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Review article

Deciphering and designing microbial communities by genome-scale metabolic modelling

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ABSTRACT

Microbial communities are shaped by the complex interactions among organisms and the environment. Genome-scale metabolic models (GEMs) can provide deeper insights into the complexity and ecological properties of various microbial communities, revealing their intricate interactions. Many researchers have modified GEMs for the microbial communities based on specific needs. Thus, GEMs need to be comprehensively summarized to better understand the trends in their development. In this review, we summarized the key developments in deciphering and designing microbial communities using different GEMs. A timeline of selected highlights in GEMs indicated that this area is evolving from the single-strain level to the microbial community level. Then, we outlined a framework for constructing GEMs of microbial communities. We also summarized the models and resources of static and dynamic community-level GEMs. We focused on the role of external environmental and intracellular resources in shaping the assembly of microbial communities. Finally, we discussed the key challenges and future directions of GEMs, focusing on the integration of GEMs with quorum sensing mechanisms, microbial ecology interactions, machine learning algorithms, and automatic modeling, all of which contribute to consortia-based applications in different fields.

1. Introduction

Microbes exist as a part of communities [1], which consist of multiple complex and highly interdependent components; together, these components drive global chemical cycles [2,3]. With the continuous advancement in gene editing technology, the application of microbial communities in various fields, such as product development, healthcare maintenance, and environmental restoration, has increased considerably [4,5]. These consortia-based applications are better than traditional metabolic engineering modifications and transformations in mono-strain cultivation [6,7]. Firstly, microbial communities reduce the metabolic burden on individual organisms through a division of labor. As such communities consist of different species and strains, complex production pathways are allocated to specific strains of bacteria that are highly specialized in those pathways [3,8]. Secondly, these communities not only achieve a synergistic effect, resulting in an overall benefit that is greater than the sum of individual contributions, but they also promote the exchange of metabolites among members, which helps mitigate the accumulation of toxic metabolites and enhances the stability of the community [9,10]. Thirdly, microbial communities maintain strong interconnections among their members, ensuring the integrity of the ecological structure and resilience of the community in response to environmental disturbances [11].

Microbial communities are the result of complex interactions among microbial components and the environment. Quantitatively predicting the composition and function of these communities across different environments is challenging [12]. Additionally, replicating natural microbial communities in the laboratory is extremely difficult due to their complex and nonlinear characteristics. Variations in metabolic pathways, preference of carbon source, and environmental adaptations among different species contribute to the overall diversity of the community. Moreover, microbial consortia often develop new traits, such as

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robustness and spatial organization, to adapt to changes in the environment [13]. These characteristics make it difficult to conduct quantitative analysis and independent assessment. Moreover, many microbial communities from natural environments are large and contain members that remain undefined. Additionally, identifying and studying some members using traditional laboratory culture techniques may not be feasible [14]. Therefore, to comprehensively investigate the structure and function of microbial communities, the principles of simplified, controllable laboratory systems need to be combined with quantitative models, such as genome-scale metabolic models (GEMs).

GEMs are systems biology tools that describe the metabolic state within cells. They link genes that encode enzymes to the reactions catalyzed by those enzymes, thus, characterizing various metabolic activities in organisms. Due to the advancements in high-throughput sequencing technology, a large amount of genomic data is available, which contributes to the construction of GEMs. The continuous updates to automated modeling tools provide software and platforms to further enhance the development of GEMs [15]. This mathematical modeling approach can significantly enhance our understanding of the complexity and ecological properties of different microbial communities, elucidating the mechanism underlying their intricate interactions [4,16]. Therefore, as GEMs can describe intracellular metabolic states, they have become essential for studying various microbial communities, which can provide a necessary and effective tool for designing and optimizing synthetic microbial communities, offering guidance for the advancement in the field of synthetic biology [17,18]. Although microbial ecological models can accurately predict community dynamics and identify emergent properties, their broader application is hindered by difficulties in obtaining parameters and a lack of biological interpretability [19].

Several review articles have systematically organized community models of metabolic networks, such as summarizing the top-down and bottom-up modeling approaches [20], mathematical frameworks, and theoretical foundations of biological control in community-level GEMs [21,22], as well as their applications across different ecosystems [23,24]. Although some researchers have investigated the predictive capabilities of these models in specific contexts, discussion on methodologies for broader application scenarios is lacking. Furthermore, with advancements in multi-omics and bioinformatics, various data dimensions need to be integrated into metabolic network models, as they can help predict microbial community properties comprehensively; such developments need to be further reviewed.

In this review, we illustrated the application of GEMs in understanding and designing interactions within microbial communities. Based on a brief introduction to the theory and development of GEMs, we found that the FBA shifted from focusing on single strains to including entire communities. We proposed a framework for constructing GEMs tailored to various microbial communities and reviewed the strengths and weaknesses of different GEM reconstruction methodologies. Then, we systematically summarized the static and dynamic GEMs at the community level. We emphasized the role of resources in shaping the assembly of the microbial communities and divided the limited resources into external environmental resources and intracellular resources to distinguish between different drivers. While external environmental resources provide the necessary conditions for the survival of the community, intracellular resources regulate the characteristics and dynamics of the communities. Finally, we discussed ways to optimize the models considering full metabolic regulation, combining automated modeling and machine learning to improve prediction accuracy and the direction of microbial community modeling.

2. Theoretical basis and development of genome-scale metabolic model

GEMs provide a mathematical description of the complete gene set of an organism, encompassing various biochemical reactions and metabolic relationships. This includes the processes of synthesis, catabolism, and substance transformation [25,26]. GEM is a critical tool for conducting system-level metabolic studies and combines gene annotation with experimental data while associating gene-protein reactions with metabolic pathways. This bottom-up construction paradigm offers highly precise metabolic insights and helps elucidate the behavior and adaptability of microorganisms across diverse environments. Using constraint-based reconstruction and analysis (COBRA) and elementary mode analysis (EMA), GEMs simulate the metabolic states of organisms across different environments, facilitating the study of interactions among organisms [21]. FBA is the most widely used algorithm for COBRA. In contrast to traditional ecological modeling based on ordinary differential equations, FBA can more precisely quantify cellular metabolism and help in evaluating the role of different metabolites in shaping microbial characteristics and functions [27,28]. Unlike other COBRA approaches, such as agent-based and graph-based methods, FBA simplifies the complex metabolic network into a linear programming problem, which significantly decreases the number of parameters and the complexity of the model. This simplification can immensely help in analyzing large-scale microbial communities. Research on FBA over the last three decades has evolved from focusing on tool development at the single-strain level to addressing broader microbial community interactions, including internal interactions and adaptations to external environments (Fig. 1) [29–59]. Studies have become more comprehensive, such as those on internal interaction and external environmental adaptability. In principle, FBA can also be classified as static and dynamic models.

Static FBA characterizes the properties and behavior of a system by introducing various constraints to realize accurate model descriptions without relying heavily on biological information, such as kinetics and thermodynamics. FBA assumes that the system maintains a pseudo-steady state and applies some constraints on external fluxes (usually based on empirical data) to obtain an objective function value and a set of flux distributions [37]. The method incorporates a "biomass reaction" for quantifying microbial growth. The accuracy of this reaction is ensured by the consumption rates of precursor substances (such as proteins, nucleic acids, and lipids) that are necessary for cell growth, which are measured through various experiments [60].

Static FBA has some major limitations. First, although static FBA can provide the distribution of metabolic flux, it cannot capture information on the changes in the concentration of exchangeable metabolites over time, which is required for studying the dynamic behavior of microorganisms [61]. Second, FBA struggles to quantitatively analyze the interactions of various components in complex microbial communities, which prevents a deeper understanding of microbial ecology. To address these issues, Mahadevan et al. [32] developed the dynamic flux balance analysis (dFBA). In dFBA, the most commonly used mode of operation is static dFBA (SOA), which uses the explicit Euler method to discretize the problem and divide time into multiple time intervals. At the beginning of each time interval, instantaneous FBA is used to predict the solution of the problem, and the results are then iteratively used to refine the outcomes of the previous intervals. Ultimately, these time intervals are dynamically integrated to predict metabolite concentrations over time. Another method of implementing dFBA involves integrating FBA with ordinary differential equations (ODEs). In this approach, ODEs supply the kinetic parameters to constrain the entire model, while FBA resolves it, thus capturing the dynamics of the biological processes with greater accuracy.

Correspondingly, GEMs of the microbial communities can also be divided into static and dynamic types, but some researchers have proposed corresponding modifications to specific requirements. Further details on the construction framework of the community-level GEMs and the introduction for the static and dynamic ones are presented in the following sections.

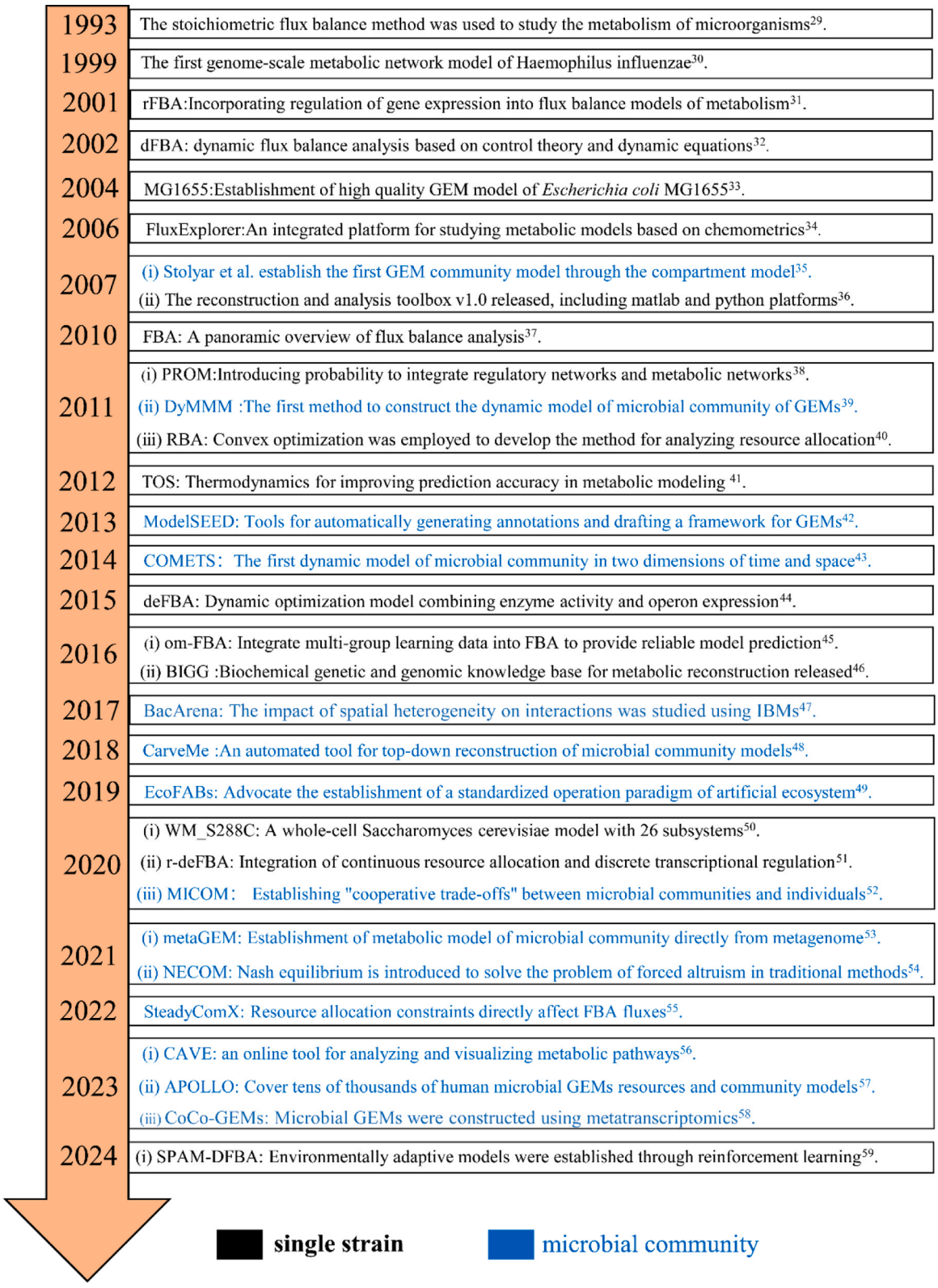

Fig. 1. Timeline of selected highlights in GEMs. Mainly, it can be divided into the levels of single strain (black) and microbial community (blue).

3. The construction framework of the GEMs for microbial communities

Synthetic biology is shifting toward consortium-based synthetic ecology. This shift necessitates substantial advancements in model-based deciphering and designing of various microbial communities. The reconstruction of microbial community models is considerably more complex than modeling a single strain. Different members of the community occupy different ecological niches, and changes in population size, settlement environment, and time, can lead to differences in metabolic states, which can alter their roles within the community. Additionally, the community must maintain a balance between the

interests of individual members and those of the whole community to maintain overall welfare. Simply concatenating individual models often fails to capture these dynamics, undermining the significance of constructing microbial community models. Therefore, we proposed a framework (Fig. 2) for constructing community-level GEMs by integrating current approaches to decipher and design different microbial consortia.

The accuracy of biochemical information in single-strain models is the foundation of further applications for microbial communities. Each microorganism has unique metabolic pathways and growth characteristics. These organisms form complex metabolic networks through interactions, which play a key role in maintaining the stability and function of the community. Various tools have been recently implemented to refine the metabolic modeling of individual strains, such as BIGG [46], ModelSEED [42], etc. Thus, by using these tools, an accurate description of the metabolic pathways of every microorganism in microbial communities can be achieved to construct high-quality community models (Fig. 2A).

Secondly, the characteristics of the microbial symbiont should be considered, e.g., the size of the community and the relationship between microorganisms (Fig. 2B). Mixed-bag community modeling is a suitable approach for building large-scale communities, in which the whole community is regarded as a single organism with all members sharing the same metabolic pool. This approach facilitates investigating community function at a holistic level without focusing on the interactions between individual members. Additionally, incorporating temporal and spatial dimensions allows dynamic changes to be tracked within microbial communities from a broader perspective.

Thirdly, more information on multi-omics and thermodynamic characteristics should be introduced into the community-level model. Multi-omics approaches allow more accurate representations of the metabolic states of community members, identification of key interactions, and comprehensive resolution of community models. Introducing kinetic and thermodynamic equations helps exclude physiologically implausible solutions and increases prediction accuracy (Fig. 2C). Kinetic equations also reflect internal resource regulation mechanisms and capture physiological dynamics like growth, death, and migration.

Finally, the selection of optimization objectives might vary to suit different applications more effectively (Fig. 2D).

(I) Internal analysis of microbial communities. To cope with varying environmental conditions, organisms undergo adaptive modifications, including changes in the intensity and distribution of metabolism. In such types of analyses, precise functions need to be implemented to accurately monitor the sensitive fluctuations in microbial metabolic flow.

(II) Nutrients cross-feeding among colonies. Community members shape collective phenotypes and exchange resources through direct and indirect interactions. Although various methods are available to accurately predict microbial interactions, a comprehensive model that integrates cellular gene regulation, interbacterial interactions, and metabolic feedback needs to be constructed. This holistic approach can substantially improve our understanding of microbial community dynamics.

(III) Consortia-based coculture fermentation. Microbial consortia are being increasingly employed for producing high-value chemicals. A reliable mixed-bacteria model needs to be established to simulate and optimize fermentation conditions to enhance production efficiency. Modeling stable communities for maximizing metabolite production necessitates a dual focus on production and growth. This balance is needed to achieve optimal results in microbial community modeling and industrial applications.

(IV) Self-regulation to environmental disturbance. Microbial communities cope with changes or disturbances in their external environment using various strategies, such as resource allocation, functional redundancy, and adaptive evolution. These communities adapt to environmental pressures through self-regulation, which aims to maximize cell growth besides meeting other objectives. Thus, this adaptive process may lead to suboptimal growth rates, reflecting a compromise between survival and optimal growth.

(V) Multilevel host-microbe-phage interactions. Pathogens grow more efficiently compared to non-pathogenic organisms by utilizing host metabolic pathways, whereas phage replication leads to resource depletion and significant disruption within the biological community. This vertical biological hierarchy needs to be examined, as it might aid in the treatment of diseases.

4. Static GEMs for microbial communities

Genome-scale metabolic models (GEMs) offer a comprehensive, interpretable, and scalable method for modeling complex interactions for different microbial communities. The modeling principles used in GEMs for microbial communities are similar to the individual FBA approach, where the entire system is maintained in a pseudo-steady state, i.e., there is no accumulation or depletion of metabolites [60]. Compartmental modeling, also known as joint-FBA [62], is a commonly used method for the static GEMs of microbial communities. Unlike certain methods that integrate the metabolic networks of multiple strains into a single hybrid network, compartmental modeling places different strains in separate compartmentalized spaces (similar to different organelles within a cell), and these compartments exchange metabolites indirectly with the external environment [63]. After Stolyar et al. [35] proposed the joint-FBA model for metabolic networks of microbial communities, this approach has been extensively implemented to manage parameters among different strains, e.g., to predict relative abundance and investigate intricate interactions [64].

Usually, in joint-FBA, the biomass flux of a microbial community is used as the objective function, often employing a non-uniform distribution of weights. This setup can yield solutions with different metabolic combinations, thus additional constraints need to be imposed to integrate the components effectively. The joint-FBA framework primarily restricts cellular growth at the population level but ignores the limitations on the growth rate of each strain; this can cause fast-growing strains to dominate the system, while slower-growing strains may become extinct. Additionally, joint-FBA often combines the concepts of exchange flux among strains with individual exchange flux, leading to confusion.

To address these limitations, several researchers, including Vera et al. [65], developed the community flux balance analysis (cFBA), which assumes a community-based equilibrium state where all microorganisms grow at a constant rate. This approach integrates metabolic activity with relative species abundance to determine the optimal community growth rate, posing a nonlinear optimization problem. However, the nonlinear multi-objective function framework of cFBA cannot be easily applied to large-scale microbial communities, as the computational demand grows exponentially with an increase in the number of microbes involved.

A gap exists in constraint-based modeling and functional analysis of metagenomic data. To bridge this gap, Baldini et al. [66] developed a comprehensive and suitable toolbox, known as the Microbiome Modeling Toolbox, for predicting metabolic functions and interactions of different microbial communities. Xiang used microbial sequencing data from fecal samples of boys with diet-induced Prader-Willi Syndrome, mapping them to the gut microbial model AGORA to demonstrate how folate production by *Bifidobacterium longum* contributes to weight loss through model-based simulations [67].

In addition to assisting in the analysis of microbial interactions, GEMs are also often used to design microbial communities. For example, Chan et al. [62] developed the SteadyCom framework to ensure the coexistence and high stability within microbial populations. In this

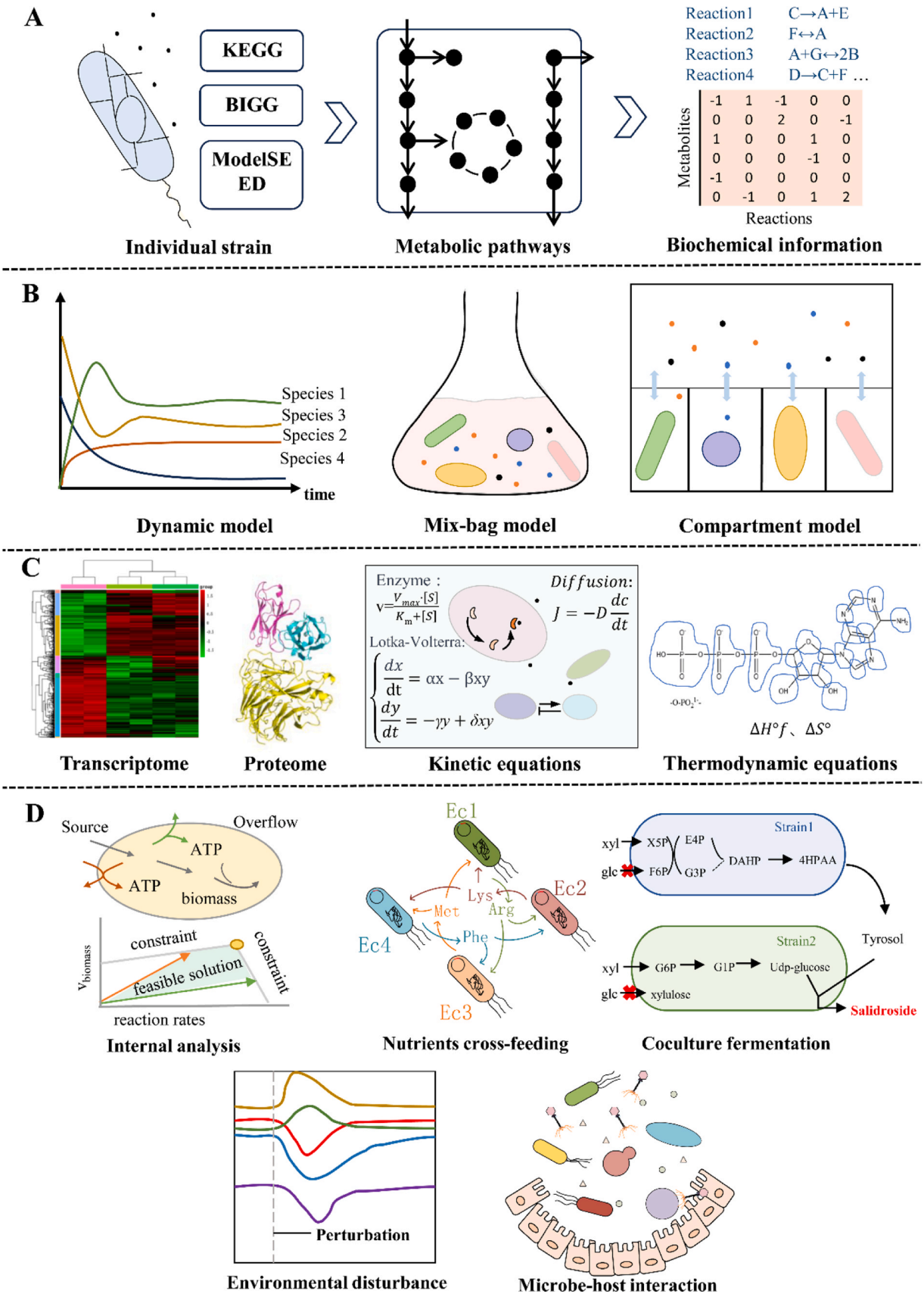

Fig. 2. Steps of reconstructing the GEMs for microbial community. A. Accurate description of the metabolic pathways and biochemical information of single strains in constructing individual high-quality GEMs. B. Selecting the right modeling method based on community characteristics. C. More information on multi-omics and thermodynamic characteristics should be introduced into the community-level model. D. Selecting the optimization objectives to suit different applications.

framework, the model system consists of four bacterial strains that participate in cross-feeding dynamics. It includes three mutants, where each mutant lacks two essential amino acids but can synthesize a different amino acid and thus rely on others for the missing nutrients. By integrating total biomass with cell growth rates, SteadyCom solves the problem of colony collapse, which is attributed to differential growth advantages among strains. This approach ensures that any mutant strain contributing to the nutrition of other strains has a minimum viable biomass in the system, which is proportional to its amino acid production capacity; thus, this approach promotes stable colony growth. The framework also predicts strong competitive and collaborative relationships among strains; specifically, colony interactions are predicted to become more cohesive as growth rates increase. Michael A. Henson used 16 S rRNA data to identify the genera within a microbial community and assess their relative abundances in samples from cystic fibrosis patients. They used SteadyCom to predict how environmental changes influence sample heterogeneity and increase pathogen prevalence [68]. Unlike cFBA, which solves nonlinear programming (NLP) problems, SteadyCom uses an iterative approach to solve linear programming (LP) problems, enabling faster convergence.

To design the metabolic processes in microbial communities, Ali et al. developed an innovative framework called OptCom [69], which introduces a multilevel, multi-objective optimization strategy that addresses suboptimal growth in communities by differentiating between the optimization processes of individual strain biomass and overall community growth. Unlike traditional methods that rely on a single objective function, OptCom uses a multi-tiered objective function to balance the internal optimization needs of individual strains with the external goals of the entire community. This approach can be used to resolve conflicts between biomass and yield optimization encountered in microbial communities. It provides a highly customizable strategy for designing efficient microbial consortia.

5. Dynamic GEMs for microbial communities

Resource utilization influences various microbial interaction, such as cooperation and competition [70]. Dynamic GEMs reveal the intricate mechanisms underlying intracellular resource allocation among microorganisms and can simulate the dynamic processes of resource competition and cooperation in different microbial communities. The survival of the community depends on the environmental conditions, including spatial structure, substrate resources, etc [71]. Competition for these external resources acts as a primary driving force and influences the assembly of microbial communities [72,73].

In microbial cells, all metabolic reactions incur a metabolic cost; they are catalyzed by a few enzymes and constrained by cellular dynamics [16,51]. Therefore, microbes must dynamically regulate resource allocation to maximize their efficiency. This regulatory mechanism allows microorganisms to adapt to different environmental conditions and makes them superior competitors in microbial communities. In natural environments, microbial communities frequently display suboptimal growth rates to support new community functions. In a study, the concept of abundance-growth space was introduced as a quantitative metric for assessing the metabolic phenotype of these communities. This metric accurately describes how microbes adjust their metabolic flux distributions in response to environmental changes [74]. In this context, we summarized the advancement in research on dynamic GEM modeling of the community from the perspectives of the external environment and intracellular resources, respectively.

5.1. External environmental resources

The dFBA is a powerful tool that can capture the dynamics of microbial communities across different time points. Additionally, incorporating spatial scales can increase our understanding of the spatial distribution and interactions of microbial communities across various environments. The spatial differentiation of resources significantly affects local microbial interactions [75,76]. In response to resource scarcity, microbial communities may expand and merge spatially to maintain stability [77,78]. Therefore, the quantitative analysis of the temporal and spatial changes in different microbial communities is necessary.

Harcombe et al. [43], introduced the COMETS tool, a novel approach related to the dynamic GEMs of microbial communities. COMETS integrates a multi-species dynamic FBA with a molecular diffusion model to illustrate the spatial distribution of microbial cells. This tool divides a given space into lattice cells, each potentially containing different species, and uses dynamic FBA to calculate the biomass and metabolite concentrations in each cell. COMETS employs standard two-dimensional diffusion equations to simulate the movement of substrates and byproducts between cells, simulating growth and diffusion steps. In a study, COMETS was used to investigate the effect of partner species and nutrients on butyrate production by *F. prausnitzii*. The results showed that butyrate production increased in the co-culture system, which matched experimental observations. However, butyrate production did not increase linearly with an increase in substrate availability, indicating the presence of a complex interaction pattern among microorganisms for the optimal production of butyric acid. This finding provided valuable insights into the intricate actions of gut microbial communities [79]. The recent update to COMETS 2.0 further refined the model, incorporating extracellular enzyme activities and the diffusive and convective movements of biomass. It also added modules for the evolution of the microbial community, cyclical environmental changes, and host simulations [80]. Michael Quintin integrated the kinetics of cellulose hydrolases into an existing model of *Saccharomyces cerevisiae* to simulate the secretion and catalytic functions of extracellular enzymes [81]. The results showed that microbes adopt an optimal enzyme production strategy to maximize the benefits derived from extracellular enzymes. Applying this method to microbial communities can provide novel insights into competition and cooperation within microbial consortia, including the dynamics of "cheaters.".

To facilitate the design of different microbial consortium models, García-Jiménez et al. [82] developed the automated integration tool FLYCOP, which utilizes metabolic models generated by COBRA and allows users to select parameter combinations of interest. It simulates the dynamic evolution of the consortium using COMETS and uses a stochastic localized search (SMAC) to iteratively refine the protocol. FLYCOP offers highly flexible parameter settings, including options for the initial inoculation ratio, cross-feeding rate, composition of the medium, and selection of members.

Based on the individual agent-based approach, Maranas et al. [47] constructed BacArena to study complex dynamics among interacting strains. In this model, a two-dimensional grid hosts individuals with distinct metabolic profiles, facilitating the flow of metabolites through diffusion. This method is effective for studying heterogeneous microbial communities that vary due to differences in spatial resources. It is used to investigate how spatial or trophic gradients influence microbial community structures, such as those induced by the distribution of mucopolysaccharides in the gut.

To address the spatiotemporal heterogeneity in the distribution of gut microbiota, Jun Geng proposed a multi-scale framework for COmputing the DYnamics of microbiota (CODY) [83]. This framework captures the dynamic processes of microbiota by establishing an enzyme-centered regulatory layer that allows microorganisms to switch between multiple metabolic modes. Additionally, CODY introduces an external resource allocation framework that translates higher-order colony interactions into exchange fluxes of small molecules of common metabolites. This approach significantly reduces the reliance on the a priori knowledge typically required by FBA tools. CODY also considers the structural variations across different regions of the colon and accurately predicts the dynamics of microbial communities in the intestinal lumen and mucus.

5.2. Intracellular resource regulation

Microbes require material and energy to perform various physiological activities. Although metabolic flux can be facilitated by sharing metabolites among microbes, the export of costly metabolites (e.g., proteins and nucleic acids) from cells is generally restricted to ensure that their interests are maintained [84,85]. These macromolecules play important roles, such as catalyzing, transporting, and regulating reactions and pathways, which are essential for the survival and proper functioning of cells.

Traditional metabolic models cannot accurately quantitatively characterize gene expression and regulation, which limits the detailed resolution of metabolic responses. Integrating multi-omics data with metabolic network modeling (GEM) is an effective approach for analyzing complex ecosystems. Metabolic network models include information on macromolecules such as DNA, RNA, proteins, and metabolites. The advancement of high-throughput biotechnologies has provided researchers with transcriptomic, proteomic, and other multi-omics data, which provide valuable tools for regulating metabolic networks, allowing researchers to perform more precise and comprehensive analyses.

There are two main approaches to using transcriptomic information for constraining metabolic networks. The first approach uses Boolean logic rules (ON/OFF) to impose constraints and discretize the growth stages for iterative analysis. PROM [38] is a novel strategy to combine metabolic networks; it describes the phenotypic associations between genes and regulatory networks incorporating regulatory constraints. PROM is used to characterize the probability of gene states or transcription factor-gene interactions by analyzing large amounts of transcriptomic data. Unlike other Boolean logic approaches, where only two cases (on and off) indicate the state of a metabolic response flux, this approach, which uses the active frequencies of genes, can quantify the strength of cellular regulation. MTBPROM 2.0, a specific model for *Mycobacterium tuberculosis*, was used to map 104 transcription factors to 810 genes in the model and construct a regulatory-metabolic knowledge base [86]. It can predict the effects of environmental factors on the phenotype of *M. tuberculosis*, including the overexpression of transcription factors and the synergistic effects between anti-tuberculosis drugs, thus providing a novel therapeutic tool for treating tuberculosis.

However, the mapping of such interactions is incomplete and often limited to only a few typical environmental conditions. IDREAM addresses this limitation by introducing statistically inferred Environmental and Gene Regulation Influence Networks (EGRIN) to enhance the function of PROM and broaden its application to more complex eukaryotic cells. Moreover, datasets often contain significant interaction noise due to the absence of large-scale data regulation [87]. The Gene Expression and Metabolism Integrated Network Inference (GEMINI) algorithm resolves the discrepancies between model predictions and experimental results by incorporating new regulatory interactions based on an iterative process [88]. TRIMER constructs Bayesian networks using a priori knowledge, such as gene expression profiles, to more efficiently capture global transcriptional regulatory relationships [89].

Another technique used to combine transcriptomic information involves analyzing the correlation between phenotype and gene expression, such as SR-FBA, which can be used to quantify changes in metabolic behavior due to constraints in metabolic and transcriptional regulation at different levels in *E. coli* [90]. This method revealed a direct connection between gene expression levels and phenotypic traits, which enhanced our understanding of how genes affect the function and identity of organisms. Similarly, OM-FBA was used to develop a "phenotype-matching" algorithm to accurately assess target function yields [45].

The abundance of mRNAs in the transcriptome does not always correlate with the level of protein expression. Proteins perform vital functions in organisms and are directly related to cellular functions, structures, and interactions; thus, proteomic data often provide more direct constraints. Steffen Waldherr integrated metabolic networks with gene expression data of enzymes to assess enzyme production costs and capacities. Using this information, they created a dynamic optimization framework, de-FBA, for predicting changes in fluxes and biomass during metabolic adaptation [44]. Additionally, Goelzer et al. developed Resource Balance Analysis (RBA), which integrates constrained proteome allocation with cellular resource management to predict optimal resource allocation for maximizing steady-state growth rates. Sanchez et al. introduced catalytic rate constants into enzyme constraints using the GECKO model, which simulates constrained metabolism based on protein abundance measurements [91]. Qiu et al. verified the interdependence of *Streptococcus thermophilus* and *Streptococcus bulgaricus* by integrating constrained proteome assignments into a community system for yogurt fermentation [92].

Multi-omics approaches are commonly integrated with metabolic networks in medicine and health, particularly for analyzing and treating diseases. Crohn's disease, a chronic inflammatory condition of the gastrointestinal tract, is a pertinent example. In a study, transcriptomic data was used along with Recon3D, a human genome-scale metabolic network, to develop an ileum-specific model for patients with Crohn's disease [93]. This model identified significant disparities in mevalonate metabolism, fatty acid oxidation, and uridine metabolic flux in patients with Crohn's disease compared to controls, highlighting the limitations in the production of precursors necessary for immune responses. In studies on cardiovascular diseases, the iCardio model used transcriptomics data to show that a decrease in the level of expression of genes involved in synthesizing nitric oxide and N-acetylneuraminic acid is a common marker of heart failure [94]. Similarly, by combining proteomic data and using the HMR 2.0 network with the tINIT algorithm, Rasmus Agren constructed a personalized metabolomic model for patients with hepatocellular carcinoma. This model can be used to assess the effectiveness of antimetabolite drugs in inhibiting tumor growth, and thus, provide crucial insights into personalized treatment strategies [24].

Note that when resources are scarce, the microbial community will spontaneously develop a complementary metabolic division of labor to maximize the efficiency of resource utilization. However, synthetic microbial consortia are often unstable and require more comprehensive GEMs to help establish and maintain a stable division of cooperative labor. Therefore, accurate and real-time dynamic GEMs will enhance the design of microbial communities in improving their controllability and efficiency. Table 1.

6. Concluding remarks and future perspectives

Quorum sensing (QS) is a key molecular communication mechanism in microbial communities and regulates their social behavior across spatial and temporal scales to adapt to environmental changes [101]. QS enables microorganisms to control key metabolic pathways, such as the TCA cycle, purine nucleotide synthesis, and the pentose phosphate pathway, through the secretion and detection of signaling molecules. Research on incorporating population sensing into GEMs is limited and primarily focused on modeling single strains. For example, some researchers used transcriptomic data from *Xanthomonas campestris* to model how resources are directly allocated for the growth and biosynthesis of xanthan gum. The findings provided insights into the mechanism of infection caused by this species [102]. In microbial communities, members continuously monitor environmental changes, displaying behaviors ranging from self-centered to altruistic depending on cell density. Understanding these behaviors is crucial for characterizing complex ecological dynamics. Additionally, while GEMs are useful, they often cannot capture the interactions beyond competition. Particularly, interactions such as the production of antibiotics and toxins also contribute to comprehensively understanding the dynamics of microbial communities.

Building multi-scale, multi-omics metabolic models is a challenging

Table 1
Summarization of the characteristics and applications for the static and dynamic community-level GEMs.

Types	Methods	Scale	Language	Advantages and limitations	Applications
Static	cFBA	Small	Python	◆ Fewer parameter assumptions. ◆ Computational cost increases with community size.	Suitable for modeling slow-growing or equilibrium microbial communities[65].
	SteadyCom	Large	MATLAB/ Python	◆ Compatible with flux variance analysis (FVA). ◆ The computational cost is independent of the number of community members. ◆ The assumption is that the community's growth rate (μ) is known.	Changes in abundance observed in a gut microbiota model consisting of nine species[62].
	Microbiome Modeling Toolbox2.0	Large	MATLAB	◆ Describe microbe-microbe and host-microbe interactions. ◆ User-friendly and easy to visualize.	Simulation and interpretation of pairwise microbe-microbe and host-microbe interactions[95].
	OptCom	Small	Python	◆ Using multi-objective optimization to weigh individual and group level conflicts of interest. ◆ Capture all types of interbacterial relationships. ◆ Requires multiple rounds of data processing.	Interaction between Bifidobacterium adolescentis and Faecalibacterium prausnitzi[96].
	Redcom	Large	MATLAB	◆ Eliminates impractical solutions through network transformations. ◆ Lacks detailed information on internal fluxes.	Construction of a simplified biogas plant community model[97].
	CASINO	Large		◆ Accounts for the uniqueness of each community member. ◆ Requires large amounts of experimental data as parameter input.	Prediction of dietary requirements to maintain normal metabolic levels in the gut[18].
	MICOM	Large	Python	◆ Incorporates additional and metagenomic information to predict biological abundance. ◆ Good expandability. ◆ Depends on a wealth of reliable functionally annotated information.	Exploration of ecological principles shaping the microbial landscape in the gut system[52].
Dynamic	DMMM	Large	MATLAB	◆ Modeling that incorporates multiple metabolic patterns. ◆ Dynamics of populations and their metabolite concentrations can be predicted.	Predicting competitive relationships between microorganisms as affected by nitrogen source[39].
	DFBAlab	Large	MATLAB	◆ Avoiding the infeasible linear programming problem associated with the ODE equation. ◆ Provide penalty functions suitable for optimization purposes.	Modeling a two-species oral biofilm[98].
	d-OptCom	Small		◆ Relative differences in the transport and utilization of metabolites among different members. ◆ Not recommended for polymicrobial models with single competition.	Assessing the dynamics and composition of uranium-reducing communities in a three-bacterial system[99].
	COMETS	Large	MATLAB/ Python	◆ Integration of knowledge in diffusion kinetics, growth kinetics, evolutionary kinetics, and enzyme kinetics. ◆ Complexity involved in the numerical integration of convection-diffusion equations. ◆ Not applicable to study phenotypic cell-to-cell variability in a population.	Examining microbial growth, competition for resources, metabolic exchange, and evolution[79].
	FLYCOP	Small	Python/R	◆ Applicable across a variety of scenarios. ◆ Many parameters to configure.	Optimizes a consortium to produce the maximum amount of bio-plastic[82].
	μbiasim	Large	MATLAB	◆ Eliminates the need to predefine functional objectives and compound assignments. ◆ Requirement for accurate microbial monoculture data.	Culturing a batch of hydrogenotrophic archaea [100].
	BacArena	Small	R	◆ Spatio-temporal multidimensional community models combining flux balance analysis and individual modeling. ◆ Need for high quality individual models.	Observing spatial variations in metabolic phenotypes within biofilms[47].
	CODY	Large		◆ Calculation of spatio-temporal specific changes in absolute and relative abundance profiles within gut microbial communities. ◆ Quantifying the effects of nutrients and hosts.	Gaining insight into the biogeographical heterogeneity of the lumen, mucus, and feces[83].

task that requires collecting, analyzing, and processing many biological datasets [103]. Machine learning aids metabolic modeling, as it can deeply analyze datasets to extract key features, thus providing reliable inputs [22]. The Data-driven Keystone Species Identification (DKI) framework uses deep-learning models trained with samples from community habitats to reveal the structure of specific colonies. Through species-removal thought experiments, DKI quantifies the keystone species within any given community (e.g., gastrointestinal tract, soil, etc.), aligning with the recognized core structure of these communities [104]. Moreover, machine learning can be integrated directly into traditional metabolic modeling techniques to increase the accuracy of algorithmic predictions. For example, the Simple Constrained Artificial Bee Colony Flux Balance Score (SCABCFBA) is a hybrid algorithm that combines colony intelligence optimization with Flux Balance Analysis (FBA) methods. This algorithm effectively addresses the overproduction of lactic acid and succinic acid in *Escherichia coli* [105]. Machine learning also trains on model predictions to elucidate intricate ecological mechanisms. Joon-Yong Lee used neural networks to model interactions and spatiotemporal variations between organisms. Using this approach, they accurately predicted the effects of spatial distribution on biotic interactions [106]. However, machine learning approaches have certain limitations. The complexity of microbial community genotype-phenotype relationships, influenced by several factors, implies that low-quality or insufficient datasets may lead to model overfitting. This can result in inaccurate predictions, and the models cannot be generalized to other ecosystems. Additionally, the "black box" nature of machine learning often obscures the underlying causal relationships and ecological structures, making it hard to interpret how model weights and parameters are interconnected. Finally, due to the strong correlation in microbial community data, meticulous data processing is required to mitigate issues such as sampling bias, data noise, and data downscaling.

Analyzing and reconstructing the synthetic microbial consortia

based on GEMs for microbial ecology and synthetic biology is challenging. By mapping the metabolic capabilities of different organisms, GEMs help design and optimize microbial communities for specific functions. While GEMs for well-studied strains, such as *E. coli* (iML1515 [107]) and *S. cerevisiae* (Yeast 8 [108]), are relatively comprehensive, tractable models for non-model strains and uncultured strains have not been developed. As non-model strains dominate the microbial community, standardized process paradigms, computational tools, and evaluation criteria need to be developed to be applicated in different community models. Many software programs for automated synthesis of GEMs have been developed, such as RAVEN2.0 [109], CarveMe [48], and AutoKEGGRec [110], which facilitate important modeling steps such as data collection, gene annotation, gap filling, evaluation, and model storage. However, automated reconstruction tools may sometimes produce incomplete or incorrect annotations, and thus, should be used with caution. In these methods, manual intervention is often required to manage the data, resolve blocking reactions, perform detailed annotations, and determine the directionality of reaction [111]. The diversity of biomarker languages, different levels of gene annotation, and different methods for judging performance all contribute to the complexity of modeling efforts. Thus, standardized processes, computational tools, and evaluation guidelines need to be established to build and expand diverse community models [112]. The absence of highly operational minimal metabolic community models and shared platforms is an additional challenge to the reconstruction of microbial community models. Some researchers have introduced the concept of reproducible fabricated ecosystems (EcoFABs) to create minimal microbial communities based on functional characteristics for developing functional models that can be utilized across different laboratories [49]. Osiel S. Gonçalves used a metabolic complementarity approach involving microbes and hosts to establish a minimal microbial community that retains plant growth-promoting features. This community was scaled down to 4.5 times its original size, and six key species were identified within the community [113]. Finally, we believe that establishing open resource-sharing platforms, such as Metabolic Atlas [114], can aid in constructing more comprehensive GEMs and extend the application field of microbial communities.

CRediT authorship contribution statement

Jianjun Qiao: Conceptualization, Project administration, Supervision. **Qinggele Caiyin:** Conceptualization, Project administration, Supervision. **Hao Wu:** Visualization, Writing – review & editing. **Shengbo Wu:** Data curation, Formal analysis, Writing – original draft. **Danlei Chen:** Visualization, Writing – review & editing. **Zheping Qu:** Data curation, Formal analysis, Writing – original draft.

Declaration of Competing Interest

The authors declare no competing interests.

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