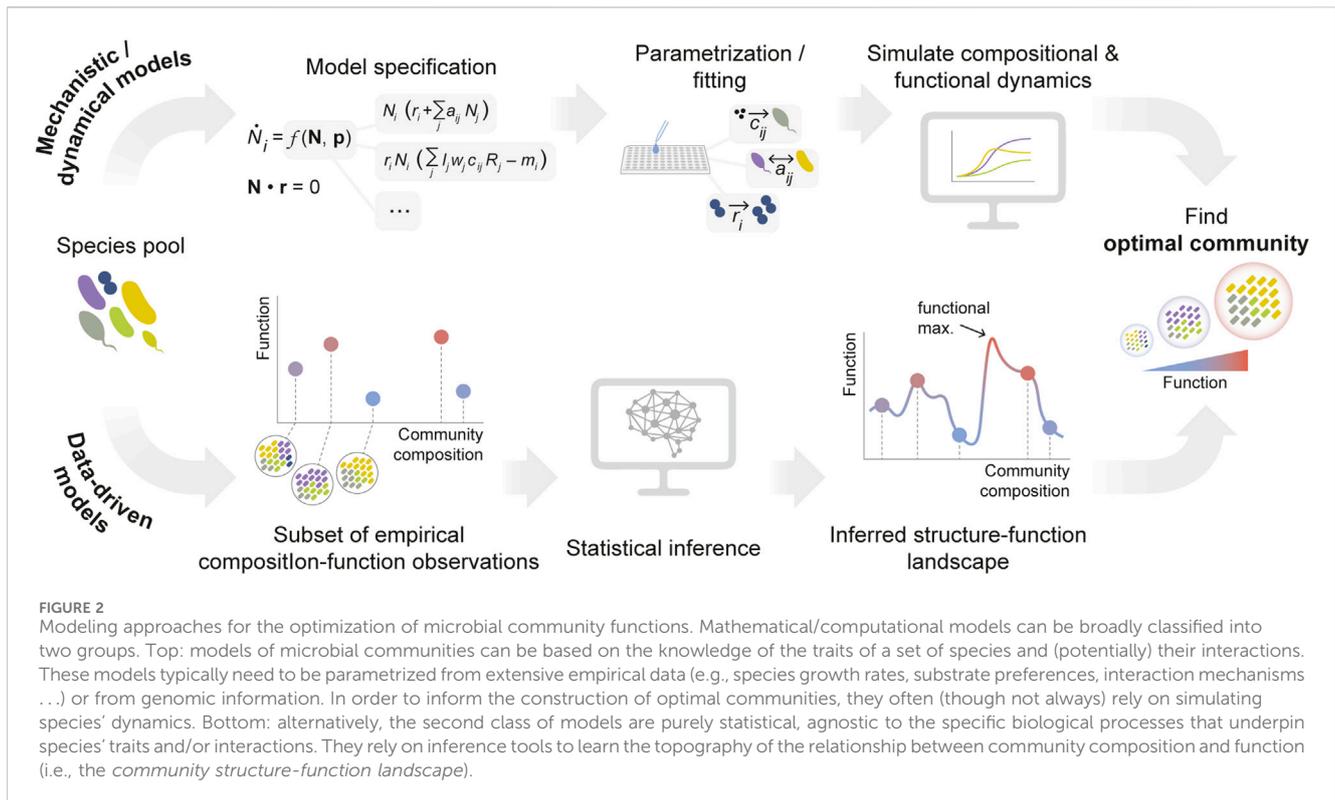
4 Environmental engineering

Most efforts for the optimization of microbial community functions have relied on manipulating its species/strain-level composition and/or the genetic architecture of community members. However, there is extensive evidence that abiotic environmental variables can strongly modulate the traits of individual microbes (Hu et al., 2021; Wasner et al., 2024; Yang, 2021), the interactions between species (Crocker et al., 2024; Ratzke and Gore, 2018), and therefore the dynamics, composition, and function of complex communities (Dal Bello et al., 2021; Estrela et al., 2021; Goldford et al., 2018; Hu et al., 2022; Silverstein et al., 2024; Sun et al., 2024). Thus, an alternative optimization strategy is to rationally engineer the environment that microorganisms inhabit (Sánchez et al., 2024; Silverstein et al., 2023; Silverstein et al., 2024) (Figure 1C).

One of the most paradigmatic examples of environmental engineering for microbial biotechnology is found in open fermentation systems (Li et al., 2014). In these, the premise is to manipulate a set of environmental conditions (e.g., pH, substrate availability, etc.) that will naturally select for microbial taxa which are able to carry out a desired function. The system is left unsterilized, open to the influx of environmental microbes, rather than inoculated with a specific set of strains previously chosen by the experimenter. This approach has been traditionally used in food and beverage fermentation, and has more recently been employed for applications such as the production of butanol from butyrate (Pinto et al., 2022) or the synthesis of enzymes (Qureshi et al., 2017) or lactic acid (Wang et al., 2016) from food waste.

The challenge of manipulating microbial environments lies in managing their highly multidimensional nature. Microbial growth and functional profiles may depend on a plethora of abiotic factors, including nutrient availability (Okano et al., 2019; Skonieczny and Yargeau, 2009; Zhu and Dai, 2024), temperature (Fu et al., 2022; Sun et al., 2024), pH (Pinto et al., 2022; Ratzke and Gore, 2018), the presence of antimicrobial compounds (Athamneh et al., 2014), or the spatial structure (or lack thereof) in their habitat (Pignon et al., 2024; Van Vliet et al., 2022). As an additional complication, the effect of these factors can often be highly non-additive. A paradigmatic example of this non-linearity is the observation that different antibiotics can act synergistically in combination (Cacace et al., 2023; Lázár et al., 2022; Yeh et al., 2006), or modify microbial responses to other environmental variables such as temperature (Cruz-Loya et al., 2021).

Ultimately, these challenges are similar to those faced by synthetic biologists when engineering single organisms (or communities) from the bottom-up. Interactions between environmental components are, at least conceptually, reminiscent of the interactions that exist between genes within an organism (or between member species within a community). These interaction networks can be very complex and of high dimensionality in all of these cases. Owing to this analogy, strategies for environmental design have drawn inspiration from the bioengineering of organisms or subcellular components (Sánchez et al., 2024). For example, genetic algorithms have been used to select optimal environments. In these cases, a set of environmental variables (pH, salinity, concentration of vitamins and minerals, etc.) were manipulated in each round of selection, and the best environments were propagated into subsequent generations. Vandecasteele et al. followed this principle to identify environments where a microbial community maximized the degradation of a synthetic dye (Vandecasteele et al., 2008). In another example, Kucharzyk et al. followed a similar approach to optimize the degradation of perchlorate, both when the function was performed by a single

FIGURE 2
Modeling approaches for the optimization of microbial community functions. Mathematical/computational models can be broadly classified into two groups. Top: models of microbial communities can be based on the knowledge of the traits of a set of species and (potentially) their interactions. These models typically need to be parametrized from extensive empirical data (e.g., species growth rates, substrate preferences, interaction mechanisms . . .) or from genomic information. In order to inform the construction of optimal communities, they often (though not always) rely on simulating species' dynamics. Bottom: alternatively, the second class of models are purely statistical, agnostic to the specific biological processes that underpin species' traits and/or interactions. They rely on inference tools to learn the topography of the relationship between community composition and function (i.e., the *community structure-function landscape*).

strain of *Dechlorosoma* sp. or by a complex microbial community (Kucharzyk et al., 2012).

Modeling tools can also be used to inform environmental design strategies. For example, Pacheco and Segrè developed a computational method combining metabolic modeling and genetic algorithms to find optimal environmental compositions for target community functions (Pacheco and Segrè, 2021). Mathematical models of community dynamics that are extensively used by microbial ecologists may explicitly incorporate environmental variables, e.g., the secretion of metabolic byproducts to the environment is specifically included in microbial consumer-resource models (Marsland et al., 2020b; Marsland et al., 2020a) or in some dynamic community-level metabolic models (Dukovski et al., 2021). These could be useful to inform the construction of environments which optimize microbial community functions such as the efficiency of substrate utilization or the production of specific secondary metabolites.

Data-driven models have also been developed which aim to infer optimal environmental compositions from partial observations (Chen et al., 2009; Jiménez et al., 2014; Kikot et al., 2010; Skonieczny and Yargeau, 2009; Zhou et al., 2023). These methods have the advantage of not requiring information on the specific biological mechanisms which may drive microbial responses to their environment. Yet, the typically vast number of potential environmental factors makes it so these models often must be trained with very limited empirical data. They have thus generally taken very simple forms, e.g., linear regressions with the variables being the effects of each environmental factor, at most incorporating pairwise interactions between environmental

components (Jiménez et al., 2014; Kikot et al., 2010). In practice, this has limited their application to relatively simple settings including few environmental components. As is the case with the bottom-up assembly of communities, recent methodological advances could facilitate the construction of environments in high throughput (Diaz-Colunga et al., 2024a; Sánchez et al., 2024) and the expansion of the scope of these models.

5 Modeling and computer-aided design

Mathematical and computational models have been widely used by microbial ecologists to address questions such as inferring species interactions from co-ocurrence networks (Faust and Raes, 2012), assessing microbial coexistence and community stability (Akjouj et al., 2024), explaining the emergence of community-level properties from complex interactions between species (Van Den Berg et al., 2022), or reproducing the relationships that exist between biodiversity and function within microbial communities (Marsland et al., 2020b). In the context of biotechnology, modeling has served to guide the construction of microbial consortia that efficiently deliver target functions (Figure 1D), such as the production of relevant metabolites (e.g., Jones et al., 2016; Clark et al., 2021).

Models can be cataloged according to different criteria (Van Den Berg et al., 2022). Here, for the sake of simplicity, we broadly classify them into two categories: (1) models based on species' traits and interaction mechanisms, and (2) models which leverage statistical features of microbial communities to reproduce and predict

community-level properties while remaining mechanism-agnostic (Figure 2).

5.1 Mechanistic models

Mechanistic, trait-based models often (though not always) represent the dynamics of the community, and can be expressed in the generic form

$$\frac{dN_i}{dt} = f\left(\mathbf{N}, \mathbf{p}\right) \qquad (1)$$

Where f is a function of some set of parameters (denoted as $\mathbf{p}$), and of a vector $\mathbf{N}$ which represents species abundances, with its i-th element (N_i) being the abundance of species i. Out of the models of the form in Equation 1, generalized Lotka-Volterra (gLV) models are arguably the most widely used, where the parameters would be the species' growth rates and the pairwise species-by-species effects on them. These models have successfully informed the construction of microbial communities for specific functions, such as the retrieval of heavy metals (Zheng and Li, 2016), the production of antibacterial food additives (Giménez-Palomares et al., 2022), or the induction of host immune responses (Stein et al., 2018). It is important to notice, however, that even when a gLV model may be able to accurately reproduce and predict species abundances, the function of the community would remain unknown unless the *per capita* contribution of each species was constant, that is, unless the total functional contribution of every species was proportional to its population size:

$$F = \sum_i \phi_i N_i \qquad (2)$$

With ϕ_i being the (constant and known) *per capita* contribution of species i (with a population size N_i) to the community function F. This is, however, very frequently not the case. Characterizing species traits in isolation or interactions in pairwise co-cultures may often not be sufficient for the models to reproduce how species will behave in a more complex community (e.g., the per capita contributions in Equation 2 may not be independent of community context) (Diaz-Colunga et al., 2024a; Guo and Boedicker, 2016; Mickalide and Kuehn, 2019; Morin et al., 2022; Sanchez-Gorostiaga et al., 2019), a limitation shared with the empirical trait-based approaches we discussed previously.

Alternatively, the dynamics of the function to optimize can be explicitly incorporated into the model. This can be the case in microbial consumer-resource models (mCRM) (Marsland et al., 2020b; Marsland et al., 2020a), which explicitly model the dynamics of metabolite exchange between community members. For this reason, mCRMs can inform community design when the function of interest is the production of specific secondary metabolites or the rate of utilization of a substrate (Gowda et al., 2022; Van Den Berg et al., 2022). Still, using this type of models for biotechnology generally requires exhaustive characterization and quantification of species traits [although in some cases these may be inferred from genomic information (Gowda et al., 2022)] and/or their interactions.

An alternative modeling approach based on species-specific traits is metabolic modeling. Unlike gLV and mCRM models,

metabolic modeling is not typically used to predict temporal changes in species abundances (although it can be adapted for this purpose, as discussed below). Instead, it leverages stoichiometric data inferred from species genomes and applies optimization criteria to predict metabolic fluxes. Given its extensive use in biotechnology, we describe this method in more detail in the following section.

5.1.1 Metabolic models

Within the field of microbial biotechnology, arguably the most prominent class of mechanistic models are metabolic models. Metabolic modeling has become a cornerstone of systems biology, enabling researchers to simulate cellular metabolism, predict phenotypes, and guide metabolic engineering. Initially, metabolic modeling was utilized to investigate clonal populations (Varma and Palsson, 1994). For this, species-specific genome-scale metabolic models (GEMs) (Gu et al., 2019; Mendoza et al., 2019) together with Flux Balance Analysis (FBA) (Orth et al., 2010b) and related graph- and constraint-based techniques were used. The use of metabolic modeling to study clonal populations resulted in successful predictions of growth, secretion profiles (Jouhten et al., 2022; Neal et al., 2024; O'Brien et al., 2013), and the impact of genetic modifications (Edwards and Palsson, 2000; Segrè et al., 2002; Shlomi et al., 2005), making it invaluable for applications in bioproduction and strain engineering (Blazeck and Alper, 2010; Jiang et al., 2022) that considered isolated species.

In recent years, the use of metabolic modeling has expanded from individual species to microbial communities, whether they are small synthetic consortia or large natural microbiomes (Giordano et al., 2024; Machado et al., 2021; Zelezniak et al., 2015). This expansion has been made possible due to the development of tools that support the rapid reconstruction of GEMs (Heinken et al., 2023) and the adaptation of existing analytical methods as well as the development of new methods for studying communities instead of clonal populations (Chan et al., 2017; Diener et al., 2020; Heinken and Thiele, 2022; Khandelwal et al., 2013; Stolyar et al., 2007; Zomorrodi and Maranas, 2012). Additionally, the increasing development of easy-to-use software packages that implement complex metabolic modeling methods (Belcour et al., 2020; Dukovski et al., 2021; Ebrahim et al., 2013; Frioux et al., 2018; García-Jiménez et al., 2018; Zelezniak et al., 2015) has expanded the use of this approach, allowing non-experts to leverage these techniques for their own interests — ranging from studying the ecology of microbes to bioremediation, bioenergy production or personalized medicine.

Metabolic modeling relies on species-specific GEMs. These represent all known metabolites, metabolic genes, and reactions within a given organism. The process of reconstructing GEMs begins with draft model reconstruction. Using annotated genomes, metabolic genes and reactions are predicted to generate an initial draft. This draft model is then refined to improve accuracy in reproducing experimental data (Orth et al., 2010a; Mendoza et al., 2019). With recent software tools (Aite et al., 2018; Arkin et al., 2018; Karlsen et al., 2018; Machado et al., 2018; Olivier, 2018; Wang et al., 2018), high-quality draft models can now be created in minutes, expanding the application of metabolic modeling beyond a limited set of well-characterized, culturable species to include uncultured or lesser-known organisms as well.

Besides GEMs, metabolic modeling leverages methods for their analysis. Typically, these methods are categorized as graph-based and constraint-based. Adapting graph-based methods to investigate communities instead of clonal population is straightforward. On top of this, graph-based simulations are computationally efficient, enabling scalability and the analysis of large, complex microbial communities (Belcour et al., 2020). However, the main limitation of this approach is the loss of stoichiometric information which results in reduced output information and prediction accuracy.

As an alternative, constraint-based methods such as FBA, rely on species' stoichiometric information. These methods rely on an optimization to find the flux through every reaction in the metabolic model which satisfies the stoichiometric and thermodynamic constraints imposed. This sets the main limitation of using metabolic modeling to investigate communities. While the optimization criteria when simulating clonal populations is straightforward - to maximize growth rate, it is far less evident when simulating communities where positive and negative interactions between community members determine total community biomass. To overcome this limitation, different methods have been developed (see for example Diener et al., 2020; Zomorrodi and Maranas, 2012) which account for potential trade-offs between species and community growth rate, improving predictions at community level.

In addition to graph and constraint-based methods, dynamic FBA (dFBA) was developed (Mahadevan et al., 2002) and soon adapted to investigate communities (Chiu et al., 2014; Hanly et al., 2012; Hanly and Henson, 2011; Salimi et al., 2010; Tzamali et al., 2011; Zhuang et al., 2011). dFBA combines FBA with ordinary differential equations to capture both environmental and growth dynamics. In essence, dFBA involves simulating a series of consecutive FBA calculations, updating species abundance and nutrient availability between simulations. This method has been further developed to model not only temporal changes but also spatial distributions of microbial populations (Bauer et al., 2017; Harcombe et al., 2014).

The application of metabolic modeling to microbial communities has opened new avenues for community design and optimization, with significant implications for industrial and environmental applications. For example, graph-based methods can be used to identify combinations of species that achieve a specific metabolic goal, such as the production of a valuable compound, by mapping metabolic pathways across community members (Belcour et al., 2020; Eng and Borenstein, 2016; Frioux et al., 2018; Julien-Laferrière et al., 2016). Constraint-based approaches, adapted for community-level analysis, enable researchers to optimize community composition and environmental conditions to enhance desired outputs (Benito-Vaquerizo et al., 2020; Jouhten et al., 2022), such as biofuel and bioplastic production, nutrient cycling, or pollutant degradation. Dynamic Flux Balance Analysis (dFBA) further extends these capabilities by incorporating temporal changes in nutrient availability and species abundance, allowing researchers to model how community function evolves over time.

Metabolic modeling is thus transforming the field of microbial community design, providing a framework for systematically engineering communities with tailored functions. Through *in silico* simulations, researchers can test multiple community configurations, explore various environmental conditions, and fine-tune community composition to achieve optimal performance.

5.2 Data-driven models and machine learning

Ultimately, the task of identifying optimal communities relies on our ability to accurately predict ecological function from composition — that is, to learn the topography of the *community structure-function* landscape (George and Korolev, 2023; Sanchez et al., 2023; Skwara et al., 2023). In the most general form, such a structure-function landscape can be expressed as a transformation (which we denote as Γ) from a compositional space, containing all potential community structures, to a scalar function F, as:

$$F = \Gamma\left(\{x_i\}\right) \qquad (3)$$

With x_i representing the compositional information of species i, where the values that this variable can take depend on how the landscape is defined (e.g., $x_i = N_i$ if x represents total population sizes, $x_i = N_i/\sum_j N_j$ if it represents relative abundances, $x_i = 1, 0$ if it represents species presence/absence, etc.). The basic premise of data-driven methods is to infer the form of the relationship between composition and function (that is, the form of Γ in Equation 3) based on some subset of empirical observations (Figure 2, bottom).

There exist many general methodologies designed for this task, with applications beyond microbial biotechnology. Perhaps some of the simplest examples are linear regression algorithms (Maulud and Abdulazeez, 2020), where the function F is expanded as

$$F = \beta_0 + \sum_i \beta_i x_i + \sum_i \sum_{j>i} \beta_{ij} x_i x_j + \sum_i \sum_{j>i} \sum_{k>j>i} \beta_{ijk} x_i x_j x_k + \dots \quad (4)$$

And the coefficients in Equation 4 (β) are estimated via linear regression. This family of models has been used by microbial ecologists to reproduce empirical biodiversity-function patterns (Kirwan et al., 2009), and has more recently been applied to synthetic consortia (Clark et al., 2021; Skwara et al., 2023). In order to make it possible to train these models on incomplete data, they have been typically truncated at low orders, e.g., including only first-order effects ($\beta_i x_i$) or up to second-order interactions ($\beta_{ij} x_i x_j$). Note that, in this context, an "interaction" is defined in a statistical sense, as the combined effect that two (or more) species may have on the community function F. This definition is therefore agnostic to the specific biological mechanisms that may drive the interaction (be it resource competition, metabolic cross-feeding, or others), a notable difference with respect to mechanistic models. Thanks to this flexibility, these methods have been successfully applied across a wide range of communities and functions (Skwara et al., 2023), and they have also served to model interactions between other agents, such as environmental components (Connors et al., 2023; Jiménez et al., 2014; Kikot et al., 2010). In addition to their predictive power, linear regression models have been proven able to identify functional groups within microbial communities (Zhao et al., 2024).

Other data-driven methods, such as random forests, Bayesian inference approaches, or neural networks, have been often used to analyze the taxonomic structure, interactions, and dynamics of

microbial communities (Baig et al., 2023; Bauer et al., 2017; DiMucci et al., 2018; Shafiei et al., 2014; Shafiei et al., 2015; Statnikov et al., 2013; Walsh et al., 2024). Yet, their use for predicting and optimizing biotechnologically relevant functions has remained more limited. A few notable exceptions include the use of random forests to predict the ability of soil microbiomes for decomposing plant litter (Thompson et al., 2019), the design of gut microbial consortia for the production of butyrate through a combination of linear regression, Bayesian inference, and gLV modeling (Clark et al., 2021), or the use of Bayesian optimization together with recurrent neural networks to predict and optimize species abundances and metabolite concentrations (Thompson et al., 2023).

If we once again turn our attention to the organismal scale and below, we find a much broader variety of data-driven methods which have been used to infer the relationship between structure and function, including in the context of protein sequence-structure-function landscapes (e.g., Barrio-Hernandez et al., 2023; Otwinowski, 2018; Romero et al., 2013) and organismal genotype-phenotype maps (e.g., Sailer et al., 2020; Tareen et al., 2022; Tonner et al., 2022). Perhaps the most notable recent example is the development of AlphaFold, a deep learning-based method for predicting protein structure from sequence (Jumper et al., 2021). These methods bypass the need for *a priori* knowledge on the mechanisms of interaction between genetic components or amino acids, allowing for the optimization of biological function much further beyond what rational design strategies can achieve.

Can we apply similar principles at the ecological scale? Microbial community functions emerge from interactions between species, similar to how protein function emerges from biochemical interactions between amino acids. The mechanistic basis underpinning interactions at these two scales is in principle very different. However, recent research has shown that the interactions between species within a community often follow similar statistical patterns than those between genetic components (Diaz-Colunga et al., 2024b; Eble et al., 2023; Gould et al., 2018; Morris et al., 2020; Sanchez-Gorostiaga et al., 2019). Furthermore, microbial communities may in many cases be well represented by low-dimensional statistical models with few parameters (Arya et al., 2023; Arya et al., 2024; Skwara et al., 2023; Zhao et al., 2024) — that is, despite the underlying complexity of species interactions, there often appears to be an *emergent simplicity* at the level of community function (Bergelson et al., 2021; Goldford et al., 2018). This strongly suggests that models akin to those used to predict function from structure at the scale of genes and proteins could also fare well for predicting microbial community function from composition, even when trained on very sparse data. Still, the application of such methodologies for the optimization of microbial community functions remains to be tested in practice.

6 Discussion and outlook

Throughout this review, we have discussed the parallels that exist between the engineering of biological systems at the ecological scale and below. It is clear that synthetic biology tools have had an enormous influence on the development of strategies for the design and optimization of community-level microbial functions. In particular, a very common strategy has been to assemble

synthetic consortia from the bottom-up, building on the available phenotypic information of the constituent parts (i.e., the species or strains). This approach has sometimes been informed by mechanistic models of ecological interactions, and/or relied on the genetic engineering of community members. While this is naturally a reasonable starting point when attempting to engineer microbiomes, it is also important to acknowledge its limitations.

Communities, like all biological entities, are complex systems, and thus their properties and functions are often difficult to explain simply from those of their parts. Applying engineering principles to their design is therefore nuanced, as in doing so there is an underlying assumption of modularity and scalability that may often not hold. This, of course, also applies at the organismal scale and below: a single cell is a very complex system in itself, and thus combining different organism types into a community means building complexity on top of complexity. Synthetic biology has arguably not yet fulfilled its promise of developing practical solutions for biotechnology on a large scale. It is thus imperative that we ask whether applying similar principles at the level of communities will yield better results, or if, on the other hand, there are fundamental limitations to this approach when it comes to biological systems.

Top-down strategies, as well as data-driven modeling, could in principle be more suitable for the task of prediction and optimization. Their main advantage lies in the fact that these methods do not rely on mechanistic information. For example, community-level directed evolution could be implemented without even characterizing the composition of the community nor the interactions between its member species. In practice, however, this strategy has only yielded modest results, and further work is necessary to identify the factors which may have limited its success. Data-driven models have been used extensively for predicting biological function below the ecological scale, but more rarely for optimizing microbial community functions. Their main limitation is the difficulty in extracting relevant biological insights from them, as these models often tend to operate as "black boxes". In any case, this may be a lesser consideration if our primary focus is to optimize biotechnological processes.

The variety of available strategies for community-level engineering underscore the importance of choosing the appropriate approach if we wish to develop practical, viable, and sustainable solutions for open challenges in biotechnology. Taking inspiration in other areas of biology beyond microbial ecology can be fruitful, but we must carefully consider the limitations we may face. Synthetic ecology emerged as an extension to synthetic biology that promised to alleviate the limitations of the latter, in particular with respect to scalability and robustness. Yet, the rational design of microbial consortia has faced similar obstacles, perhaps because it has been approached using similar bottom-up thinking. As the field of synthetic ecology develops, it will be important to devise new optimization strategies that embrace and deal with the underlying complexity of microbial communities — and of biological systems at all scales.

Author contributions

MS: Investigation, Methodology, Writing–original draft, Writing–review and editing. AA: Investigation, Methodology, Writing–original draft, Writing–review and editing. BB-D: Investigation, Methodology, Writing–original draft,

Writing–review and editing. IQ-R: Investigation, Methodology, Writing–original draft, Writing–review and editing. JD-C: Funding acquisition, Investigation, Methodology, Supervision, Visualization, Writing–original draft, Writing–review and editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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