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The sum is greater than the parts: exploiting microbial communities to achieve complex functions

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Multi-species microbial communities are ubiquitous in nature. The widespread prevalence of these communities is due to highly elaborated interactions among their members thereby accomplishing metabolic functions that are unattainable by individual members alone. Harnessing these communal capabilities is an emerging field in biotechnology. The rational intervention of microbial communities for the purpose of improved function has been facilitated in part by developments in multi-omics approaches, synthetic biology, and computational methods. Recent studies have demonstrated the benefits of rational interventions to human and animal health as well as agricultural productivity. Emergent technologies, such as *in situ* modification of complex microbial community and community metabolic modeling, represent an avenue to engineer sustainable microbial communities. In this opinion, we review relevant computational and experimental approaches to study and engineer microbial communities and discuss their potential for biotechnological applications.

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Introduction

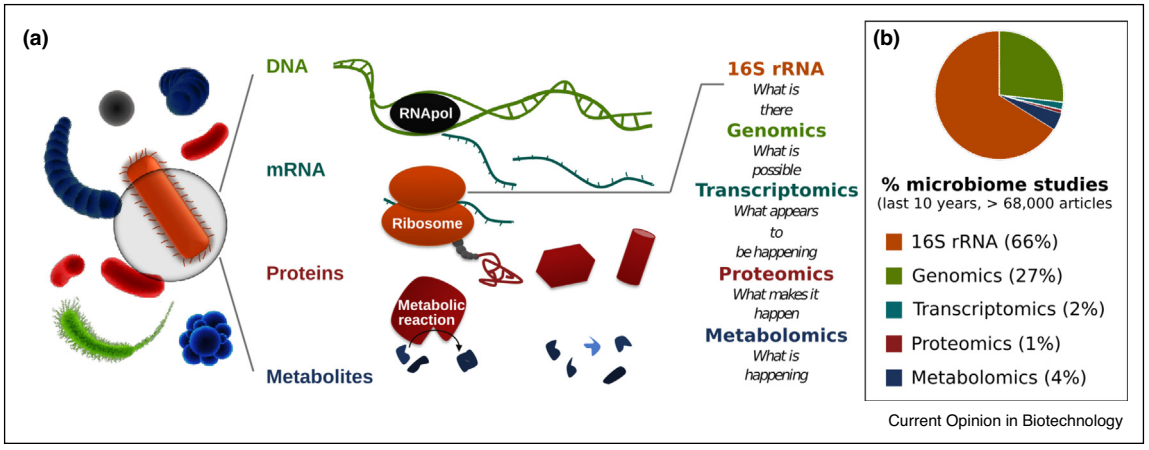
Life as we know it is not possible in the absence of microorganisms. Essential biogeochemical processes are only possible due to the interaction of many different microbes involved in carbon-, nitrogen-, and sulfur-cycling [1]. Many biotechnological processes, such as the production of fermented foods [2], or of biofuels [3] also rely on the concerted effort of different microorganisms. Additionally, higher animals and plants depend on the metabolism of the microbial communities associated with them [4]. All these processes rely on the ability of microbial communities to perform complex functions, for instance, the sequential metabolism of macro-molecules, nitrogen fixation, and microbial fermentation. The importance of the microbiome, that is, the sum of all microbial genetic material, in health and disease has gained substantial recognition over the last decade and has led to various therapeutic developments [5]. These developments range from the use of single microbes with beneficial features, that is, probiotics, to the transfer of entire communities, that is, fecal microbial transplantation [6] to sustain or improve health.

Exploiting microbial co-cultures or whole communities for biotechnological and medical purposes requires the identification of relevant microbes serving as microbial cell factories or therapeutic agents. Many approaches to distinguish the right microbes for the job from all the other community members have been based on correlations, such as composition, coexistence, and co-exclusion [7,8•]. However, detailed mechanistic insights into microbe-microbe and microbe-host interactions often remain undetermined [9•]. Understanding the mechanisms and rules of metabolic exchanges are critical i) to unravel community architecture and dynamic, ii) to maintain a stable community, and iii) to design and build community-based biotechnological or medical applications.

Determining the parts list for microbial communities

Designing, building, and manipulating microbial communities require detailed knowledge of their taxonomic composition. Over the past decade, an increasing number of studies have characterized the composition (i.e. microbiota) and the genomic features (i.e. microbiome) of microbial ecosystems [7,10]. Most of these studies have deployed 16S rRNA amplicon or shotgun metagenomic sequencing to evaluate relative changes in community composition (Figure 1). Retrieving partial or complete genome sequences by metagenomic analyses revealed

Figure 1



Overview of widely used meta-omics approaches for microbiome studies.
(a) Microbial communities can be studied at the rRNA, DNA, mRNA, protein and/or metabolite level [7,11–14]. Each of these meta-omics methods provides a representation of what taxa are present (16S rRNA) [12], what genes and what set of possible biochemical reactions are present (metagenomics) [7], which of those genes are transcribed (metatranscriptomics) [14], or have been translated (metaproteomics) [13], or defining the metabolic state of the organisms (metabolomics) [7,15]. **(b)** Proportions of PubMed-referenced studies containing the terms "16S rRNA", "metagenom*, metatranscriptom*, metaproteom*, or metabolom*" AND microbiome (a total of 68 377 articles published between 2010 and 2020).

biochemical pathways and provided insight into microbial metabolism [7]. However, these genome-centric analyses are often blind to downstream biological regulation and have a limited ability to yield a functional, condition-resolved, and time-resolved view, that is, ecological succession, of community organization. Thus, post-genomic approaches have been developed that aim at providing functional profiling in microbiome studies [7,11–14] (Figure 1).

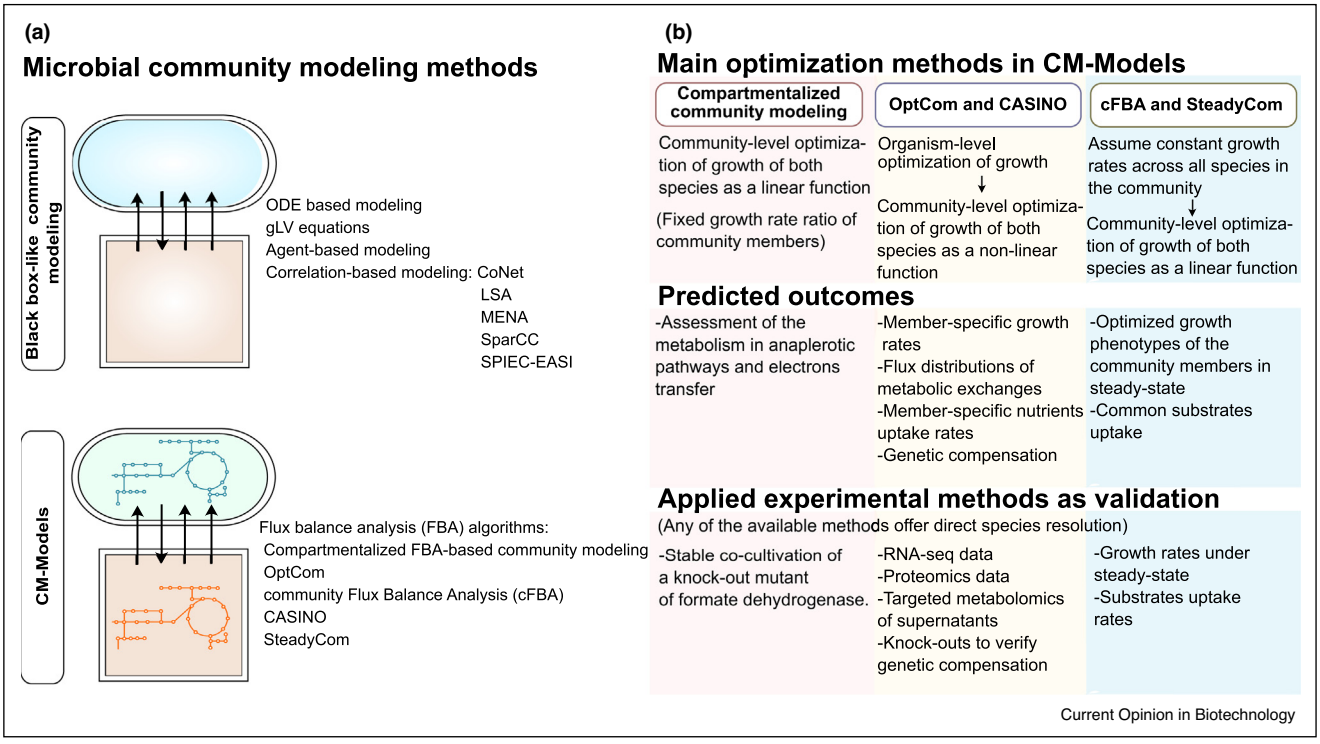
These post-genomic analyses are focused on measuring the levels of messenger RNAs (mRNA) (metatranscriptomics), proteins (metaproteomics), and/or metabolites (metabolomics) [13–15]. Metatranscriptomics allows to identify differential gene expression in response to changing environmental conditions and has been applied, for example, to study the mechanism of biogas production [14] or to identify lignocellulose degrading enzymes [16]. While metatranscriptomics is a good proxy of biological activity [17], metaproteomics provides a readout of biological function independent of post-transcriptional and post-translational regulation [17]. Although comprehensive metaproteomics studies are still technically challenging due to relatively low coverage compared to sequencing-based methods, this approach was successfully deployed to study the detrimental effect of phage-induced cell lysis in the production of biogas [13]. In turn, metabolomics aims at measuring all metabolites present in a sample [7]. A recent example demonstrated how the gut microbiota affects the chemistry of its human host by revealing previously uncharacterized bile acid conjugations that are enriched in patients with inflammatory diseases [15]. However, distinguishing if a metabolite is associated with the host or the microbiota or assigning metabolites to a certain species is currently still a chalenge.

Mathematical modeling: reconstructing the sum from its parts

After the parts of a microbial community have been identified we often face the challenge of how to contextualize this data. Multi-omics data integration offers an opportunity to untangle complex microbial interactions that shape and constrain a microbial community. Additionally, multi-omics approaches in combination with computational approaches allows to overcome one of the biggest challenge of studying highly diverse microbial communities, that is our inability to cultivate most of the microorganisms in the laboratory. The increasing amount of information makes the development of computational methods essential for the analysis of different types of datasets and for interpretation of results. Mathematical modeling of biological systems has been a resourceful tool to elucidate underlying drivers of cellular metabolism in several fields of research, including medical [18,19], environmental [18], agricultural [20], and biotechnological sciences [18].

Different mathematical models are currently available to analyze biological systems and to identify and quantify metabolite exchanges in microbial communities [9•]. For example, kinetic models have been used to describe metabolites consumption, enzyme synthesis, growth rates, and abundance patterns in microbial cultures [21], while sequence-based models have been deployed

Figure 2



Microbial community modeling.
(a) Black box-like [21,28–34] and community metabolic (CM)-models methods [25–27,35,36] to understand the role of different species in a given community. **(b)** CM-models methods use different optimization algorithms to simulate the microbial communities for exploring metabolic interactions among different species in a given community. Main outcomes and experimental validation were included by optimization method. Arrows between species 1 and species 2 represent known or possible metabolic exchanges. ODE - Ordinary Differential Equation. gLV - generalized Lotka–Volterra. CoNet - Cytoscape app version. LSA - Local Similarity Analysis. MENA - Molecular Ecological Network Analyses. SPIEC-EASI - SParse InversE Covariance Estimation for Ecological Association Inference. FBA - Flux Balance Analysis. OptCom - Optimization Framework for the Metabolic Modeling and Analysis of Microbial Communities. cFBA - community Flux Balance Analysis. CASINO - Community And Systems-level INteractive Optimization. SteadyCom - optimization framework for predicting metabolic flux distributions consistent with the steady-state requirement. Please refer Table 1 for a complete overview of microbial community modeling methods.

to identify microbial associations [9•] (Figure 2a). While many methods rely on abundances to predict associations, the elucidation of what metabolic exchanges contribute to community metabolism is often challenging. These black box-like community modeling approaches are in general incapable of revealing the underlying mechanism of interactions between microbes (Figure 2a). Lack of mechanistic insight makes it problematic to design interventions in a predictable fashion, which result in reproducible outcomes. Robust, stable, and predictable microbial communities are crucial for any industrial application. So far only genome-scale community metabolic models (CM-models) have been able to offer quantitative insight into metabolite exchanges and microbial interactions [9•,22,23••] (Figure 2b, Box 1; Table 1). Similar to other constraint-based models, CM-models use flux balance analysis (FBA) to calculate metabolic flux distributions [24••]. FBA-based computational approaches were developed to model microbial communities and to evaluate the

Box 1 Genome-scale metabolic modeling

A genome-scale metabolic (GSM) model is a mathematical representation of the metabolic reaction network of an organism, directly defined by its annotated genome. The significant increase in the wealth of omics data in recent years opened the door for GSM models [24••], with ever-increasing quality as new data integration methods arise. These models contain a steady-state algebraic system of metabolite mass balance equations, which are solved as a linear programming (LP) problem. This approach, called Flux Balance Analysis (FBA), determines the fluxes throughout the network that maximize an objective function, widely defined as the biomass production, to describe the exponential growth of single organisms [Feist2010]. However, several other objective functions have successfully been used to describe sub-optimal and non-growth-oriented phenotypes [24••]. As a result, GSM models are tightly tailored models that predict the response of one or multiple organisms to perturbations and changes in nutrient levels, organism abundances, and genome content [9•], thus rendering GSM models suitable to study multi-cell systems.

Table 1

An overview of modeling approaches for studying microbial communities

Modeling framework	Scale	Remarks	References
Black box-like modeling			
ODE-based modeling	Microbial community of a few members	• This method is limited to model a simple microbial community of a few members. • It depends significantly on metabolic (e.g. fermentation) data to estimate modeling parameters. • This dependence makes this method less desirable to explore a large microbial community since many of the microorganisms in natural communities cannot be cultivated in the laboratory yet.	[61–63]
gLV equations	Simplified microbial community (e.g. genus-level)	• This method relies on longitudinal abundance datasets and can capture the time-dependent variations in the communities. • However, it cannot explain the underlying interaction mechanisms between members, such as metabolite exchanges.	[64]
Agent-based modeling (ABM)	Microbial community of a few members	• ABM can be used to explore temporal and spatial interactions between species. • It can predict the particular behavior of microorganisms as a group, but not of individual microorganisms. • It can be computationally expensive for analyzing the large communities. • No study has been published yet that employed ABM for a diverse microbial community.	[65]
Correlation-based modeling: CoNet, LSA, MENA, SparCC, SPIEC-EASI	Large microbial community	• These methods can predict associations between species/operational taxonomic units in terms of co-occurrence and co-exclusion. • These methods can be applied to large communities but cannot determine the interactions between different community members. • These methods are also incapable of providing any functional similarities or dissimilarities responsible for co-occurrence or co-exclusion of community members.	[30,31,33,34]
Community Metabolic (CM)-Modeling-based methods			
Compartmentalized FBA-based community modeling	Pairwise analysis	• Although, in general, CM-models-based methods are advantageous for investigating metabolic exchanges between members, this approach has only been used to perform pairwise analyses. • This concept was used to develop other CM-models-based methods by defining different biological objective functions (see below).	[35]
OptCom	Microbial community of two to four species	• This approach involves multi-level and multi-objective optimization. • The growth of individual microorganisms and the whole community is optimized.	[25]
dOptCom	Microbial community of three species	• dOptCom is advantageous over OptCom, as it can capture the temporal dynamics of growth members and the whole community, as well as metabolite exchanges between members.	[66]
CASINO	Microbial community of six species	• Unlike OptCom and dOptCom, CASINO optimizes a biological objective function iteratively at microorganism-level and community-level.	[26]

Table 1 (*Continued*)

Modeling framework	Scale	Remarks	References
cFBA	Pairwise analysis	• This method includes a non-linear, multi-objective function assuming a fixed growth rate of microorganisms in the community. • It was tested on a synthetic community of two different strains of *E. coli*.	[36]
SteadyCom	Microbial communities of four and nine species	• SteadyCom simulates the community-level problem at a steady-state, making this method easy to implement for larger communities requiring less computational power than cFBA. • Unlike other CM-models-based methods, this method includes flux variability analysis to capture the variation in microbial abundances due to perturbations in nutrient availability.	[27]
Computation of Microbial Ecosystems in Time and Space (COMETS)	Microbial communities up to three species	• Like other CM-models-based methods, this approach can capture the growth of individual members and the whole community, and metabolic exchanges. • This method can help predict the temporal dynamics and spatial organization of members in the community.	[67]
MICOM (Microbial Community)	Large microbial community	• MICOM simulates microbial community based on the linear dependency of growth rate on the abundance of individual members in the community. • In an initial attempt, the growth rates of microorganisms in a large gut microbial community were found in agreement with the replication rates.	[19]

interactions between members. These approaches are primarily different from each other based on what function is used to simulate the models [25–27] (Figure 2b). An overview of these methods, highlighting their differences, advantages, and limitations, is summarized in Table 1.

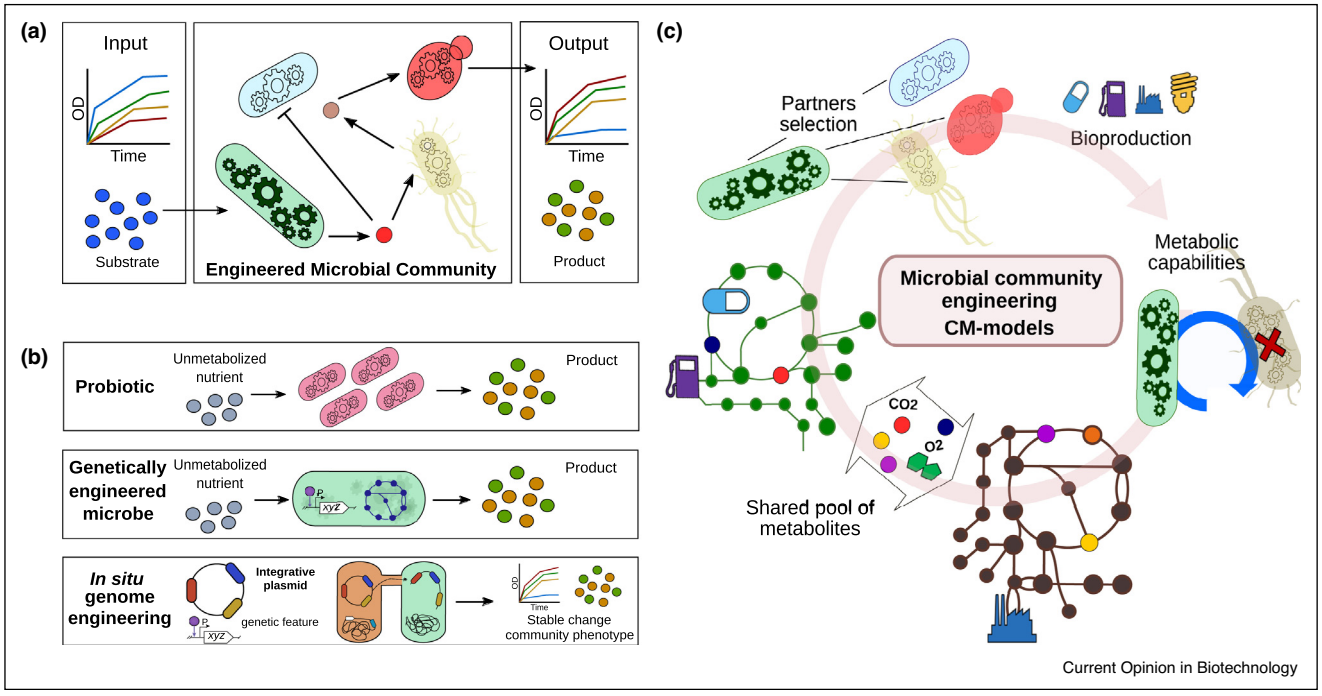
Initial modeling approaches were applied to communities of a few species [23••,26,27] and so far no further developments of these tools using complex communities, that is, highly diverse microbial communities, have been published. One reason why these tools have not been applied to model diverse microbial communities is because of the infinite number of possible metabolite exchanges between species that result in a computational infeasible solution space. This underlines a need for future efforts for optimizing existing tools and to develop new methods to analyze highly diverse microbial communities. One promising development in this field has been the integration of experimental measurements (growth phenotype and meta-omics data) to constrain community-scale metabolic models, which enables simulations of large communities but also enhances the capabilities of the modeling framework [19,37–39]. Combining these efforts with recently developed tools, such as adaptive laboratory evolution of auxotrophic strains [40,41] and synthetic microbial communities, will yield more effective ways to predict community outcomes in the future and thus open the door to engineer complex communities for stability or desired metabolic tasks [42,43].

Engineering of microbial communities by changing their parts

The idea of modifying microbial communities for health benefits or biotechnological applications is not new (Figure 3a). For instance, probiotics, that is, beneficial live microbes, as part of fermented food, have been consumed and associated with health benefits for thousands of years [44] (Figure 3b). However, probiotics are highly species- or strain-dependent, and often have a narrow host-spectrum, rendering individual responses, for example, in different humans, unpredictable [45].

Advances in synthetic biology, metabolic engineering, and computational biology have been utilized to overcome these limitations. The constant improvement of molecular biology and genetic engineering tools, such as DNA assembly, cloning strategies, synthetic DNA synthesis have enabled the designing and optimization of new metabolic pathways for biotechnological applications [8•]. In turn, computational approaches can narrow down the solution space to select more promising targets, for instance, identifying highly conserved promoter regions to improve gene expression or optimizing codon usage for protein translation. Over the past years, these tools have

Figure 3



Engineering of microbial communities.
(a) Rational engineering of microbes will allow us to control growth and metabolism of complex microbial communities. **(b)** Approaches to rationally intervene in microbial community composition. Probiotics can be used for short-term alterations by producing metabolites of interest, such as short chain fatty acids [44]. The use of genetically engineered microbes can provide a more controlled intervention, targeting specific host cells or community members [47•]. *In situ* genome engineering provides a unique opportunity to community engineering by modifying bacterial genomes *in situ*, without the need of growing them in the laboratory [53,54••,55]. **(c)** Computational framework and applications of community metabolic (CM) modeling. CM-models can be used to design interventions (e.g. partner selection) and to improve the production of value-added compounds [9•,18,23••,25,27].

enabled the rapid engineering of single organisms to improve the production of enzymes, to synthesize bio-products; such as biofuels, solvents and polymers; and to improve food and animal feedstock production [8•,46].

Although the use of genetically modified microorganisms is widespread in the biotech industry, their application in humans and their unintentional release into the environment is a matter of current debate. Over the past few years, there has been an increase in studies using engineered probiotics for therapeutic interventions in humans and in agriculture (Figure 3b). Among the different strains, the probiotic *E. coli* Nissle 1917 has been used extensively for applications that prevent or treat diseases [47•], with notable examples in cancer therapy [48,49••]. Other microorganisms, such as *Azorhizobium caulinodans* ORS571 and *Rhizobium* sp. IRBG74, have been engineered to improve nitrogen-fixation in the rhizosphere to benefit crops [50].

Albeit great advances have been made in engineering single microbes for biotechnological applications, the incorporation of large or complex heterologous pathways can still be challenging. Furthermore, heterologous expression represents an additional metabolic burden and can result in metabolic impairments, rendering these strains less competitive in a community setting or in nature [51•]. Most importantly, many microbes of potential interest cannot be readily cultivated in the laboratory and controlled genetic manipulation *in vitro* is thus limited [8•,52]. These limitations jeopardize the rational intervention of microbial communities, once they prevent to cultivate and genetically modify diverse microbial communities in the laboratory. Advances are being made to mimic environmental conditions and reproduce natural microbial communities. Some of these efforts will be discussed later.

A recent approach with remarkable potential is the *in situ* engineering of microbial communities using integrative and conjugative platforms [53,54••,55]. These methods enable direct modification of microbes with desired genetic features and circumvents propagation of so far uncultured microbes in the laboratory (Figure 3b). In

addition, *in situ* engineering allows the genetic modification of microbes already adapted to the environment, instead of introducing a new one. Recent methods involve donor strains and transfer of replicative or integrative vectors into heterogeneous microbial communities, for example, in soil or in the mammalian gut [54••,55]. A CRISPR-based genome editing method demonstrated that genetic material could be transferred across numerous microbial genera, including bacteria not previously genetically modified, such as *Prevotella* and *Campylobacter* [54••]. A study using integrative and conjugative elements from *Bacillus subtilis*, successfully transferred the gene cluster for nitrogen-fixation (*nif*) to soil bacteria [55]. Moreover, Hsu *et al.* demonstrated that oral delivery of the temperate phage λ, expressing a programmable dCas9, could modulate the expression of a specific bacterial gene in the gut [53]. While these studies have exemplified the great biotechnological potential of manipulating microbial communities *in situ*, several questions remain. For example, can we predict a priory, which microbes should be modified and what effect such modification will have on the entire community, its activity, and its stability over time? Can we rationally design communities and will these designs be reproducible and applicable to different hosts, for example, different human subjects or different plants?

Many of these questions are not easily addressable experimentally because they require i) highly reproducible ecosystems or ii) the ability to screen an extremely large number of samples [40,56]. However, computational approaches, such as CM-model simulations, can assist in answering these questions in remarkably short timeframes. CM-models scan a myriad of possibilities and select for environmental conditions and genotypes that trigger a specific phenotype (Figure 3c). Adjusting for different environmental conditions allows testing community responses at large scale. For example, when the metabolic networks of the alga *Chlorella vulgaris* and the yeast *Saccharomyces cerevisiae* were combined into a CM-model, simulations identified specific environmental conditions that were driving either cooperative or competitive coexistence of the alga and the yeast [23••]. Additionally, these CM-models are able to unravel detailed interactions by simulating the exchange of various metabolites [57•]. CM-model simulations can also explore how communities respond to stress and how metabolic exchanges are adjusted under such conditions. For example, *C. vulgaris* increases the exchange of amino acids in co-culture to compensate for nitrogen starvation [23••,58].

CM-models have recently been applied to study bioproduction in phototrophic microbial communities. These communities consist of a phototropic and a heterotrophic partner and hold great promise for sustainable biotechnology by producing value-added compounds from carbon dioxide [7,10,59]. A study containing the engineered cyanobacterium *Synechococcus elongatus* cscB+ and various heterotrophic partners showed that communities with phylogenetically distant members were able to exchange larger numbers of metabolites [57•]. CM-models for these co-cultures not only identified the optimal heterotrophic partner for *Synechococcus* but also predicted strain designs and growth conditions that would lead to increased growth and higher production of value-added compounds [57•] (Figure 3c). These computational models allow for the intricate design of improved community stability and productivity, potentially facilitating the rise of community-based biotechnology, eliminating current metabolic limitations of monocultures.

Future directions

Integration of experimental and computational methods are essential to understand microbe-microbe and microbe-host interactions in complex biological systems. The recent development of synthetic biology tools applied to community engineering creates new opportunities for biotechnological applications. However, the dynamic nature of microbial communities requires mechanistic knowledge of interactions to rationally re-program community function. This dynamic includes for example fluctuations in community composition, genetic stability, and condition-specific phenotypes. The use of synthetic communities to unravel the basis of these interactions and gain mechanistic insight is crucial to lay the foundation for future studies and ultimately for rational design of communities for biotechnological applications. The recent development of synthetic communities circumvents some of the essential problems in microbiome research, that is, standardization and reproducibility [57•]. Reproducible fabricated ecosystems, that is, EcoFABs [56], are an attractive solution to study microbial communities. EcoFABs enable researchers to design and create model microbial ecosystems and couple them to standardized workflows, computational tools, and computational models. These standardized systems facilitate inter-laboratory comparisons [60] and have been applied to study microbial interactions in gut and plant synthetic environments [56,60]. Integration of standardized ecosystems with new genetic manipulation tools and computational modeling will ultimately develop stable consortia and communities for the biotech industry, and expedite broad applications by providing new metabolic capabilities.

Conflict of interest statement

Nothing declared.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Trivedi P, Anderson IC, Singh BK: **Microbial modulators of soil carbon storage: Integrating genomic and metabolic knowledge for global prediction**. *Trends Microbiol* 2013, **21**:641-651.

2. Reese AT, Madden AA, Joossens M, Lacaze G, Dunn RR: **Influences of ingredients and bakers on the bacteria and fungi in sourdough starters and bread**. *mSphere* 2020, **5**:1-15.

3. Grady KL, Sorensen JW, Stopnisek N, Guittar J, Shade A: **Assembly and seasonality of core phyllosphere microbiota on perennial biofuel crops**. *Nat Commun* 2019, **10**:1-10.

4. Zengler K, Zaramela LS: **The social network of microorganisms - how auxotrophies shape complex communities**. *Nat Rev Microbiol* 2018, **16**:383-390.

5. FitzGerald MJ, Spek EJ: **Microbiome therapeutics and patent protection**. *Nat Biotechnol* 2020, **38**:798-811.

6. Brugman S, Ikeda-Ohtsubo W, Braber S, Folkerts G, Pieterse CMJ, Bakker PAHM: **A comparative review on microbiota manipulation: lessons from fish, plants, livestock, and human research**. *Front Nutr* 2018, **5**:1-15.

7. Zuñiga C, Zaramela L, Zengler K: **Elucidation of complexity and prediction of interactions in microbial communities**. *Microb Biotechnol* 2017, **10**:1500-1522.

8. • Lawson CE, Harcombe WR, Hatzenpichler R, Lindemann SR, Löffler FE, O'Malley MA, García Martín H, Pfleger BF, Raskin L, Venturelli OS *et al.*: **Common principles and best practices for engineering microbiomes**. *Nat Rev Microbiol* 2019, **17**:725-741
This review presents key elements of an iterative approach for rational engineering of microbial communities.

9. • Kumar M, Ji B, Zengler K, Nielsen J: **Modelling approaches for studying the microbiome**. *Nat Microbiol* 2019, **4**:1253-1267
This paper provides a comprehensive review of the latest developments and current approaches in community modeling, and highlights how different modelling strategies have been applied to understand the highly dynamic nature of microbial communities.

10. Tan J, Zuniga C, Zengler K: **Unraveling interactions in microbial communities - from co-cultures to microbiomes**. *J Microbiol* 2015, **53**:295-305.

11. Chen LX, Anantharaman K, Shaiber A, Murat Eren A, Banfield JF: **Accurate and complete genomes from metagenomes**. *Genome Res* 2020, **30**:315-333.

12. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F *et al.*: **Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2**. *Nat Biotechnol* 2019, **37**:852-857.

13. Heyer R, Schallert K, Siewert C, Kohrs F, Greve J, Maus I, Klang J, Klocke M, Heiermann M, Hoffmann M *et al.*: **Metaproteome analysis reveals that syntrophy, competition, and phage-host interaction shape microbial communities in biogas plants**. *Microbiome* 2019, **7**:1-17.

14. Stolze Y, Bremges A, Maus I, Pühler A, Sczyrba A, Schlüter A: **Targeted *in situ* metatranscriptomics for selected taxa from mesophilic and thermophilic biogas plants**. *Microb Biotechnol* 2018, **11**:667-679.

15. Quinn RA, Melnik AV, Vrbanac A, Fu T, Patras KA, Christy MP, Bodai Z, Belda-Ferre P, Tripathi A, Chung LK *et al.*: **Global chemical effects of the microbiome include new bile-acid conjugations**. *Nature* 2020, **579**:123-129.

16. Calusinska M, Marynowska M, Bertucci M, Untereiner B, Klimek D, Goux X, Sillam-Dussès D, Gawron P, Halder R, Wilmes P *et al.*: **Integrative omics analysis of the termite gut system adaptation to Miscanthus diet identifies lignocellulose degradation enzymes**. *Commun Biol* 2020, **3**:1-12.

17. Buccitelli C: **mRNAs, proteins and the emerging principles of gene expression control**. *Nat Rev Genet* 2020:1-15. Pre-print.

18. Ang KS, Lakshmanan M, Lee N-R, Lee D-Y: **Metabolic modeling of microbial mommunity interactions for health, environmental and biotechnological applications**. *Curr Genomics* 2018, **19**:712-722.

19. Diener C, Gibbons SM, Resendis-Antonio O: **MICOM: metagenome-scale modeling to infer metabolic interactions in the gut microbiota**. *mSystems* 2020, **5**:1-17.

20. Chiu HC, Levy R, Borenstein E: **Emergent biosynthetic capacity in simple microbial communities**. *PLoS Comput Biol* 2014, **10**:1-18.

21. Resat H, Bailey V, McCue LA, Konopka A: **Modeling microbial dynamics in heterogeneous environments: growth on soil carbon sources**. *Microb Ecol* 2012, **64**:883-897.

22. Zengler K, Palsson BO: **A road map for the development of community systems (CoSy) biology**. *Nat Rev Microbiol* 2012, **10**:366-372.

23. •• Zuñiga C, Li CT, Yu G, Al-Bassam MM, Li T, Jiang L, Zaramela LS, Guarnieri M, Betenbaugh MJ, Zengler K: **Environmental *stimuli* drive a transition from cooperation to competition in synthetic phototrophic communities**. *Nat Microbiol* 2019, **4**:2184-2191
This study integrates meta-omics data with community metabolic models to reveal condition-dependent interactions in a phototrophic community.

24. •• Thiele I, Palsson B: **A protocol for generating a high-quality genome-scale metabolic reconstruction**. *Nat Protoc* 2010, **5**:93-121
This protocol describes a detailed methodology to reconstruct genome-scale metabolic models. It includes several procedures to improve the quality of metabolic models to achieve biologically meaningful predictions.

25. Zomorrodi AR, Maranas CD: **OptCom: a multi-level optimization framework for the metabolic modeling and analysis of microbial communities**. *PLoS Comput Biol* 2012, **8**:1-13.

26. Shoaie S, Ghaffari P, Kovatcheva-Datchary P, Mardinoglu A, Sen P, Pujos-Guillot E, De Wouters T, Juste C, Rizkalla S, Chilloux J *et al.*: **Quantifying diet-induced metabolic changes of the human gut microbiome**. *Cell Metab* 2015, **22**:320-331.

27. Chan SHJ, Simons MN, Maranas CD: **SteadyCom: Predicting microbial abundances while ensuring community stability**. *PLoS Comput Biol* 2017, **13**:1-25.

28. Venturelli OS, Carr AV, Fisher G, Hsu RH, Lau R, Bowen BP, Hromada S, Northen T, Arkin AP: **Deciphering microbial interactions in synthetic human gut microbiome communities**. *Mol Syst Biol* 2018, **14**:1-19.

29. Bonabeau E: **Agent-based modeling: methods and techniques for simulating human systems**. *Proc Natl Acad Sci U S A* 2002, **99**:7280-7287.

30. Faust K, Sathirapongsasuti JF, Izard J, Segata N, Gevers D, Raes J, Huttenhower C: **Microbial co-occurrence relationships in the human microbiome**. *PLoS Comput Biol* 2012, **8**:1-17.

31. Ruan Q, Dutta D, Schwalbach MS, Steele JA, Fuhrman JA, Sun F: **Local similarity analysis reveals unique associations among marine bacterioplankton species and environmental factors**. *Bioinformatics* 2006, **22**:2532-2538.

32. Deng Y, Jiang YH, Yang Y, He Z, Luo F, Zhou J: **Molecular ecological network analyses**. *BMC Bioinformatics* 2012, **13**:1-20.

33. Friedman J, Alm EJ: **Inferring correlation networks from genomic survey data**. *PLoS Comput Biol* 2012, **8**:1-11.

34. Kurtz ZD, Müller CL, Miraldi ER, Littman DR, Blaser MJ, Bonneau RA: **Sparse and compositionally robust inference of microbial ecological networks**. *PLoS Comput Biol* 2015, **11**:1-25.

35. Stolyar S, Van Dien S, Hillesland KL, Pinel N, Lie TJ, Leigh JA, Stahl DA: **Metabolic modeling of a mutualistic microbial community**. *Mol Syst Biol* 2007, **3**:1-14.

36. Khandelwal RA, Olivier BG, Röling WFM, Teusink B, Bruggeman FJ: **Community flux balance analysis for microbial consortia at balanced growth**. *PLoS One* 2013, **8**:1-10.

37. Zimmermann J, Kaleta C, Waschina S: **gapseq: Informed prediction of bacterial metabolic pathways and reconstruction of accurate metabolic models**. *bioRxiv* 2020 http://dx.doi.org/10.1101/2020.03.20.000737.

38. Seif Y, Choudhary KS, Hefner Y, Anand A, Yang L, Palsson BO: **Metabolic and genetic basis for auxotrophies in gram-negative species**. *Proc Natl Acad Sci U S A* 2020, **117**:6264-6273.

39. Garza DR, Van Verk MC, Huynen MA, Dutilh BE: **Towards predicting the environmental metabolome from metagenomics with a mechanistic model**. *Nat Microbiol* 2018, **3**:456-460.

40. Zhalnina K, Zengler K, Newman D, Northen TR: **Need for laboratory ecosystems to unravel the structures**. *mBio* 2018, **9**:1-8.

41. Mee MT, Collins JJ, Church GM, Wang HH: **Syntrophic exchange in synthetic microbial communities**. *Proc Natl Acad Sci U S A* 2014, **111**.

42. Lloyd CJ, King ZA, Sandberg TE, Hefner Y, Olson CA, Phaneuf PV, O'Brien EJ, Sanders JG, Salido RA, Sanders K *et al.*: **The genetic basis for adaptation of model designed syntrophic co-cultures**. *PLoS Comput Biol* 2019, **15**:1-28.

43. García-Jiménez B, García JL, Nogales J: **FLYCOP: metabolic modeling-based analysis and engineering microbial communities**. *Bioinformatics* 2018, **34**:i954-i963.

44. Gasbarrini G, Bonvicini F, Gramenzi A: **Probiotics history**. *J Clin Gastroenterol* 2016, **50**:S116-S119.

45. Kolodziejczyk AA, Zheng D, Elinav E: **Diet–microbiota interactions and personalized nutrition**. *Nat Rev Microbiol* 2019, **17**:742-753.

46. Chubukov V, Mukhopadhyay A, Petzold CJ, Keasling JD, Martín HG: **Synthetic and systems biology for microbial production of commodity chemicals**. *NPJ Syst Biol Appl* 2016, **2**:1-11.

47. • Aggarwal N, Breedon AME, Davis CM, Hwang IY, Chang MW: **Engineering probiotics for therapeutic applications: recent examples and translational outlook**. *Curr Opin Biotechnol* 2020, **65**:171-179

This review highlights recent advances in the engineering of probiotics for therapeutic applications. It also lists numerous examples of engineered probiotics.

48. Gurbatri CR, Lia I, Vincent R, Coker C, Castro S, Treuting PM, Hinchliffe TE, Arpaia N, Danino T: **Engineered probiotics for local tumor delivery of checkpoint blockade nanobodies**. *Sci Transl Med* 2020, **12**:1-12.

49. •• Ho CL, Tan HQ, Chua KJ, Kang A, Lim KH, Ling KL, Yew WS, Lee YS, Thiery JP, Chang MW: **Engineered commensal microbes for diet-mediated colorectal-cancer chemoprevention**. *Nat Biomed Eng* 2018, **2**:27-37

This study describes the development of an engineered probiotic that can specifically bind to the target cell and release an anticancer compound.

50. Ryu MH, Zhang J, Toth T, Khokhani D, Geddes BA, Mus F, Garcia-Costas A, Peters JW, Poole PS, Ané JM *et al.*: **Control of nitrogen fixation in bacteria that associate with cereals**. *Nat Microbiol* 2020, **5**:314-330.

51. • Roell GW, Zha J, Carr RR, Koffas MA, Fong SS, Tang YJ: **Engineering microbial consortia by division of labor**. *Microb Cell Fact* 2019, **18**:1-11

This review highlights limitations and opportunities to genetically manipulate microbial communities.

52. Zengler K: **Central role of the cell in microbial ecology**. *Microbiol Mol Biol Rev* 2009, **73**:712-729.

53. Hsu BB, Plant IN, Lyon L, Anastassacos FM: ***In situ* reprogramming of gut bacteria by oral delivery**. *bioRxiv Bioeng* 2020 http://dx.doi.org/10.1101/2020.04.15.043232.

54. •• Ronda C, Chen SP, Cabral V, Yaung SJ, Wang HH: **Metagenomic engineering of the mammalian gut microbiome *in situ***. *Nat Methods* 2019, **16**:167-170

This study describes the development of a genetic platform to engineering microbial communities *in situ*. It demonstrated the ability to genetically engineer unculturable microbes in the mammalian gut.

55. Brophy JAN, Triassi AJ, Adams BL, Renberg RL, Stratis-Cullum DN, Grossman AD, Voigt CA: **Engineered integrative and conjugative elements for efficient and inducible DNA transfer to undomesticated bacteria**. *Nat Microbiol* 2018, **3**:1043-1053.

56. Zengler K, Hofmockel K, Baliga NS, Behie SW, Bernstein HC, Brown JB, Dinneny JR, Floge SA, Forry SP, Hess M *et al.*: **EcoFABs: advancing microbiome science through standardized fabricated ecosystems**. *Nat Methods* 2019, **16**:567-571.

57. • Zuñiga C, Li T, Guarnieri MT, Jenkins JP, Li C, Bingol K, Kim Y, Betenbaugh MJ, Zengler K: **Synthetic microbial communities of heterotrophs and phototrophs facilitate sustainable growth**. *Nat Commun* 2020, **11**:1-13

In this study, community metabolic models are used to simulate phototrophic microbial communities for sustainable bioproduction and succeed to identify optimal conditions for increased growth and production.

58. Tibocha-Bonilla JD, Kumar M, Richelle A, Godoy-Silva RD, Zengler K, Zuñiga C: **Dynamic resource allocation drives growth under nitrogen starvation in eukaryotes**. *NPJ Syst Biol Appl* 2020, **6**:1-9.

59. Li T, Jiang L, Hu Y, Paul JT, Zuniga C, Zengler K, Betenbaugh MJ: **Creating a synthetic lichen: mutualistic co-culture of fungi and extracellular polysaccharide-secreting cyanobacterium Nostoc PCC 7413**. *Algal Res* 2020, **45**:1-8.

60. Sasse J, Kant J, Cole BJ, Klein AP, Arsova B, Schlaepfer P, Gao J, Lewald K, Zhalnina K, Kosina S *et al.*: **Multilab EcoFAB study shows highly reproducible physiology and depletion of soil metabolites by a model grass**. *New Phytol* 2019, **222**:1149-1160.

61. Amaretti A, Bernardi T, Tamburini E, Zanoni S, Lomma M, Matteuzzi D, Rossi M: **Kinetics and metabolism of Bifidobacterium adolescentis MB 239 growing on glucose, galactose, lactose, and galactooligosaccharides**. *Appl Environ Microbiol* 2007, **73**:3637-3644.

62. Van Wey AS, Cookson AL, Roy NC, McNabb WC, Soboleva TK, Shorten PR: **Monoculture parameters successfully predict coculture growth kinetics of Bacteroides thetaiotaomicron and two Bifidobacterium strains**. *Int J Food Microbiol* 2014, **191**:172-181.

63. Van Wey AS, Lovatt SJ, Roy NC, Shorten PR: **Determination of potential metabolic pathways of human intestinal bacteria by modeling growth kinetics from cross-feeding dynamics**. *Food Res Int* 2016, **88**:207-216.

64. Buffie CG, Bucci V, Stein RR, McKenney PT, Ling L, Gobourne A, No D, Liu H, Kinnebrew M, Viale A *et al.*: **Precision microbiome reconstitution restores bile acid mediated resistance to *Clostridium difficile***. *Nature* 2015, **517**:205-208.

65. Bauer E, Zimmermann J, Baldini F, Thiele I, Kaleta C: **BacArena: individual-based metabolic modeling of heterogeneous microbes in complex communities**. *PLoS Comput Biol* 2017, **13**:1-22.

66. Zomorrodi AR, Islam MM, Maranas CD: **D-OptCom: dynamic multi-level and multi-objective metabolic modeling of microbial communities**. *ACS Synth Biol* 2014, **3**:247-257.

67. Harcombe WR, Riehl WJ, Dukovski I, Granger BR, Betts A, Lang AH: **Metabolic resource allocation in individual microbes determines ecosystem interactions and spatial dynamics**. *Cell* 2010, **7**:1104-1115.