The optimization of microbial community functions through rational environmental manipulations.

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Abstract

Microbial communities are gaining ground in biotechnology, as they offer many advantages over single-organism monocultures. To make microbial communities competitive as a biotechnological platform, it is essential that we develop strategies to engineering and optimizing their functionality. To this end, most efforts have focused on genetic manipulations. An alternative and also very promising strategy is to optimize the function of microbial communities by rationally engineering their environment and culture conditions. A major challenge is that the combinatorial space of environmental factors is enormous. Furthermore, environmental factors such as temperature, pH, nutrient composition, etc., generally combine their effects in complex, non-additive ways. In this piece, we overview the origins and consequences of these "interactions" between environmental factors, and discuss how they have been built into statistical models of microbial community function to identify optimal environmental conditions. We also overview alternative "top-down" approaches, such as genetic algorithms, to finding combinations of environmental factors that optimize the function of microbial consortia. By providing a brief summary of the state of this field, we hope to stimulate the development of novel methodologies to rationally manipulate and optimize microbial communities through their environment.

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Abstract. Microbial communities are gaining ground in biotechnology, as they offer many advantages over single-organism monocultures. To make microbial communities competitive as a biotechnological platform, it is essential that we develop strategies to engineering and optimizing their functionality. To this end, most efforts have focused on genetic manipulations. An alternative and also very promising strategy is to optimize the function of microbial communities by rationally engineering their environment and culture conditions. A major challenge is that the combinatorial space of environmental factors is enormous. Furthermore, environmental factors such as temperature, pH, nutrient composition, etc., generally combine their effects in complex, non-additive ways. In this piece, we overview the origins and consequences of these "interactions" between environmental factors, and discuss how they have been built into statistical models of microbial community function to identify optimal environmental conditions. We also overview alternative "top-down" approaches, such as genetic algorithms, to finding combinations of environmental factors that optimize the

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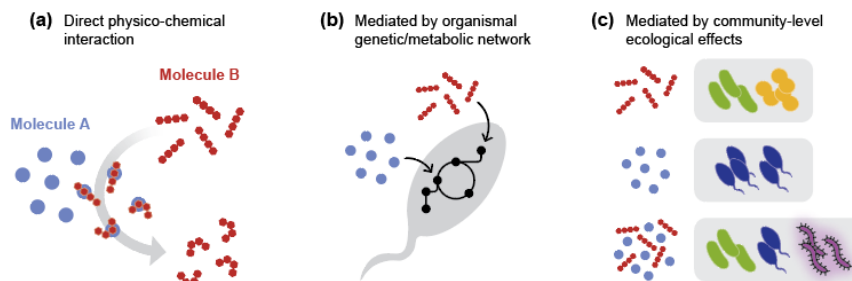
Introduction

Synthetic biology is gaining ground as a means to engineer and optimize microorganisms for desirable biotechnological applications, from the production of high-value chemicals to bioremediation (Voigt, 2020). A major focus of this field has been the genetic manipulation of microbial cells and the communities they form, through the introduction of synthetic genetic circuits and metabolic pathways (McCarty and Ledesma-Amaro, 2019; San León and Nogales, 2022). Somewhat less attention has been paid to the abiotic environment on which microorganisms grow, yet the environment is a key element that controls the traits and properties of microorganisms, and one that can be exploited to optimize microbial functions in a straightforward manner (Eng and Borenstein, 2019).

A main challenge for engineering the abiotic environment is its highly multidimensional nature, which includes physical (Temperature, volume, flow rate) and biochemical (pH, concentration of all molecular species) factors. In general, the effect of these different environmental factors on the behavior of microbial cultures is complex and non-additive. Microbiologists have long known that the fitness and phenotypic effects of a particular environmental factor will differ in various environmental contexts. To provide just one iconic example, Monod famously showed that the effect of lactose on the growth rate of *E. coli* is initially negligible when glucose is also present, despite its strong growth-promoting effects when glucose is absent (Monod, 1942). Glucose and lactose do not combine additively, as the former masks the effect of the latter.

In situations like the one above, when the effect of a particular environmental axis on microbial function is modulated by other environmental axes, we can say that environmental factors "interact". This definition of an "interaction" between two environmental factors may seem to be unusual. After all, glucose and lactose do not chemically affect one another. Yet, it is in fact the same definition of interactions (epistasis) that is used in genetics to describe the analogous situation where the fitness effect of a mutation is altered by the presence of another (Sanchez, 2019).

Of course, these environmental interactions have their basis in concrete molecular mechanisms. Interactions between two environmental factors may have a direct origin, for instance when an extracellular molecule chemically modifies another (Fig. 1a). Alternatively, interactions between environmental factors may be indirect and mediated by the changes they induce in the state of the genetic network of a cell, as was the case for the glucose-lactose interaction described above (Fig. 1b). In the case of microbial consortia, an environmental change may affect collective community functions through a variety of additional mechanisms. For instance, the presence of an extracellular molecule may inhibit or stimulate the growth or the gene expression patterns of one member species, thus influencing its ability to carry out the role it plays in the community (Fig. 1c).

Figure 1: Examples of interactions between environmental factors. We represent two factors as two different environmental molecules, A and B. These two environmental factorsinteract if these have a non-

additive effect on the behavior of microbial cultures. Diverse mechanisms can give rise to interactions, for instance,(a) they can be direct due to physico-chemical modifications, such as through a chemical reaction between them. They may also have an indirect origin, for instance (b) when their combined effect on population attributes is mediated by the genetic or metabolic network of an organism. Their origin may also be ecological (c) mediated by ecological effects between member species of a community, such as when the combination of two different nutrients leads to community compositions that differ from those of the sum of the monocultures.

Given their potential complexity, interactions between environmental axes may substantially complicate the task of identifying culture conditions that will optimize microbial functions, and thus may be seen as a detriment to engineering the environment in a rational manner. Despite this complication, past work in microbial biotechnology has found ways to deal with interactions and has met success at finding environmental manipulations that will move a target function to more desirable values, both in monocultures and in microbial consortia.

In the following, we will overview the current state of these efforts. We will begin by briefly overviewing recent work on disentangling the effect of combinatorial interactions between environmental factors in both monocultures and consortia. We will then overview the current state of the field in exploring combinatorial spaces of environmental factors, in search for environments that optimize the function of monocultures and consortia. We finish with an outlook where we propose potential future directions that may help move the field forward.

Interactions between environmental factors shape the fitness and traits of individual microorganisms

Environmental factors often combine non additively to shape the phenotypes and fitness of microorganisms (Kaplan et al., 2008; Okano et al., 2020; Wood et al., 2012). Interactions between environmental factors on microbial growth and phenotypes have been a major focus of microbiology since the early days of the discipline (Blaiseau and Holmes, 2021). Among the most iconic examples we find diauxic growth, whereby microorganisms consume nutrients in succession, rather than simultaneously. The effect of a nutrient on the growth of a microorganism will thus be masked by the presence of another nutrient whose hierarchy in the diauxic choice is higher (Aidelberg et al., 2014; Bajic and Sanchez, 2020). This interaction between nutrients is akin to the phenomenon of dominance in genetics, an interaction whose result is the masking of the phenotypic effect of an allele by the presence of another (dominant) allele in a diploid organism.

Diauxic growth is implemented by genetic networks that respond to the presence of more than one input nutrient by activating the metabolic pathways required to metabolize just one of them, but not the others. Beyond the specific example of diauxie, many microbial gene-regulatory networks integrate multiple environmental inputs (Espinar et al., 2013; Kaplan et al., 2008), and bacterial promoters commonly contain binding sites for multiple different transcription factors (Bintu et al., 2005; Rydenfelt et al., 2014). The combinatorial nature of gene regulatory networks and their ability to integrate multiple environmental signals provides a basis for the emergence of complex interactions between environmental factors.

Interactions between environmental factors do not need to have a gene regulatory basis. An example are the well-characterized interactions between antibiotics. It has been shown that antibiotics targeting different cellular processes often act synergistically (Cacace et al., 2023; Yeh et al., 2006), whereby the detrimental effect of an antibiotic is higher than expected when a second antibiotic is present too (Yeh et al., 2009). Systematic screens of interactions between antibiotics have found that they are pervasive in both Gram positive and Gram negative bacterial species (Wood et al., 2012), and they can exhibit substantial complexity (Lázár et al., 2022). Antibiotics can also interact with physical environmental factors, and their very presence in the medium can alter the optimal growth temperature of bacteria, and modulate the effect of stressful temperatures (Cruz-Loya et al., 2021). The idea that two or more stressors may act non-additively is an old concept in microbial ecology (for a recent example see (Smith et al., 2023)), which in fact extends well beyond the realm of microorganisms (Côté et al., 2016).

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Interactions between environmental factors may affect biotechnologically significant traits. For instance, Skonieczny & Yargeau examined the combined effect of the initial pH and initial glucose concentration on the rate of H_2 produced by populations of the bacterium *Clostridium beijerinckii* (Skonieczny and Yargeau, 2009). At low initial glucose concentrations, H_2 production peaked at pH=6.1. Yet, when higher initial concentrations of glucose were provided, the peak shifted to higher values of pH, close to 6.5. In other words, an increase in glucose concentration changes the response of H_2 production to pH, and this affects the optimal pH in the culture. This particular case exemplifies the potential significance of interactions between environmental factors in biotechnology, and we did not have to dig deeply to find it. Most environmental factors exhibit a non-additive effect even when they are the only ones being changed. Take for instance the example of Temperature. Microbial Temperature response curves are not only non-linear but also non-monotonic. A 2°C increase in Temperature will have a positive effect in the carbon use efficiency of microorganisms below their optimal temperature (Smith et al., 2021), but a detrimental effect when it takes place above that point. This well-known example illustrates the many challenges of predicting the effect of the environment on organismal traits, a challenge that extends to the community level.

Interactions between environmental factors shape the composition and function of microbial communities

Environmental factors often combine non additively to shape the functions and taxonomic composition of microbial communities and the traits and phenotypes of microbial populations (Dal Bello et al., 2021; Estrela et al., 2021a, 2021b; Fonte et al., 2013; Lázár et al., 2022; Pacheco et al., 2021; Pacheco and Segrè, 2021; Replansky and Bell, 2009; Smith et al., 2023; Wood et al., 2012). In what follows, we provide a few recent examples of environmental interactions in the context of community composition and function, with the aim of illustrating this point.

Nutrients interact non additively to determine community composition. Microbial communities assemble in complex environments that contain multiple resources. These resources can have different effects in different members of the community: a molecule that is discarded by a microorganism is often useful for others (D'Souza et al., 2018; Goldford et al., 2018; Mataigne et al., 2021). In a recent study (Estrela et al., 2021b), we have systematically investigated how nutrients combine to shape the taxonomic composition of microbial communities. Through an enrichment approach from soil and plant communities (described in detail elsewhere (Estrela et al., 2021a)), we studied the taxonomic composition of stable, serially passaged communities in media containing various sets of two growth limiting nutrients, and compared these with the community compositions that formed in each nutrient separately. We found that interactions between nutrients were often strong. Increasing the number of supplied resources from one to two or even three had no effect on the total diversity of our enrichment communities (Estrela et al., 2021a), and this was often due to an effect we termed "nutrient dominance", where one of the two nutrients masked the ability of the other to recruit taxa into the community (Estrela et al., 2021b). For example, the taxonomic composition of enrichment communities where both glycerol and succinate were supplied externally as the only carbon sources were nearly indistinguishable from the composition of the communities assembled in glycerol alone. The majority of the taxa that were coexisting stably when succinate was the only supplied nutrient were excluded from the community when glycerol was also supplied in the culture medium at the beginning of every passage (Estrela et al., 2021b).

These results echo similar findings from other groups. Pacheco and Segre (Pacheco et al., 2021) found strong non-additive interactions between nutrients in serially passaged, defined communities, and Dal Bello et al (Dal Bello et al., 2021) found that community richness increased very weakly as we increase the number of supplied resources, barely doubling as we went from 1 to 32. Together with older evidence (Replansky and Bell, 2009) these studies support the idea that nutrients often interact non additively in their effect on community composition. It stands to reason that such non-additivity will extend to function also.

Environmental variables interact non additively to determine community functions. In addition to their non-additive effect on the taxonomic composition of a community, the effect of two or more environmental variables on community functions is also often non additive. For instance, Jimenez et al studied the combined

effect of different environmental variables on the methanogenic output of an anaerobic digestor community (Jiménez et al., 2014). Specifically, these authors examined the combined effect of three substrates (pig manure, rice straw and clay residual) at different concentrations on the specific methanogenic activity of the digester community, and employed a fractional factorial design followed by a linear regression with interaction terms to predict out of sample. Their approach revealed the existence of strong pairwise interactions between the substrates, particularly for clay residual and rice straw under mesophilic conditions. The interactions were strong enough to change the sign effect of increasing clay concentration at high vs low manure concentrations: increasing clay concentration had a positive effect on the methanogenic activity when manure concentration was low, but a negative effect when manure concentration was high. Sign-effect interactions (akin to sign epistasis in genetics) are common in microbial consortia (Sanchez et al., 2023).
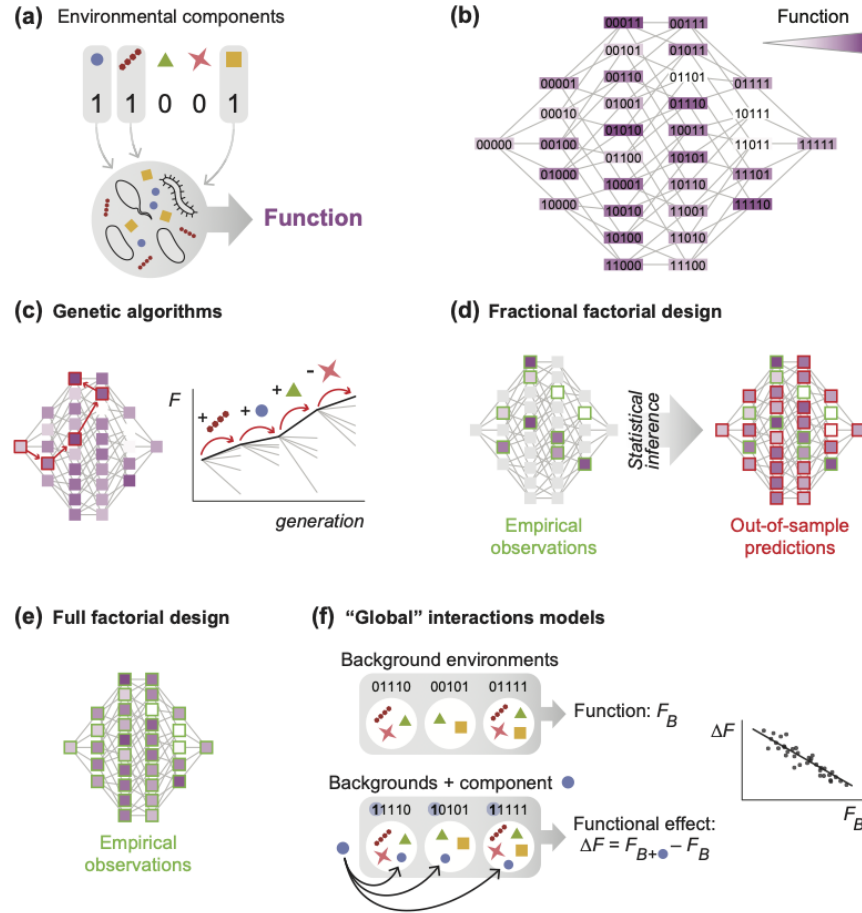
In another study, Molina-Barahona et al sought to optimize the environmental conditions for diesel removal in contaminated soils through biostimulation (Molina-Barahona et al., 2004). To this end, they investigated the combinatorial effects of various environmental factors that are known to individually have an effect on diesel removal rate: C:N ratio, moisture, and the amount and type of crop residue (CR). These factors were individually toggled between two values and combinations were generated through a fractional factorial design approach. The authors then found that interactions between moisture and CR type had a modest but significant effect on the amount of diesel removed in microcosms, as the amount of diesel removed in the presence of both factors at the same time was higher than expected by the product of each in isolation (Molina-Barahona et al., 2004) .

On a similar vein, Zhou et al, studied the combined effect of three binary variables: glucose addition, sodium dodecyl benzene sulfonate (SDBS) addition, and immobilization of the bacteria on biochar (or lack thereof) on the biodegradation of polycyclic aromatic hydrocarbons (PAH) by a microbial consortium (Zhou et al., 2023). While glucose alone stimulated PAH biodegradation, it had a negligible effect when combined with SDBS unless the consortia was also immobilized. In this instance, glucose had a modest (but seemingly significant) positive effect on the degradation of low molecular weight PAH. The authors propose a mechanistic explanation for this three-way interaction: Glucose had been previously found to stimulate the metabolism of PAHs and it is also known to promote microorganism to absorb more PAHs. In turn, SDBS is believed to increase the bioavailability of PAHs by enhancing their mobility and bringing them closer to the immobilized bacteria (Zhou et al., 2023).

All of these examples illustrate the potential effect of environmental interactions in the performance of single and multi-strain microbial consortia. On the one hand, the functional response of microbial consortia to environmental manipulations creates opportunities for their optimization. On the other hand, the combinatorial complexity of these environmental interaction effects may appear to be dissuasive. While it is indeed undoubtedly challenging, as we will see below there exists clear and convincing evidence that it is far from impossible.

Engineering microbial consortia by manipulating the environment.

As the presented examples illustrate, interactions between environmental factors are common and can be strong. These interactions may complicate the task of predicting optimal culture conditions. Despite this, as we show below, there exist strategies (Fig. 2) to navigate this complexity and optimize the environment.

Figure 2: Strategies used to find optimal environmental conditions. (a) Schematic showing five environmental components that can be combined (1-present, 0-absent) to design an optimal environment that maximizes a target microbial community function. (b) Map between environmental composition and function. The diagram shows all 32 (2^5) combinations that can be constructed from five environmental components. Color gradient from white to purple indicates increasing function level.(c-f) We show different strategies used to design the environment that maximizes a function of interest. (c) Genetic Algorithms (GA) begin by empirically quantifying the function ('fitness') in a subset of environments selected at random. Environments are then ranked by decreasing function. Environments mapping to highest function values are used to design a new 'population' of environments. Top environments are carried over to the new population and more variation is added by recombining and mutating those top environments. A new round of function quantification and selection of top environments begins. The whole process can be repeated until the function no longer increases or for a fixed number of predefined generations. (d)In fractional factorial design, a carefully selected subset of environments is used to quantify function. The results are then used to build regression models which allow predicting the function of out-of-sample environments. (e) Full factorial design consists in quantifying function in every possible environmental combination, therefore revealing the full composition-function map. This method is restricted to environments made up of few components as the number of environments increases exponentially with the number of environmental components. (f) Patterns of global epistasis observed in genetics allow predicting the effect a mutation has on organismal fitness, knowing the fitness of the background where the mutation occurred (see (Diaz-Colunga et al., 2023; Johnson et al., 2023) and references therein) and. An ecological parallel to global epistasis has also been demonstrated, where the effect of a species addition on a community-level function is predictable from the

function of the original community without the species (Diaz-Colunga et al., 2023; Ruiz et al., 2023; Sanchez et al., 2023). Here we propose that global patterns akin to global epistasis may also allow us to predict the change in function induced by the addition of a new component to an environment. If these patterns did exist, this would open up the opportunity for a new method for predicting function.

Genetic algorithms can find optimal environments that maximize desirable traits of clonal and multi-genotype communities. One approach to identify optimal combinations of environmental factors is the use of genetic algorithms (GA) to explore their combinatorial functional landscape (Fig. 2c) (Kucharzyk et al., 2012; Pacheco et al., 2021; Vandecasteele et al., 2008). As an example, Kucharzyk et al demonstrated the utility of a generational GA for identifying optimal environmental conditions for the degradation of perchlorate in both enrichment communities and in pure cultures of *Dechlorosoma sp.* Strains (Kucharzyk et al., 2012). In this study, the authors formed a multidimensional functional landscape including environmental variables such as the pH, salinity ([KCl]), buffering capacity ([NaH2PO4] and [NaHCO3]), concentration of electron donor (acetate) and electron acceptor (the perchlorate itself), and the concentrations of other microbial nutrients such as vitamins and trace minerals. Environments were represented by a 9-string vector, containing in each entry the concentration of each variable, and the fitness of each individual environment (i.e. its probability to be selected for reproduction in the next generation) was given by the perchlorate degradation rate, which was determined empirically. The strings that made up the next generation were derived by recombination (via uniform crossover with probability 0.5) from those that were selected. In the daughter strings, each environmental variable could also randomly "mutate", i.e. increase or decrease by a magnitude equal to a pre-determined step size. The mutation rate was set so that, on average, one variable would change per individual per generation. The GA was applied for 11 generations, and its outcome was remarkably successful. The authors found environmental conditions that increased perchlorate degradation by over 16-fold for individual strains and over 5-fold for the consortia.

This work followed previous attempts to use genetic algorithms to optimize community functions through environmental manipulations. In the earliest such study we are aware of, Vandecasteele et al used a microbial community derived from human saliva as an inoculum, and built a genetic algorithm to optimize a collective function consisting of azo dye decoloration (Vandecasteele et al., 2008). Rather than manipulating the concentration of a set of resources, the environmental space that was explored in this work was the presence or absence of 10 different chemical supplements, including nutrients (i.e. glucose or glycerol) as well as various buffers, acids, and bases. Each combination of chemicals was added to an 12.5x dilution of a saliva sample and the fitness of the environment was given by the amount of dye decoloration over 24hr of culture. Dye decoloration increased after 15 optimization steps, and the authors convincingly demonstrated that besides the average increase in the metapopulation, the function of the best environments in each generation also responded to selection (an important benchmark in artificial community-level selection experiments (Chang et al., 2021)). A strength of this study is that the authors examined the ability of their approach to find a better ecosystem than the one they started with.

An exciting prospect for manipulating microbial community functions through their metabolic environment consists of combining evolutionary algorithms with genome-scale metabolic models. In pioneering work (Harcombe et al., 2014; Pacheco and Segrè, 2021), Pacheco and Segre used dynamical flux balance analysis (Dukovski et al., 2021) to model the growth and other metabolic properties of *in silico* microbial communities. Using this computational platform, they were able to combinatorially generate thousands of different environments, each containing combinations of up to 20 different resources, which were then inoculated with the same microbial consortium consisting of 13 microorganisms. The same approach was then expanded to a larger combinatorial space of over 150 limiting carbon sources. Similar to previous work, the authors implemented a genetic algorithm where a subset of environments were selected with a fitness score determined by their proximity to a community-level objective function. The selected environments were then used to generate the next generation through cross-over recombination and mutation (addition of new metabolites or removal of existing ones). Among the objective functions, the authors included compositional metrics such as community evenness, the abundance of target bacteria, as well as metabolic traits such as the secretion of particular metabolites or the degree of metabolic exchange among coexisting species (Pacheco and Segrè,

2021).

Altogether, these experiments support the feasibility and promise of evolutionary engineering approaches to explore the combinatorial space of environmental factors, in search for optimal culture conditions for microbial consortia. Importantly, these approaches do not require us to have a mechanistic understanding of environmental interactions. As we will see, however, bottom-up models that are built up from these interactions can be promising too.

Learning the landscape of environmental effects to infer optimal habitats (e.g. diets) for microbial communities. An alternative approach to finding optimal environments is to statistically learn the relationship between environment and community function for a given inoculum community. This is essentially the reverse problem to that of inferring the relationship between composition and function in a given environment (Eng and Borenstein, 2019; Sanchez et al., 2023). Because both problems are so similar, they are plagued by the same problems (chiefly, the presence of interactions between components, which we overview in previous sections), and the approaches that have been followed to solve them are similar also (Eng and Borenstein, 2019). Due to their combinatorial nature, a full factorial assessment of environmental factors (**Fig. 2d-e**) has been challenging to execute experimentally. Most studies have instead focused on fractional factorial design (**Fig. 2d**), where a subset of all possible environmental combinations is used to train a statistical model (Chen et al., 2009; Jiménez et al., 2014; Kikot et al., 2010; Skonieczny and Yargeau, 2009; Zhou et al., 2023). Typically, these models consisted of linear regression to either the presence/absence or the magnitude of different environmental factors, with the occasional inclusion of interaction terms. Once a statistical model of the community function landscape is available, it can be used to predict out of sample and thus locate environments that optimize the target function.

This approach was employed successfully by various authors. In the aforementioned work by Jimenez et al, for instance the authors were able to successfully predict the effect of the concentrations of three input substrates in the methanogenic activity of an anaerobic digester, thus identifying an optimal operation point (Jiménez et al., 2014). The other studies employing this strategy also focused on small combinatorial spaces with up to six different variables. Ideally, one would like to extend the approach to larger combinatorial spaces, but as the dimensionality increases so does the number of potential interactions, and therefore the number of measurements one must make to estimate their effect. A potential approach to handle this combinatorial explosion of interactions is to, once again, draw inspiration from genetics (Sanchez et al., 2023). In genetics, it has been found that simple quantitative patterns often emerge from myriad microscopic interactions between genes, allowing us to predict the fitness effect of a mutation without having to first parameterize its pairwise and higher-order epistatic interactions (see (Diaz-Colunga et al., 2023; Johnson et al., 2023) and references therein). We have recently shown that this idea extends as well to ecological communities, so that the functional effect of a species often follows simple quantitative patterns that do not require one to parameterize all possible interactions with every other member of the consortia (Diaz-Colunga et al., 2023; Ruiz et al., 2023; Sanchez et al., 2023). Future work will have to determine whether these global epistasis - like patterns also describe the effects of environmental factors in different contexts (**Fig. 2f**).

Discussion and Outlook

The field of synthetic biology has grown remarkably in the past two decades, and it has made substantial progress in engineering gene regulatory and metabolic networks in microorganisms. In recent years the field has put a spotlight on engineering microbial consortia (Brenner et al., 2008), with a focus again on their composition and genetic make up. Notwithstanding the many successful examples we cite above, and the many others we have surely missed, there is much room to be explored in engineering the environment where microorganisms grow. Among the most promising directions we would like to highlight is the development of evolutionary engineering approaches. While the examples we discuss above were executed in parallel, evolutionary engineering of the environment can also be implemented in time, where multiple environmental changes are introduced serially and the best environment is retained. The development of highly controllable milifluidic devices for continuous culture of microorganisms offer a promising platform for identifying optimal culture conditions for organisms and communities (Mancuso et al., 2021; Wong et al., 2018).

After a slow start, the field of artificial community-level selection is experiencing growing attention (Blouin et al., 2015; Chang et al., 2021; Doulcier et al., 2020, 2020; Panke-Buisse et al., 2015; Swenson et al., 2000; Vessman et al., 2023; Williams and Lenton, 2007; Xie et al., 2019; Xie and Shou, 2021). There exist obvious parallels between the process of optimizing the composition of an inoculum in a fixed environment, and that of optimizing an environment for a fixed inoculant. These may lead to a dialog between both fields, allowing the transfer of successful methodologies from one to another. Encouragingly, a wealth of novel evolutionary algorithms are being developed for the directed evolution of microbial communities (Chang et al., 2021; Vessman et al., 2023; Xie et al., 2019). These could be fruitful when applied to finding optimal combinations of environmental factors.

In a bottom-up manner, genome-scale metabolic models have been used for some time to rationally manipulate microbial interactions and to predict environments where microorganisms may coexist (Harcombe et al., 2014; Klitgord and Segrè, 2010), and they have been used to find culture conditions that optimize the growth of single strains or the production of target molecular products (e.g. (Swayambhu et al., 2020)). Constraint based models are being developed to quantitatively predict the behavior of microbial consortia (e.g. see (Heinken et al., 2021) and references therein), paving the way for their application to finding optimal environments.

Rather than being two parallel pursuits, bottom-up and top-down approaches can actually benefit from one another. By gaining a deeper understanding of the topology of the map (i.e. the response surface) between environment and function, researchers will be better equipped to design evolutionary algorithms that are capable of efficiently navigating those maps and finding optimal culture conditions. We hope that our review will stimulate efforts on this front.

References

Aidelberg, G., Towbin, B.D., Rothschild, D., Dekel, E., Bren, A., Alon, U., 2014. Hierarchy of non-glucose sugars in Escherichia coli. BMC Syst. Biol. 8, 133. https://doi.org/10.1186/s12918-014-0133-z

Bajic, D., Sanchez, A., 2020. The ecology and evolution of microbial metabolic strategies. Curr. Opin. Biotechnol., Energy Biotechnologyy * Environmental Biotechnology 62, 123–128. https://doi.org/10.1016/j.copbio.2019.09.003

Bintu, L., Buchler, N.E., Garcia, H.G., Gerland, U., Hwa, T., Kondev, J., Kuhlman, T., Phillips, R., 2005. Transcriptional regulation by the numbers: applications. Curr. Opin. Genet. Dev., Chromosomes and expression mechanisms 15, 125–135. https://doi.org/10.1016/j.gde.2005.02.006

Blaiseau, P.L., Holmes, A.M., 2021. Diauxic Inhibition: Jacques Monod's Ignored Work. J. Hist. Biol. 54, 175–196. https://doi.org/10.1007/s10739-021-09639-4

Blouin, M., Karimi, B., Mathieu, J., Lerch, T.Z., 2015. Levels and limits in artificial selection of communities. Ecol. Lett. 18, 1040–1048. https://doi.org/10.1111/ele.12486

Brenner, K., You, L., Arnold, F.H., 2008. Engineering microbial consortia: a new frontier in synthetic biology. Trends Biotechnol. 26, 483–489. https://doi.org/10.1016/j.tibtech.2008.05.004

Cacace, E., Kim, V., Varik, V., Knopp, M., Tietgen, M., Brauer-Nikonow, A., Inecik, K., Mateus, A., Milanese, A., Marli, M.T., Mitosch, K., Selkrig, J., Brochado, A.R., Kuipers, O.P., Kjos, M., Zeller, G., Savitski, M.M., Gottig, S., Huber, W., Typas, A., 2023. Systematic analysis of drug combinations against Gram-positive bacteria. Nat. Microbiol. 1–17. https://doi.org/10.1038/s41564-023-01486-9

Chang, C.-Y., Vila, J.C.C., Bender, M., Li, R., Mankowski, M.C., Bassette, M., Borden, J., Golfier, S., Sanchez, P.G.L., Waymack, R., Zhu, X., Diaz-Colunga, J., Estrela, S., Rebolleda-Gomez, M., Sanchez, A., 2021. Engineering complex communities by directed evolution. Nat. Ecol. Evol. 5, 1011–1023. https://doi.org/10.1038/s41559-021-01457-5

Chen, Y., Lin, C.-J., Jones, G., Fu, S., Zhan, H., 2009. Enhancing biodegradation of wastewater by microbial consortia with fractional factorial design. J. Hazard. Mater. 171, 948–953. https://doi.org/10.1016/j.jhazmat.2009.06.100

Cote, I.M., Darling, E.S., Brown, C.J., 2016. Interactions among ecosystem stressors and their importance in conservation. Proc. R. Soc. B Biol. Sci. 283, 20152592. https://doi.org/10.1098/rspb.2015.2592

Cruz-Loya, M., Tekin, E., Kang, T.M., Cardona, N., Lozano-Huntelman, N., Rodriguez-Verdugo, A., Savage, V.M., Yeh, P.J., 2021. Antibiotics Shift the Temperature Response Curve of Escherichia coli Growth. mSystems 6, 10.1128/msystems.00228-21. https://doi.org/10.1128/msystems.00228-21

Dal Bello, M., Lee, H., Goyal, A., Gore, J., 2021. Resource–diversity relationships in bacterial communities reflect the network structure of microbial metabolism. Nat. Ecol. Evol. 5, 1424–1434. https://doi.org/10.1038/s41559-021-01535-8

Diaz-Colunga, J., Skwara, A., Vila, J.C.C., Bajic, D., Sanchez, A., 2023. Global epistasis and the emergence of ecological function. https://doi.org/10.1101/2022.06.21.496987

Doulcier, G., Lambert, A., De Monte, S., Rainey, P.B., 2020. Eco-evolutionary dynamics of nested Darwinian populations and the emergence of community-level heredity. eLife 9, e53433. https://doi.org/10.7554/eLife.53433

D'Souza, G., Shitut, S., Preussger, D., Yousif, G., Waschina, S., Kost, C., 2018. Ecology and evolution of metabolic cross-feeding interactions in bacteria. Nat. Prod. Rep. 35, 455–488. https://doi.org/10.1039/C8NP00009C

Dukovski, I., Bajić, D., Chacón, J.M., Quintin, M., Vila, J.C.C., Sulheim, S., Pacheco, A.R., Bernstein, D.B., Riehl, W.J., Korolev, K.S., Sanchez, A., Harcombe, W.R., Segrè, D., 2021. A metabolic modeling platform for the computation of microbial ecosystems in time and space (COMETS). Nat. Protoc. 16, 5030–5082. https://doi.org/10.1038/s41596-021-00593-3

Eng, A., Borenstein, E., 2019. Microbial community design: methods, applications, and opportunities. Curr. Opin. Biotechnol., Systems Biology * Nanobiotechnology 58, 117–128. https://doi.org/10.1016/j.copbio.2019.03.002

Espinar, L., Dies, M., Cağatay, T., Süel, G.M., Garcia-Ojalvo, J., 2013. Circuit-level input integration in bacterial gene regulation. Proc. Natl. Acad. Sci. 110, 7091–7096. https://doi.org/10.1073/pnas.1216091110

Estrela, S., Sánchez, Á., Rebolleda-Gómez, M., 2021a. Multi-Replicated Enrichment Communities as a Model System in Microbial Ecology. Front. Microbiol. 12.

Estrela, S., Sanchez-Gorostiaga, A., Vila, J.C., Sanchez, A., 2021b. Nutrient dominance governs the assembly of microbial communities in mixed nutrient environments. eLife 10, e65948. https://doi.org/10.7554/eLife.65948

Fonte, E.S., Amado, A.M., Meirelles-Pereira, F., Esteves, F.A., Rosado, A.S., Farjalla, V.F., 2013. The Combination of Different Carbon Sources Enhances Bacterial Growth Efficiency in Aquatic Ecosystems. Microb. Ecol. 66, 871–878. https://doi.org/10.1007/s00248-013-0277-1

Goldford, J.E., Lu, N., Bajić, D., Estrela, S., Tikhonov, M., Sanchez-Gorostiaga, A., Segrè, D., Mehta, P., Sanchez, A., 2018. Emergent simplicity in microbial community assembly. Science 361, 469–474. https://doi.org/10.1126/science.aat1168

Harcombe, W.R., Riehl, W.J., Dukovski, I., Granger, B.R., Betts, A., Lang, A.H., Bonilla, G., Kar, A., Leiby, N., Mehta, P., Marx, C.J., Segrè, D., 2014. Metabolic Resource Allocation in Individual Microbes Determines Ecosystem Interactions and Spatial Dynamics. Cell Rep. 7, 1104–1115. https://doi.org/10.1016/j.celrep.2014.03.070

Heinken, A., Basile, A., Thiele, I., 2021. Advances in constraint-based modelling of microbial communities. Curr. Opin. Syst. Biol. 27, 100346. https://doi.org/10.1016/j.coisb.2021.05.007

Jiménez, J., Guardia-Puebla, Y., Romero-Romero, O., Cisneros-Ortiz, M.E., Guerra, G., Morgan-Sagastume, J.M., Noyola, A., 2014. Methanogenic activity optimization using the response surface methodology, during the anaerobic co-digestion of agriculture and industrial wastes. Microbial community diversity. Biomass Bioenergy 71, 84–97. https://doi.org/10.1016/j.biombioe.2014.10.023

Johnson, M.S., Reddy, G., Desai, M.M., 2023. Epistasis and evolution: recent advances and an outlook for prediction. BMC Biol. 21, 120. https://doi.org/10.1186/s12915-023-01585-3

Kaplan, S., Bren, A., Zaslaver, A., Dekel, E., Alon, U., 2008. Diverse Two-Dimensional Input Functions Control Bacterial Sugar Genes. Mol. Cell 29, 786–792. https://doi.org/10.1016/j.molcel.2008.01.021

Kikot, P., Viera, M., Mignone, C., Donati, E., 2010. Study of the effect of pH and dissolved heavy metals on the growth of sulfate-reducing bacteria by a fractional factorial design. Hydrometallurgy, 18th International Biohydrometallurgy Symposium, IBS2009, Bariloche-Argentina, 13-17 September 2009 104, 494–500. https://doi.org/10.1016/j.hydromet.2010.02.026

Klitgord, N., Segrè, D., 2010. Environments that Induce Synthetic Microbial Ecosystems. PLOS Comput. Biol. 6, e1001002. https://doi.org/10.1371/journal.pcbi.1001002

Kucharzyk, K.H., Crawford, R.L., Paszczynski, A.J., Soule, T., Hess, T.F., 2012. Maximizing microbial degradation of perchlorate using a genetic algorithm: Media optimization. J. Biotechnol. 157, 189–197. https://doi.org/10.1016/j.jbiotec.2011.10.011

Lázár, V., Snitser, O., Barkan, D., Kishony, R., 2022. Antibiotic combinations reduce Staphylococcus aureus clearance. Nature 610, 540–546. https://doi.org/10.1038/s41586-022-05260-5

Mancuso, C.P., Lee, H., Abreu, C.I., Gore, J., Khalil, A.S., 2021. Environmental fluctuations reshape an unexpected diversity-disturbance relationship in a microbial community [WWW Document]. eLife. https://doi.org/10.7554/eLife.67175

Mataigne, V., Vannier, N., Vandenkoornhuyse, P., Hacquard, S., 2021. Microbial Systems Ecology to Understand Cross-Feeding in Microbiomes. Front. Microbiol. 12.

McCarty, N.S., Ledesma-Amaro, R., 2019. Synthetic Biology Tools to Engineer Microbial Communities for Biotechnology. Trends Biotechnol. 37, 181–197. https://doi.org/10.1016/j.tibtech.2018.11.002

Molina-Barahona, L., Rodrıguez-Vázquez, R., Hernández-Velasco, M., Vega-Jarqun, C., Zapata-Pérez, O., Mendoza-Cantú, A., Albores, A., 2004. Diesel removal from contaminated soils by biostimulation and supplementation with crop residues. Appl. Soil Ecol. 27, 165–175. https://doi.org/10.1016/j.apsoil.2004.04.002

Monod, J., 1942. Recherches sur la croissance des cultures bactériennes. Hermann & cie, Paris.

Okano, H., Hermsen, R., Kochanowski, K., Hwa, T., 2020. Regulation underlying hierarchical and simultaneous utilization of carbon substrates by flux sensors in Escherichia coli. Nat. Microbiol. 5, 206–215. https://doi.org/10.1038/s41564-019-0610-7

Pacheco, A.R., Osborne, M.L., Segrè, D., 2021. Non-additive microbial community responses to environmental complexity. Nat. Commun. 12, 2365. https://doi.org/10.1038/s41467-021-22426-3

Pacheco, A.R., Segrè, D., 2021. An evolutionary algorithm for designing microbial communities via environmental modification. J. R. Soc. Interface 18, 20210348. https://doi.org/10.1098/rsif.2021.0348

Panke-Buisse, K., Poole, A.C., Goodrich, J.K., Ley, R.E., Kao-Kniffin, J., 2015. Selection on soil microbiomes reveals reproducible impacts on plant function. ISME J. 9, 980–989. https://doi.org/10.1038/ismej.2014.196

Replansky, T., Bell, G., 2009. The relationship between environmental complexity, species diversity and productivity in a natural reconstructed yeast community. Oikos 118, 233–239. https://doi.org/10.1111/j.1600-0706.2008.16948.x

Ruiz, J., de Celis, M., Diaz-Colunga, J., Vila, J.C., Benitez-Dominguez, B., Vicente, J., Santos, A., Sanchez, A., Belda, I., 2023. Predictability of the community-function landscape in wine yeast ecosystems. Mol. Syst. Biol. 19, e11613. https://doi.org/10.15252/msb.202311613

Rydenfelt, M., Garcia, H.G., Iii, R.S.C., Phillips, R., 2014. The Influence of Promoter Architectures and Regulatory Motifs on Gene Expression in Escherichia coli. PLOS ONE 9, e114347. https://doi.org/10.1371/journal.pone.0114347

San León, D., Nogales, J., 2022. Toward merging bottom–up and top–down model-based designing of synthetic microbial communities. Curr. Opin. Microbiol. 69, 102169. https://doi.org/10.1016/j.mib.2022.102169

Sanchez, A., 2019. Defining Higher-Order Interactions in Synthetic Ecology: Lessons from Physics and Quantitative Genetics. Cell Syst. 9, 519–520. https://doi.org/10.1016/j.cels.2019.11.009

Sanchez, A., Bajic, D., Diaz-Colunga, J., Skwara, A., Vila, J.C.C., Kuehn, S., 2023. The community-function landscape of microbial consortia. Cell Syst. 14, 122–134. https://doi.org/10.1016/j.cels.2022.12.011

Skonieczny, M.T., Yargeau, V., 2009. Biohydrogen production from wastewater by Clostridium beijerinckii: Effect of pH and substrate concentration. Int. J. Hydrog. Energy 34, 3288–3294. https://doi.org/10.1016/j.ijhydene.2009.01.044

Smith, T.P., Clegg, T., Bell, T., Pawar, S., 2021. Systematic variation in the temperature dependence of bacterial carbon use efficiency. Ecol. Lett. 24, 2123–2133. https://doi.org/10.1111/ele.13840

Smith, T.P., Clegg, T., Ransome, E., Martin-Lilley, T., Rosindell, J., Woodward, G., Pawar, S., Bell, T., 2023. Bacterial responses to complex mixtures of chemical pollutants. https://doi.org/10.1101/2023.02.18.529059

Swayambhu, G., Moscatello, N., Atilla-Gokcumen, G.E., Pfeifer, B.A., 2020. Flux Balance Analysis for Media Optimization and Genetic Targets to Improve Heterologous Siderophore Production. iScience 23, 101016. https://doi.org/10.1016/j.isci.2020.101016

Swenson, W., Wilson, D.S., Elias, R., 2000. Artificial ecosystem selection. Proc. Natl. Acad. Sci. 97, 9110–9114. https://doi.org/10.1073/pnas.150237597

Vandecasteele, F.P.J., Crawford, R.L., Hess, T.F., 2008. Using a genetic algorithm to drive a microbial ecosystem in a desirable direction. Environ. Microbiol. 10, 1823–1830. https://doi.org/10.1111/j.1462-2920.2008.01603.x

Vessman, B., Guridi-Fernández, P., Arias-Sánchez, F.I., Mitri, S., 2023. Novel artificial selection method improves function of simulated microbial communities. https://doi.org/10.1101/2023.01.08.523165

Voigt, C.A., 2020. Synthetic biology 2020–2030: six commercially-available products that are changing our world. Nat. Commun. 11, 6379. https://doi.org/10.1038/s41467-020-20122-2

Williams, H.T.P., Lenton, T.M., 2007. Artificial selection of simulated microbial ecosystems. Proc. Natl. Acad. Sci. 104, 8918–8923. https://doi.org/10.1073/pnas.0610038104

Wong, B.G., Mancuso, C.P., Kiriakov, S., Bashor, C.J., Khalil, A.S., 2018. Precise, automated control of conditions for high-throughput growth of yeast and bacteria with eVOLVER. Nat. Biotechnol. 36, 614–623. https://doi.org/10.1038/nbt.4151

Wood, K., Nishida, S., Sontag, E.D., Cluzel, P., 2012. Mechanism-independent method for predicting response to multidrug combinations in bacteria. Proc. Natl. Acad. Sci. 109, 12254–12259. https://doi.org/10.1073/pnas.1201281109

Xie, L., Shou, W., 2021. Steering ecological-evolutionary dynamics to improve artificial selection of microbial communities. Nat. Commun. 12, 6799. https://doi.org/10.1038/s41467-021-26647-4

Xie, L., Yuan, A.E., Shou, W., 2019. Simulations reveal challenges to artificial community selection and possible strategies for success. PLOS Biol. 17, e3000295. https://doi.org/10.1371/journal.pbio.3000295

Yeh, P., Tschumi, A.I., Kishony, R., 2006. Functional classification of drugs by properties of their pairwise interactions. Nat. Genet. 38, 489–494. https://doi.org/10.1038/ng1755

Yeh, P.J., Hegreness, M.J., Aiden, A.P., Kishony, R., 2009. Drug interactions and the evolution of antibiotic resistance. Nat. Rev. Microbiol. 7, 460–466. https://doi.org/10.1038/nrmicro2133

Zhou, H., Gao, X., Wang, S., Zhang, Y., Coulon, F., Cai, C., 2023. Enhanced Bioremediation of Aged Polycyclic Aromatic Hydrocarbons in Soil Using Immobilized Microbial Consortia Combined with Strengthening Remediation Strategies. Int. J. Environ. Res. Public. Health 20, 1766. https://doi.org/10.3390/ijerph20031766