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# Community-scale models of microbiomes: articulating metabolic modelling and metagenome sequencing

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**RUNNING TITLE.** Metagenomics and metabolic modelling in microbiomes

**ABSTRACT.** Building models is essential for understanding the functions and dynamics of microbial communities. Metabolic models built on genome-scale metabolic network reconstructions (GENREs) are especially relevant as a means to decipher the complex interactions occurring among species.

Model reconstruction increasingly relies on metagenomics, which permits direct characterisation of naturally-occurring communities that may contain organisms that cannot be isolated or cultured. In this review, we provide an overview of the field of metabolic modelling and its increasing reliance on and synergy with metagenomics and bioinformatics. We survey the means of assigning functions and reconstructing metabolic networks from (meta-)genomes, and present the variety and mathematical fundamentals of metabolic models that foster the understanding of microbial dynamics. We emphasise the characterisation of interactions and the scaling of model construction to large communities, two important bottlenecks in the applicability of these models.

We give an overview of the current state of the art in metagenome sequencing and bioinformatics analysis, focusing on the reconstruction of genomes in microbial communities. Metagenomics benefits tremendously from third generation sequencing, and we discuss the opportunities of long read sequencing, strain-level characterisation, and eukaryotic metagenomics. We aim at providing algorithmic and mathematical support, together with tool and application resources, that permit bridging the gap between metagenomics and metabolic modelling.

**KEYWORDS.** Metagenomics - Metabolic networks - Modelling - Microbial community - Third generation sequencing

## 1. Introduction

The roles of microbiomes in the environment and in health-associated, agricultural or biotechnological applications is widely acknowledged [Peixoto et al., 2022, Foundation et al., 2022, Wilmes et al., 2022]. These assemblies of microorganisms are governed by the interactions they have with their environment, and by those occurring among species [Zengler and Zaramela, 2018]. Such ecological interactions can generally be classified as neutral, positive, or negative; and can be inferred through network analysis [Faust and Raes, 2012]. Mechanistically, they operate at a similar level, called the *metabolism*. Metabolism encompasses all of the functions of cells, defined by biochemical reactions and by molecular exchanges between cells and the environment. Microbiomes are characterised by their biosynthetic potential and by the taxonomic diversity that they harbour [Heintz-Buschart and Wilmes, 2018]. While some microbiomes such as the gastro-intestinal tract in humans have been extensively studied, thereby revealing insights into the putative cooperation and competition networks among bacteria [Coyte and Rakoff-Nahoum, 2019], in general the precise mechanisms, dynamics and control levers of metabolic interactions remain poorly understood. These difficulties are even more prevalent in complex environmental microbiomes where the massive diversity of micro-organisms and their poor representation in public databases impede taxonomic and functional surveys [Brown et al., 2021].

Culture-independent methods can be used to broadly characterise microbiomes [Zhang et al., 2019].

55 Prominent among these is metagenomics, the random sequencing of all DNA in a given sample. Metage-  
56 nomics is by now ubiquitously applied to decipher both the taxonomic and functional diversity of samples;  
57 a rich resource of public available metagenomes can support large scale studies available to even small  
58 labs with the required bioinformatics expertise [Gurbich et al., 2023]. Analysis of metagenomic data by  
59 bioinformatic tools can generate surveys of the functions embodied by the community [Kindler et al., 2021].  
60 Metabolic models, either mechanistic models such as those reconstructed from genome-scale metabolic net-  
61 work reconstructions (GENREs), or phenomenological models describing phenotypic traits, go further than  
62 these surveys. The latter have been the modeller’s choice for narrowing down the complexity of community-  
63 wide behaviour [van den Berg et al., 2022], whereas the former could originally only be applied to single  
64 species [Varma and Palsson, 1994]. However the range of modelling tools has strongly increased during the  
65 past years, and now provides a broad collection of methods for answering critical questions about microbial  
66 communities [Kumar et al., 2019]: what are the roles of bacterial species in the ecosystem? What functions  
67 are carried and how redundant are they? How will the community behave over time? And, can we alter  
68 this behaviour, or modify the community composition to reach a desired state?

69 Recent developments in metabolic model inference facilitate this procedure and make it worthwhile  
70 to consider systematic reconstruction for collections of genomes [Frioux et al., 2020]. Concurrently, al-  
71 gorithmic resources for obtaining such genomes from metagenomes have increased, and the technological  
72 development of third generation sequencing (TGS) has paved the way to a major change of paradigm in the  
73 study of microbiomes [Sereika et al., 2022]. Our goal here is twofold: (1) to review to what extent metabolic  
74 modelling and metagenomics can interact in service of better comprehending microbial ecosystems, (2) to  
75 provide comprehensive lists of practical tools that can be used by non-specialists for metagenomics assem-  
76 bly, metabolic reconstruction, and microbiome modelling. We describe the different kinds of models that  
77 can be built from metagenomic data to predict the functions and the dynamics of a microbial community,  
78 and how they can be used to infer interactions among species. As these models rely on reconstructions of  
79 metagenomes, we present as well the latest sequencing techniques, bioinformatics tools and open challenges  
80 related to metagenomics, in an attempt to draw together these two domains of computational biology.

## 81 2. From gene functions to metabolic reconstructions

### 82 2.1 Aggregating functions into metabolic networks

83 Models of microbial communities can be derived from a subset of functions, reactions, or metabolic path-  
84 ways; or on the contrary by analysing the entire metabolic potential of the genomes. In the latter case,  
85 the exploration of the link between the genome, enzymes, and the metabolic reactions they catalyse  
86 provides a system-wide understanding of the physiology of the cell and its links with its environment.  
87 *Genome-scale metabolic network reconstructions* (GENREs) contain metabolic reactions predicted from  
88 the entire genomic content through gene-protein-reaction (GPR) relationships, but also spontaneous reac-  
89 tions and transport reactions connecting the cell to its environment or even to intracellular components  
90 [Thiele and Palsson, 2010]. In this section, we present the basis of such metabolic networks, whose fun-  
91 damental concept is the association of genes to functions. Since GENREs are at the heart of mechanistic  
92 models of metabolism, the first step of community metabolic modelling is typically the acquisition of a  
93 GENRE for each of its members.

94 GENREs have been built for more than twenty years, with a spectacular acceleration of the publications  
95 over the past years, as a result of both the increased availability of genomes, and of the efforts of the  
96 community for tools development. In 2019, a review tallied more than 6,000 published GENREs, 94% of  
97 which were for bacteria [Gu et al., 2019]. However, the numbers rise even more when including systematic  
98 and automatic reconstructions performed on publicly available genomes by platforms such as BioCyc  
99 [Caspi et al., 2019], or AGORA2 for human-associated micro-organisms [Heinken et al., 2023]. Table 1  
100 gathers databases that can be used as general or specialised resources to functionally annotate genomes  
101 prior to GENRE generation, as well as collections of metabolic reconstructions. In addition, *de novo*  
102 reconstruction is facilitated by a variety of software programmes and toolboxes created for high throughput  
103 processing of genomes, an important criterion when tackling large-scale communities of micro-organisms.  
104 While many of tools, catalogued in dedicated reviews [Mendoza et al., 2019], enable this task, only a small  
105 number can do so fully automatically, without user interaction. We hereafter present a sample of tools  
106 that we believe are suitable for systematic metabolic reconstruction of GENREs starting from a list of  
107 annotated genomes.

108 Gapseq [Zimmermann et al., 2021] builds GENREs using a curated reaction database and a dedicated  
109 gap-filling algorithm. The tool was notably applied to a four-strain microbial community whose associated

**Table 1.** Main generalist and specialised functional annotation databases and resources of existing metabolic network reconstructions

Category	Name	Content	Application for metabolic modelling
Generalist databases	KEGG	Several databases including biochemical reactions [Kanehisa et al., 2021]	General annotation of genes and genomes that can be directly or indirectly (via reconstruction tools) associated to metabolic reactions
	COG	Clusters of orthologous genes [Galperin et al., 2020]	
	Gene Ontology	Knowledge base of gene functions [Galperin et al., 2020]	
	UniProt	Association of proteins to functions [Consortium et al., 2021]	
	Pfam	Classification of proteins into families and domains [Mistry et al., 2020]	
	BRENDA	Collection of enzymes and their metabolic reactions [Chang et al., 2020]	
Metabolic reconstruction collections	eggNOG	Orthologous groups and functional annotation [Huerta-Cepas et al., 2018]	Ready-to-use reconstructions and models
	MetaCyc	Encyclopaedia of metabolic reactions [Caspi et al., 2019]	
	BioCyc	More than 20,000 pathway-genome databases [Karp et al., 2019a]	
	BiGG	High quality metabolic network reconstructions [King et al., 2016]	
	BioModels	Literature -associated (metabolic) models [Malik-Sheriff et al., 2019]	
Specialised databases	AGORA2	GENREs of 7,302 human-associated micro-organisms [Heinken et al., 2023]	Curation, validation, inspection of model predictions
	EMBL GEMS	GENREs reconstructed with Carveme for 5587 reference and representative bacterial genomes of NCBI RefSeq [Machado et al., 2018]	
	CAZy	Carbohydrate-active enzymes [Drula et al., 2022]	
	CARD	Antibiotic resistance [Alcock et al., 2020]	
	TCDB	Transporter classification [Saier et al., 2021]	
	Norine	Non-ribosomal peptides [Flissi et al., 2020]	
	antiSMASH	Natural products of the secondary metabolism [Blin et al., 2020]	
MEROPS	Proteolytic enzymes and their substrates [Rawlings et al., 2017]		

110 models reproduced expected behaviours of the gut microbiome metabolism. Carveme [Machado et al., 2018]  
111 uses a top down approach consisting in pruning (carving) a universal curated model of the microbial  
112 metabolism for rapid generation of GENREs starting from genomes. While construction with Gapseq  
113 takes up to two hours, a Carveme-generated reconstruction can be obtained in a few minutes. Both  
114 computation times are compatible with a systematic generation of GENREs for microbiomes. Pathway  
115 Tools [Karp et al., 2019b] builds pathway-genome databases relying on the MetaCyc database of genomes  
116 [Caspi et al., 2019] and on functionally annotations, e.g. from EggNOG-mapper [Cantalapiedra et al., 2021],  
117 Prokka [Seemann, 2014] or RAST [Overbeek et al., 2014]. While Pathway Tools is originally based on an in-  
118 teractive single-organism interface, which can impede its use at the scale of large communities, it is possible  
119 to automatise the process using, for instance, the Python package mpwt [Belcour et al., 2020]. The latter  
120 was applied to a collection of 336 reference genomes obtained after metagenomic read mapping, in order to  
121 identify the presence of pathways of interest [Ruuskanen et al., 2021]. The latest developments of Pathway  
122 Tools additionally permit importing a set of genomes or metagenome-assembled genomes (MAGs) (see sec-  
123 tion 7) into the interface to run the reconstruction on each organism. AutoKEGGRec [Karlsen et al., 2018]  
124 is a Matlab programme that creates draft GENREs by retrieving reactions and GPR relationships from  
125 the KEGG database for one or a set of organisms, making it suitable for community modelling. In addi-  
126 tion, online platforms provide reconstruction services, such as KBase [Arkin et al., 2018], which proposes a  
127 large variety of analyses including metabolic reconstruction, with extensive efforts towards reproducibility

128 through the use of narratives; or ModelSEED [Seaver et al., 2020], which performs metabolic annotation  
129 and GENRE reconstruction for microbes, plants and fungi.

130 A general limitation of GENREs lies in the metabolic landscape covered in reaction databases; many  
131 proteins have no attributed functions even in well-studied organisms, and experimental evidence of func-  
132 tions are even scarcer [Monk et al., 2014]. It must be noted that automatically reconstructed GENREs  
133 cannot compete with manually curated ones with respect to the quality of the contents and the accuracy of  
134 the simulations they permit. High-quality GENREs exist mostly for model organisms; automatically recon-  
135 structed GENREs may suffer from erroneous GPR relationships or reversibility constraints, and inaccuracy  
136 in biomass composition [Gu et al., 2019]. Most automated tools produce GENREs that are gap-filled in  
137 order for the associated model to simulate the production of biomass [Feist and Palsson, 2010], which  
138 is a common step for flux prediction (see section 3.1). Unfortunately, automated gap-filling, while use-  
139 ful, is not as reliable as manual curation for producing high quality GENREs [Latendresse and Karp, 2018,  
140 Karp et al., 2018]. Additionally, growth is simulated in media that likely do not sustain the auxotrophies of  
141 non-cultivable organisms, which may depend on complex metabolic interactions with other microbes to grow  
142 [West et al., 2007, Zengler and Zaramela, 2018]. On the other hand, it is possible to work with non-gap-  
143 filled GENREs, for example using Pathway Tools or AutoKEGGRec. Overall, the use of simulation-ready  
144 GENREs creates a risk of missing interactions occurring in communities (false negative interaction) while  
145 using non-gap-filled GENREs may falsely predict interactions to sustain certain functions that could actu-  
146 ally be performed by individual microbes (false positive interaction). We refer to [Bernstein et al., 2021] for  
147 a description of all the steps of the GENRE reconstruction and simulation processes where uncertainty may  
148 occur and lead to inconsistent results. More generally, with the increasing use of metagenomic-assembled  
149 genomes (see section 7), more attention is paid to the effect of genome incompleteness on the inference of  
150 functions and metabolic potentials [Eisenhofer et al., 2023, Belcour et al., 2020]. The examination of mul-  
151 tiple GENREs when building models of microbiomes must therefore consider these potential shortcomings,  
152 and address them through a careful choice of model paradigm and a cautious interpretation of results.

153 Obtaining a GENRE for each member of a microbial community is already *per se* a valuable resource for  
154 querying and comparing the metabolic processes that can occur within species, even before modelling and  
155 simulation. It can consist in identifying functional differences between GENREs: strain-specific patterns,  
156 gene essentiality or biosynthetic capacity [Biggs et al., 2015]. In [Weiss et al., 2022], GENREs correspond-  
157 ing to the twelve bacterial members of a synthetic bacterial community were compared at the level of

158 metabolic pathway, as a complement to results of *in vitro* interaction experiments. More generally, any  
159 approach aiming at comparing metabolic networks could be applied to GENREs of co-occurring species  
160 [Ay et al., 2012]. Likewise, studying the pan-reactome and accessory reactome of species using GENREs  
161 is valuable [Prigent et al., 2018, Belcour et al., 2023] and these approaches can be applied to ecosystems.

### 162 **3. Metabolic models for microbial communities: from GENREs to** 163 **dynamic simulations**

164 GENREs can be the input of metabolic modelling at the genome or community scale. In this section, we  
165 describe and focus on mechanistic constraint-based models. Alternative GENRE-based models and their  
166 relationship to other modelling fields are presented in section 5.

167 When modelling a community, the first step is defining a collection of individual GENREs representative  
168 of the community. The selection can be ‘reductionist’, meaning a reduced number of genomes is selected  
169 as functionally representative of the community and a GENRE is obtained for each, either from *de novo*  
170 reconstruction, or from databases (Table1). A second ‘exhaustive’ approach consists in building a GENRE  
171 for each genome encountered in the community e.g. from metagenomic analysis. Then, two alternative  
172 conceptual views exist to build a community model, with methodological and computational pros and cons  
173 (Fig. 1): one consisting in merging GENREs to build a *a community-wide model*, also termed *bag-of-genes*  
174 *model* and the other articulating individual GENREs into a *community of GENREs (bag-of-genomes)*  
175 [Frioux et al., 2020].

#### 176 **3.1 Genome-scale metabolic modelling**

**Metabolic networks, metabolic models and dynamic simulations** A GENRE has a matrix rep-  
resentation termed the *stoichiometric matrix*  $\mathcal{S}$ , with as many rows as metabolites and as many columns  
as reactions (Fig. 1). The coefficient  $\mathcal{S}_{ij}$  is the stoichiometric coefficient of the metabolite  $i$  in the reaction  
 $j$ , with a negative or positive value indicating whether  $i$  is a substrate or a product of  $j$ , or a zero value  
if the metabolite  $i$  does not appear in reaction  $j$  [Orth et al., 2010]. The matrix  $\mathcal{S}$  allows modelling the  
dynamics of the metabolite concentrations  $c$  ( $\text{mmol L}^{-1}$ )  $b$  ( $\text{g L}^{-1}$ ) through the mass-balance equation

$$\partial_t c = \mathcal{S}\nu(c, b) \quad (1)$$



177 where  $\partial_t$  is the time derivative operator and  $\nu(c, b)$  ( $\text{mmol h}^{-1} \text{L}$ ) is a vector of the reaction fluxes that  
178 depends both on  $c$  and on the bacterial population  $b$  [Jamshidi and Palsson, 2010]. Equation (1) highlights  
179 that modelling concentration variation comprises two distinct components: (1) the metabolic network,  
180 i.e. the static functional potential carried by the micro-organism genome represented by  $\mathcal{S}$ , and (2) the  
181 distribution of the metabolic fluxes on this network, i.e. the activated functions in the current state of the  
182 system, represented by  $\nu$ . The resulting model is referred to as a *genome-scale metabolic model* (GEM).  
183 The algebraic analysis of  $\mathcal{S}$  already gives biological insights into the metabolic graph, through the use of  
184 approaches such as elementary flux modes (EFM) [Stelling et al., 2002].

185 **Constraint-based models for flux prediction** To predict  $\nu$  for a single species or a merged com-  
186 munity (see fig. 1), several approaches are available. *Kinetic modelling* adds the enzymes catalysing  
187 the reactions to the system and evaluates  $\nu$  using Michaelis-Menten-like equations that link substrate  
188 and enzyme concentrations to the reaction kinetics [Costa et al., 2016]. However, due to the difficulty of  
189 model parametrisation, which requires enzyme kinetic rates, practical use is limited at a genome scale  
190 [van Rosmalen et al., 2021] and even more so at metagenome scale. *Constraint-based metabolic models* are  
191 a widely used alternative for predicting flux distributions, that assumes a quasi steady-state of the intra-  
192 cellular metabolites in eq. 1. *Flux Balance Analysis* (FBA) [Orth et al., 2010] solves a linear optimisation of  
193 an objective function—usually the biomass production—and taking into account thermodynamic constraints  
194 modelled as flux bounds.

195 Known limitations of FBA are the need for high-quality or curated GENREs as input, and its sensitivity  
196 to the definition of the biomass function, which is organism-dependent [Xavier et al., 2017] and is hard to  
197 establish for a community-wide model. Another limitation is the existence of many alternative optimal so-  
198 lutions to the problem. Numerous variations exist, such as Parsimonious FBA (pFBA) [Lewis et al., 2010],  
199 which focuses on sparse flux distributions; Flux Variability Analysis (FVA) [Mahadevan and Schilling, 2003],  
200 which explores the range of alternative solutions to identify essential and blocked reactions; Thermody-  
201 namic Flux Analysis (TFA) [Henry et al., 2007], which identifies thermodynamically feasible flux distribu-  
202 tion; dynamic FBA (dFBA) [Mahadevan et al., 2002], which iterates flux evaluations and concentration  
203 updates to model the dynamics of the system; and Resource Balance Analysis (RBA) [Goelzer et al., 2015],  
204 which extends the optimisation problem to take into account the internal machinery of protein produc-  
205 tion. We refer to [León and Nogales, 2022] for an extensive review of constraint-based methods discussing  
206 the biological trade-offs they achieve. The Cobra toolbox [Heirendt et al., 2019] and its Python interface

207 [Ebrahim et al., 2013] provide numerous practical tools and implementations for constraint-based mod-  
208 elling.

209 Several methods integrate -omics data into constraint-based models, such as transcriptomic [Jenior et al., 2020]  
210 or metabolomic data [Toya and Shimizu, 2013]. Metatranscriptomics data can support the creation of  
211 condition-specific community metabolic models such as in the work of [Zampieri et al., 2023]. Going be-  
212 yond the analysis of functional potential—which may not be expressed—this approach takes into account  
213 whole transcriptome abundance of MAGs and gene transcript abundance, to scale and adapt metabolic  
214 reaction bounds. Such integrated solutions bring metabolic simulations closer to the biological reality.

215 Building constraint-based models using GENREs of individual microbiome members is a first step in  
216 the comparison of their biosynthetic potential [Bartell et al., 2014]. As one approach, CONGA compares  
217 GENREs pairwise to identify essential genes [Hamilton and Reed, 2012]. Pangenome analysis combined  
218 with flux analysis can also be used to detect strain specific metabolic features [Bosi et al., 2016] (see also  
219 section 6.3). A further step in the analysis is to combine individual GEMs in order to model the community  
220 and its interactions.

### 221 **3.2 From genome to metagenome-scale metabolic modelling**

222 **Bag-of-genomes models** A batch of GENREs can first be considered as a set of independent individuals  
223 interacting through extracellular metabolites. The community model is then obtained by the juxtaposition  
224 of the individual GENREs, which are used to predict individual metabolic fluxes, the sum of which deter-  
225 mines the fate of the extracellular metabolites (Fig. 1). This paradigm is also termed a *bag-of-genomes*  
226 model [Frioux et al., 2020].

227 However, the prediction of individual fluxes can require new methodological developments, in particular  
228 in a multi-species FBA framework where interactions are studied in a static regime [Gottstein et al., 2016].  
229 Indeed, the growth rate of an individual only depends on the availability of extracellular metabolites,  
230 whereas that of a community constraint-based model also depends on the instantaneous use of metabolites  
231 by organisms that limits metabolic availability for others. Individual flux prediction in communities is  
232 thus the result of a trade-off of metabolite uptake between the community members. Furthermore, the  
233 hypothesis of individual optimal growth, which is sound in monoculture, may not be valid in communities  
234 where complex ecological processes may occur. For example, symbiosis can induce sub-optimal growth for  
235 a symbiont but improve long-term persistence in the community. To predict this trade-off, several meth-

**Table 2.** Main methods and corresponding framework to solve community FBA and dFBA models

Framework	Method	Description	platform and references
FBA	multi-level optimisation	An inner optimisation is made for each individual GENRE and coupled to an outer community-wide optimisation: trade-off between individual and total biomass growth	Optcom[Zomorodi and Maranas, 2012] CASINO[Shoae et al., 2015]
	weighted squared sum	A weighted square sum of individual flux weighted by microbe abundance as community-scale objective function	MICOM [Diener et al., 2020]
	multi-objective FBA	A Pareto front is computed as set of solutions of a multi-objective optimisation problem. A solution on the Pareto front is chosen according to additional criteria (e.g. resource use)	MO-FBA [Budnich et al., 2017] [Heinken and Thiele, 2015] [Heinken et al., 2013]
	EFM-based	EFM-based individual models the kinetic rates of which is determined by a community-scale model	CODY[Geng et al., 2021]
dFBA	sum of fluxes	Straightforward sum of individual fluxes (see fig.1). Flux bounds are computed from extracellular compound concentrations that change over time according to consumption and production by community members.	$\mu$ BialSim [Popp and Centler, 2020] COMETS [Dukovski et al., 2021] IndiMeSH [Borer et al., 2019]

ods have been developed, that are conceptually very different in a static or a dynamic FBA framework. These methods and example of corresponding toolboxes are recapitulated in table 2. Extensive reviews of community-wide metabolic models can be found in [Colarusso et al., 2021], including comparison between FBA and EFM-based methods, in [León and Nogales, 2022], for a comparison between top-down and bottom-up modelling approaches, or in [García-Jiménez et al., 2021, Zaramela et al., 2021], for a parallel between computational methods and engineered microbial communities.

The Microbiome Modelling Toolbox [Heinken and Thiele, 2022a] derives community models from metagenomics data for the human gut microbiome. This platform facilitates the extraction of GEMs corresponding to OTUs identified by metabarcoding from large curated collections such as AGORA 2 [Heinken et al., 2023] or the Virtual Metabolic Human [Noronha et al., 2018], and provides efficient computation and result visualisation. The main drawbacks of such large bag-of-genomes models are the computational cost, which scales with the number of GEMs taken into account [Heinken and Thiele, 2022a], and the difficulty of ensuring GENRE quality and accuracy for a large number of genomes. In particular, the presence of draft models inferred by automatic pipelines may impair the overall quality of the simulations [Babaei et al., 2018]. Bag-of-genomes models can be embedded in an outer optimisation problem in order to find an optimal configuration of the microbial community according to a dedicated fitness function, like in FLYCOP [García-Jiménez et al., 2018].

**Bag-of-genes models** A community-wide metabolic model can be built by assembling all the functions carried by the individual GENREs and factoring the common functions shared by different organisms (Fig. 1). In this paradigm, the community is seen as a meta or supra organism, an *enzyme soup*, whose functional potential results from the gathering of the whole set of functions [Frioux et al., 2020]. The corresponding

257 stoichiometric matrix  $\mathcal{S}_{com}$  is the concatenation of the individual stoichiometric matrices after removal of  
258 duplicated metabolites and functions.

259 Bag-of-genes models have been compared to the bag-of-genomes approach in the context of natural  
260 microbial communities in hot spring mats [Taffs et al., 2009]. A community-wide metabolic model has been  
261 reconstructed with KBase and compared with expression data in a bacterial photoautotroph–heterotroph  
262 consortium [Henry et al., 2016]. The same method was reused to reconstruct a bag-of-genes model of child  
263 gut microbiota for a modelling study of malnutrition-associated dysbiosis [Kumar et al., 2018].

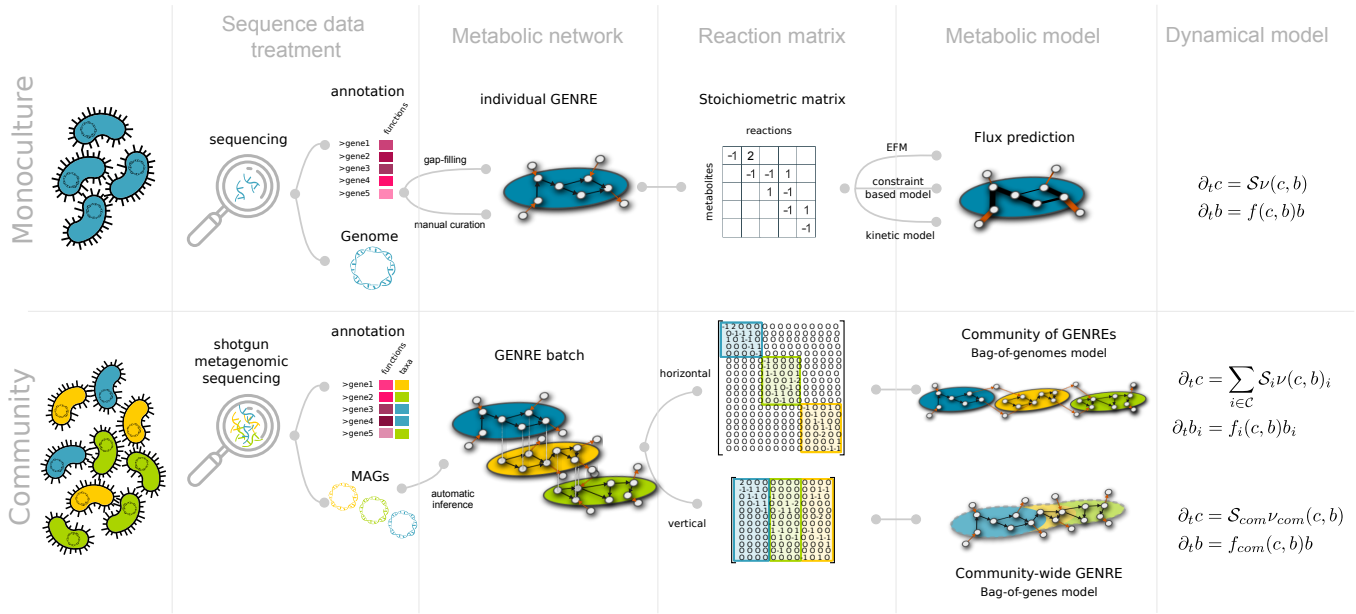
264 Carveme [Machado et al., 2018] provides an alternative between a community-wide GEM and a bag-  
265 of-genomes model by assigning a compartment to each species and adding an extracellular compartment  
266 to connect them. A community biomass equation is inferred, enabling the use of the constraint-based  
267 models described above while keeping the knowledge of metabolite producers. HUMAnN2 can also help  
268 navigating between bag-of-genomes and bag-of-genes models by providing both community and species-  
269 resolved functional potentials from metagenomic data [Franzosa et al., 2018].

## 270 **4. Models for large-scale metabolic exploration of microbial** 271 **communities**

272 The recurring goals, when considering a microbial community, are first to identify putative interactions  
273 among its members, then to globally assess the role of all species in the community. We focus here on  
274 models based on GENREs dedicated to the direct inference of putative interactions, and describe how  
275 discrete models of metabolism can be an alternative to constraint-based GEMs for scalable screening of  
276 metabolic potential in microbial communities.

### 277 **4.1 Assessing competition and cooperation potentials of microbial communities**

278 Cooperation through metabolic exchanges and competition for shared nutrients are the main metabolic  
279 interactions that occur in microbial communities. As a result, being able to estimate cooperation and  
280 competition potentials is essential for characterising, comparing and selecting communities. Approaches  
281 dedicated to this purpose can be separated in two categories, the first one composed of methods that  
282 structurally analyse and compare individual GENREs, and the second comparing the individual growth  
283 rates of bacterial species to those obtained in community using GEMs (Fig. 2 upper right panel).



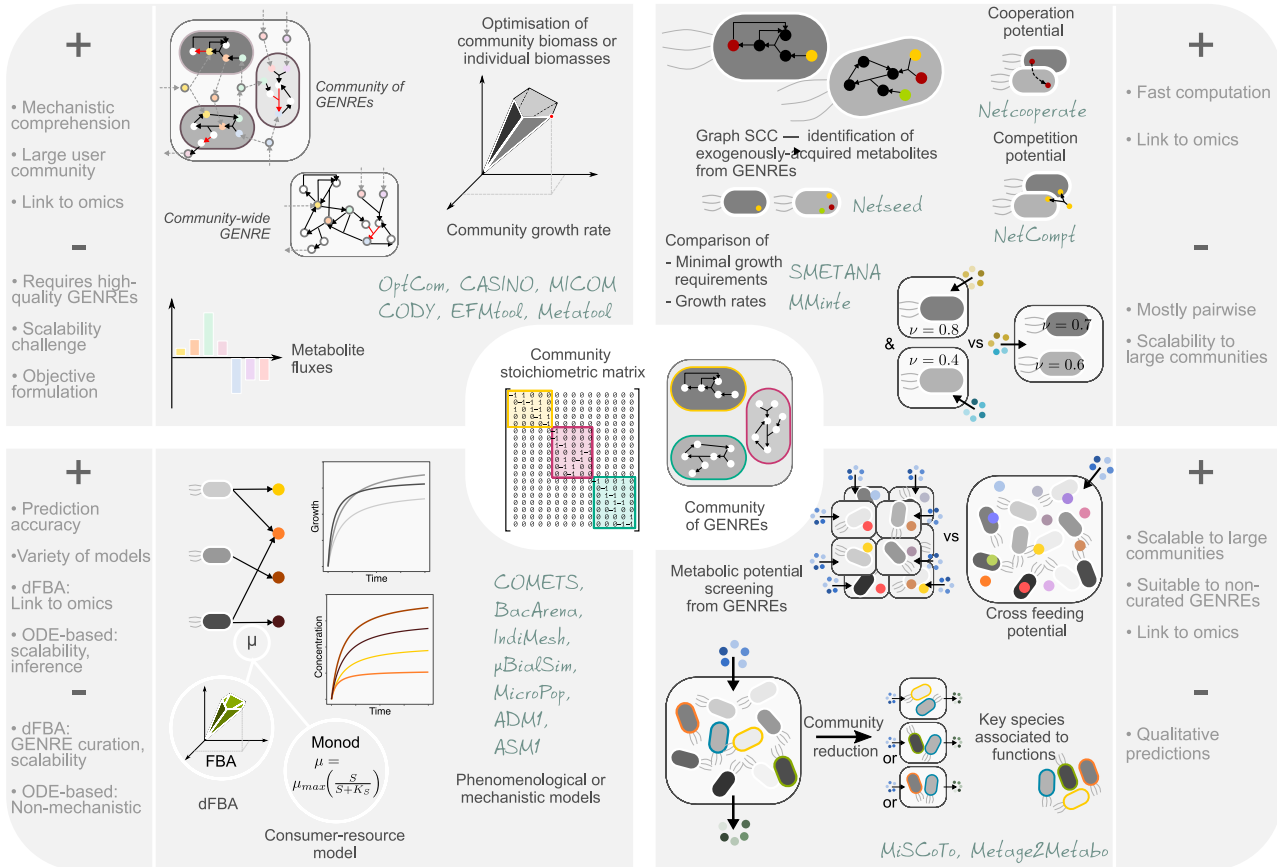
**Figure 1. From mono-organism to community function-based model of microbial populations.** *Monoculture model* (upper panel): In monoculture, a GENRE is obtained from the annotated genome, with semi-automatic tools (e.g. gap-filling) and manual curation. A matricial representation of the metabolic network, the *reaction matrix*, is an input for flux prediction models. Metabolic fluxes can be computed iteratively to estimate the time evolution of a dynamical model of the population. *Community model* (lower panel): In a microbial community, the metagenome makes it possible to obtain MAGs and GENREs for members of the community. GENREs may share some common functions and metabolites (vertical gray vertices in the GENRE batch). A first paradigm for building a community-wide metabolic model is a *bag-of-genome* model: a set of independent GENREs interacting through common external metabolites. The corresponding reaction matrix is the concatenation of the individual matrices, and the corresponding dynamical model is the sum of the fluxes computed for each unique genome. A second paradigm, termed *bag-of-gene* model consists in merging all the GENREs through their common functions and metabolites, resulting in a global metabolic network for the whole community. The corresponding reaction matrix is the concatenation of the individual matrices after merging or removal of common reactions (columns) and metabolites (rows), resulting in a lower dimension matrix and a dynamical system with a unique equation. With this paradigm analysis can be performed at the scale of a community, but the contribution of each taxon is fuzzier.

284 *Reverse ecology* [Levy and Borenstein, 2012] aims at inferring ecological mechanisms through the char-  
285 acterisation of the composition of the environment and the interactions occurring in it. A core step is the  
286 detection of exogenously-acquired compounds, or *seeds*, that NetSeed [Borenstein et al., 2008] computes  
287 using GENRE’s strongly-connected components. NetCooperate [Levy et al., 2015] quantifies the potential  
288 for pairwise syntrophic relationships. The *biosynthetic support score* quantifies the proportion of an en-  
289 dosymbiont’s seeds occurring in a host’s GENRE. The *metabolic complementarity index* of two bacterial  
290 species is the fraction of one GENRE’s seeds that are products of the second GENRE, and not in its  
291 own seed set. Comparing GENREs pairwise can also be used to determine a competition potential. In  
292 [Levy and Borenstein, 2013], the *metabolic competition index* is the fraction of an organism’s seeds that  
293 also belong to the seeds of another. NetCompt [Kreimer et al., 2012] calculates the *effective metabolic*  
294 *overlap*, combining seed calculation with the network expansion algorithm (see section 4.2), and estimating  
295 the reduction of a species’s metabolic potential in the absence of source compounds that are shared with  
296 other species. These metrics have been used for determining a large-scale evolutionary trend in the loss of  
297 biosynthetic capacity over time in parasites [Borenstein and Feldman, 2009], and detecting enzymes oper-  
298 ating on exogenously-acquired compounds in diseased individuals [Greenblum et al., 2012]. They were also  
299 integrated in analysis frameworks such as RevEcoR [Cao et al., 2016] or PhyloMint [Lam et al., 2020]. It  
300 is worth noticing that the seed set is not only the set of nutrients required for growth but includes com-  
301 pounds that may theoretically enable the production of any metabolite in the network, and can therefore  
302 underestimate or overestimate interaction potentials [Lam et al., 2020].

303 Other methods use the computation of flux distributions with constraint-based modelling to determine  
304 scores of competition and cooperation. [Freilich et al., 2011] calculate the *potential competition score* and  
305 the *potential cooperation score* to characterise metabolic interactions between nearly 7000 combinations  
306 of 118 bacterial GEMs. The authors additionally infer win-lose and give-and-take relationships between  
307 bacterial pairs by assessing their growth and co-growth rates in competition- and cooperation-inducing  
308 media. MMinte [Mendes-Soares et al., 2016] compares the growth rates of species in pairwise interacting  
309 communities to their individual growth rates in order to predict whether one species can benefit from or be  
310 negatively impacted by the presence of another. SMETANA overcomes the limitation of pairwise simulation  
311 and computes interaction scores for microbial communities by solving mixed integer linear programming  
312 problems [Zelezniak et al., 2015]. It computes the *metabolic interaction potential* which represents the  
313 extent to which metabolites produced by the community can be reused by species for their growth. The

Predict small-scale community metabolism

Decipher cooperation and competition potentials



**Figure 2. Approaches for numerical simulation and reasoning on community metabolism.** Methods and tools are sorted according to the functional characterisation of the microbial community metabolism they can provide: predicting small-scale community metabolism (upper left panel), assessing community dynamics (lower left panel), deciphering cooperation and competition potentials (upper right panel) and characterising large-scale community metabolism (lower right panel). For each class, the main methods and dedicated software (gray italic names) are recapitulated. The main “pros” (+) and “cons” (-) are listed on the lateral box associated to each panel.

314 nutrients required for growth in a non-interacting community can be compared between species, leading  
315 to the *metabolic resource overlap*, an estimation of competition potential. [Machado et al., 2021] uses  
316 these scores and describes a polarisation of microbial communities between competitive and cooperative  
317 metabolisms. Finally, the use of taxon knock-outs in MICOM [Diener et al., 2020] can also be a means to  
318 evaluate the positive or negative impacts of species on the growth of other community members.

## 319 4.2 A Boolean abstraction of metabolic producibility to scale up to large communities

320 As depicted in the above sections, most approaches for modelling interactions in microbial communities  
321 are applied pairwise, and more generally, constraint-based models are not applied to large communities  
322 due to both the curation needs for the individual GENREs and the computational costs associated with  
323 simulations. This motivates the use of alternative paradigms for modelling. One approach is *metabolic*  
324 *network percolation* [Bernstein et al., 2019], a probabilistic concept that randomly samples environmental  
325 compositions to assess the consistency of metabolite production in microbiomes. Applied to the human  
326 oral microbiome, it permitted the creation of an atlas describing metabolite biosynthesis potentials while  
327 being more tolerant to missing reactions than the classical constraint-based simulations.

328 Discrete and reasoning-based models are further approaches for estimating the ability of a species  
329 to synthesise, or not, metabolic compounds from available substrates in its environments. The *network*  
330 *expansion algorithm* [Ebenhöh et al., 2004] provides a Boolean approximation that converts the stoichio-  
331 metric matrix into a matrix of incidence of 0, 1 and -1, representing solely the participation of molecules  
332 in metabolic reactions, hence discarding mass-conservation. The metabolic potential of a species, called  
333 *scope*, is calculated from a set of available nutrients, the *seeds*: metabolic reaction activation depends on the  
334 availability of its reactants either from the seeds or as the products of other activated reactions. MeneTools  
335 implements network expansion to query metabolic capabilities of GENREs [Aite et al., 2018].

336 Metabolic scope can be used to determine the metabolic potential of several organisms in a community  
337 [Christian et al., 2007]. [Ofaim et al., 2017] built a framework considering the relationship between taxon-  
338 omy, function and environment, and attempted to use metabolic modelling to explain the co-occurrence  
339 of species in the plant rhizosphere or the soil. A similar approach applies to deciphering symbiont-host  
340 interactions: [Opatovsky et al., 2018] simulated the metabolism of obligatory and facultative symbionts  
341 associated with a phloem-feeding whitefly. As a microbiota can involve hundreds of species living in  
342 a shared environment, the scalability of network expansion is a property accounting for the associated



343 metabolic diversity (Fig. 2 bottom right panel). The community-wide implementation of network expansion in MiSCoTo [Frioux et al., 2018] permits computing metabolic potentials of communities and selecting minimal size communities exhibiting functions of interest. Metage2Metabo integrates such analyses together with GENRE reconstruction to systematically compare the metabolic potential of metagenomic samples or genome collections [Belcour et al., 2020]. Discrete models of this kind were used to screen microbial metabolic potentials [Ramalho et al., 2022], to predict potential symbiotic relationships between MAGs from patients with Crohn’s disease and healthy individuals [Sabater et al., 2022], and to analyse metabolic complementarity [Karimi et al., 2021].

## 351 **5. Towards genomics-informed phenomenological models of microbial** 352 **communities**

353 The use of mechanistic GEMs in the context of large microbial ecosystems can be impeded by poorly-reconstructed GENREs [Babaei et al., 2018], or by issues related to the high dimension of the system, such as uncertainties, accumulations, analysis of massive outputs or computational load. On the other hand, a large number of phenomenological microbial community models, has proved to be efficient in capturing macroscopic features without involving genome-scale inputs. In this section, we discuss opportunities for coupling GEMs with phenomenological and hybrid models to achieve scalability for complex microbiome, while allowing genome-resolved –omics integration.

### 360 **5.1 Existing models that could benefit from metabolic modelling**

361 Microbial community models other than GEMs have recently been divided into 4 types: **(1)** consumer-resource, **(2)** generalised Lotka-Volterra (gLV), **(3)** trait based models and **(4)** individual based model (IBM) [van den Berg et al., 2022]. Models **(1)** to **(3)** are population-dynamic models based on a system of ordinary differential equations (ODE), describing the rate of change of the population density depending on other species (**(2)** directly, **(1)** indirectly), available resources **(1)**, and environmental factors **(3)**. Model type **(4)** describes the fate of individual cells, whose juxtaposition models the population. IBMs have been used on a wide range of ecosystems in order to gain knowledge of the underlying ecology and for decision making [DeAngelis and Grimm, 2014]. Models of type **(4)** are not opposed to models **(1)**-**(3)**, as arguably they can be complementary to one another [Hellweger et al., 2016], and since ODE models

370 can be derived as large-population approximations of IBM models [Darrigade et al., 2022]. Due to their  
371 computational efficiency, models (1)-(4) can be spatialised, using e.g. partial differential equations (PDEs)  
372 as [Labarthe et al., 2019, Moorthy et al., 2015, Smith et al., 2021] did for gut microbiota.

373 GEMs can be considered not only as a category by themselves, but can also be included in models of  
374 the form (1)-(4).

375 **Coupling GEMs with consumer-resource models** It must be stressed that bag-of-genomes models  
376 (see Fig. 1) can be interpreted as particular cases of consumer-resource models (Fig. 3, Fig. 2 bottom  
377 left panel), where resource consumption, metabolite production and growth rates are modelled through  
378 FBA fluxes. Hence, existing consumer-resource models can be easily supplemented with a genome-scale  
379 metabolic model. As such, a consumer-resource model that qualitatively reproduces experimentally ob-  
380 served ecological patterns of the Earth Microbiome Project by considering the exchange of secondary  
381 metabolites [Marsland et al., 2020] could be refined with GEMs: export fluxes for the metabolites included  
382 in the consumer-resource model could be computed dynamically with the GEM while remaining essential  
383 metabolites would be given a steady-state value.

384 **Deciphering gLV interactions with GEMs** gLV models proved to be efficient in analysing interac-  
385 tions across the gut microbiome by fitting time-series of population counts [Stein et al., 2013]. Population  
386 data obtained from high-throughput sequencing reports relative abundances, inducing inference difficul-  
387 ties [Remien et al., 2021]. That drawback can be overcome by providing *priors* to the inference methods  
388 [van den Berg et al., 2022], for instance from GEMs. In an example coming from anaerobic digestion (AD),  
389 GEMs were reconstructed for each of the 800 species present in the community, and FBA was used to under-  
390 stand the syntrophic relations between micro-organisms [Basile et al., 2020], that could be used as *priors*  
391 of gLV model parameters. Conversely, a pairwise interaction identified in time-series with a gLV model  
392 could be interpreted using pairwise GEMs.

393 **Supplementing trait-based models with GEMs.** Bioreactor modelling is a good example that re-  
394 lates to both trait-based models (since they often involve abiotic parameters such as pH or temperature)  
395 and consumer-resource models. Two examples of widely implemented bioreactors are wastewater treat-  
396 ment plants based on activated sludge (AS) [Tchobanoglous et al., 2003], and biogas production from AD  
397 of organic waste [Moletta, 2015]. AS [Leslie Grady Jr et al., 1986] and AD [Batstone et al., 2002] systems

398 have been modelled in the framework of community-wide ODE systems (see Fig. 1), representing micro-  
399 bial species as functional guilds, i.e. a population of microbes responsible for a metabolic pathway. AD  
400 models have been used in various contexts such as ruminal [Muñoz-Tamayo et al., 2016], environmental  
401 [Desmond-Le Quémener et al., 2021] or gut microbiota [Kettle et al., 2018, Muñoz-Tamayo et al., 2010].  
402 Since considerable efforts have been made to sequence microbes present in AD and AS at a global scale  
403 [Dueholm et al., 2022], one could readily envision enhancing parts of the AD and AS with metabolic mod-  
404 els. However, FBA analysis alone may not suffice to represent abiotic inhibition arising in AD systems  
405 [Basile et al., 2023b].

406 **Coupling IBM with GEMs.** In an IBM model, each individual can be provided with a GEM, which can  
407 be used to predict its metabolism with FBA. Such a framework has been already included in platforms like  
408 BacArena [Bauer et al., 2017], ACBM [Karimian and Motamedian, 2020], IndiMeSH [Borer et al., 2019] or  
409 CROMICS [Angeles-Martinez and Hatzimanikatis, 2021], that were recently compared [Scott et al., 2023].  
410 These frameworks allow spatialisation: the IBM can include movement functions modelling microbial  
411 motility, and complex multi-physics features, e.g. the complex diffusive pattern in the rhizospheric area for  
412 IndiMesh.

413 Since coupling system dynamics, IBM and GEMs involve different mathematical formalisms, hybrid  
414 models provide a unifying framework that can help implementing model coupling, as described in the next  
415 section.

## 416 5.2 Extending the scope of community modelling with hybrid models

417 The complex behaviours of microbiomes arise from the interaction of different functional processes with  
418 different dynamics, that can be considered as continuous, discrete or random, depending on their time  
419 scale, dictating appropriate mathematical formalisms [Assar et al., 2014]: *numerical ODEs* for continuous  
420 dynamics, *automata* for discrete instantaneous changes, *nondeterministic* or *stochastic models* for uncertain  
421 or random transitions. This highlights the need for a flexible framework to explore conjointly these processes  
422 at the community scale.

423 *Hybrid models* are such a framework, by assembling modules modelled using different formalisms. In  
424 [Liu et al., 2022] five complementary combinations of modelling formalisms are used for biological sys-  
425 tems: *hybrid discre-continuous* methods, often used for intracellular modelling as above; *hybrid stochastic-*  
426 *deterministic* methods, introducing random delays; *hybrid flux balance analysis* methods, integrating

427 metabolic, regulatory, and signalling networks; *hybrid logic-quantitative* methods, combining quantitative  
428 methods with Boolean networks or fuzzy logic; and *spatial hybrid* methods, combining spatial stochastic  
429 and deterministic models to bridge three scales: time, abundance, and space [Widmer and Stelling, 2018].

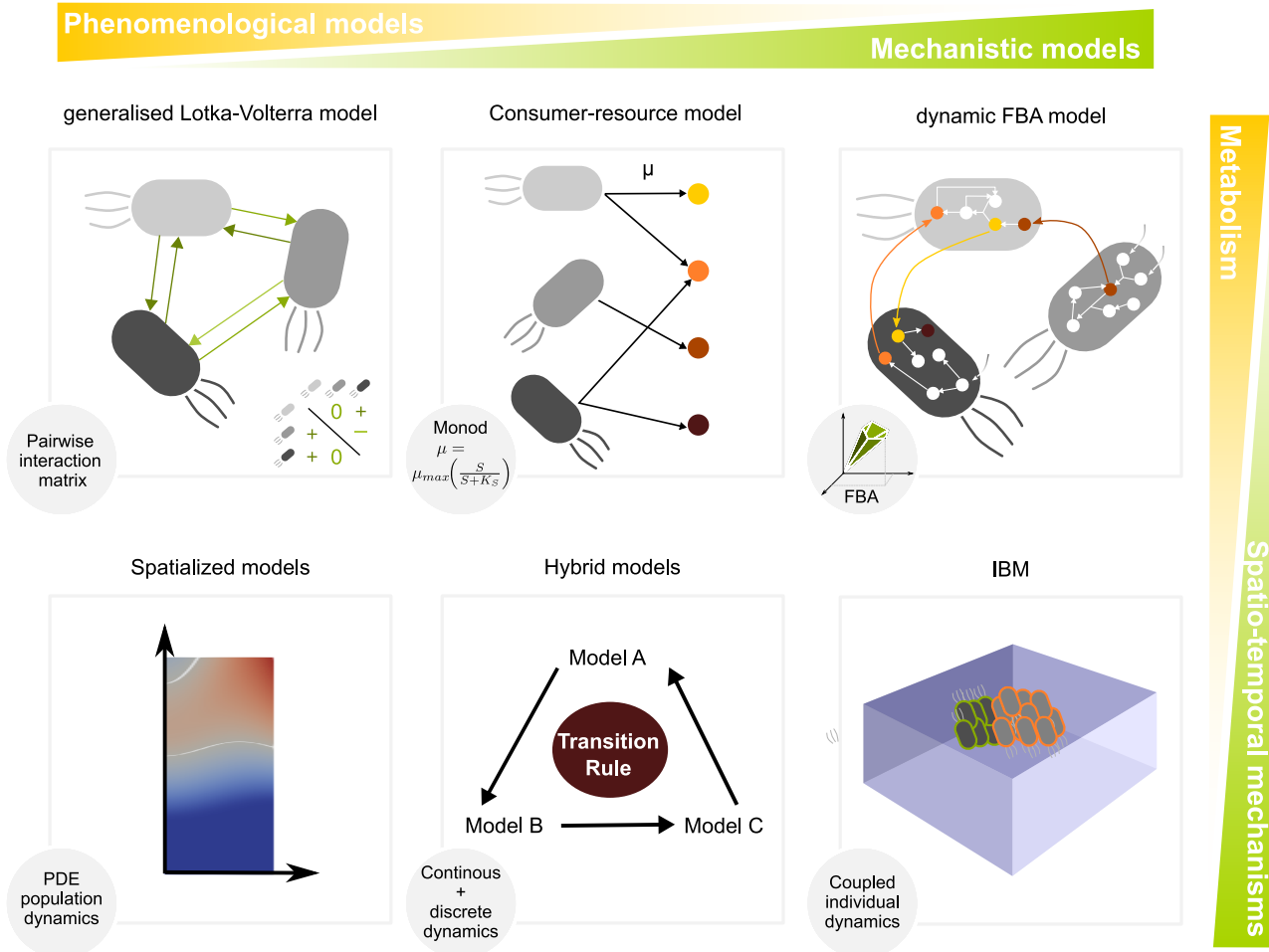
430 More formally, *hybrid dynamical systems* often combine continuous *state* and *control* variables plus  
431 a discrete *mode* variable, the transition of which is determined by *guard* conditions defined on the con-  
432 tinuous variables [Henzinger, 1996]. In *switched systems*, the mode activates, deactivates, and combines  
433 modules of a hierarchical model [Branicky, 1994, Shorten et al., 2007]. In this framework, phenomenolog-  
434 ical models and GEMs can be coupled in a flexible and rigorous way. A good example can be found in  
435 [Assar et al., 2014], that switches between two ODE models [Coleman et al., 2007, Scaglia et al., 2009] and  
436 a dFBA model of wine fermentation kinetics [Pizarro et al., 2007] according to the system state.

## 437 **6. From environmental samples to functional and taxonomic diversity**

438 The preceding has taken a high-level view of the modelling process and the insights models can provide,  
439 and has assumed that the collection of models for a community accurately reflects the taxonomic com-  
440 position of its members and the biomolecular functions they provide. These will vary greatly between  
441 environments and from sample to sample: different species provide different functions, and within those  
442 species strain differences and horizontal gene transfer will further affect gene content. In this section we  
443 consider how molecular identification of taxonomic and functional diversity is employed to build accurate  
444 characterisations of real communities.

445 Two DNA sequencing approaches are used when building metabolic models from environmental sam-  
446 ples [Pérez-Cobas et al., 2020]. *Amplicon sequencing* of marker genes characterises taxonomic diversity  
447 within a sample and can be used to infer functional profiles, map community members to existing mod-  
448 els [Heinken and Thiele, 2022b], and select genomes prior to GENRE reconstruction. *Whole metagenome*  
449 *shotgun sequencing* characterises both taxonomic and functional diversity within a sample by determin-  
450 ing the gene content of community members, and can be used to infer new GENREs of novel species  
451 and strains. In both approaches, *third generation sequencing* (TGS) using long reads can complement, or  
452 advantageously replace, NGS using short reads.

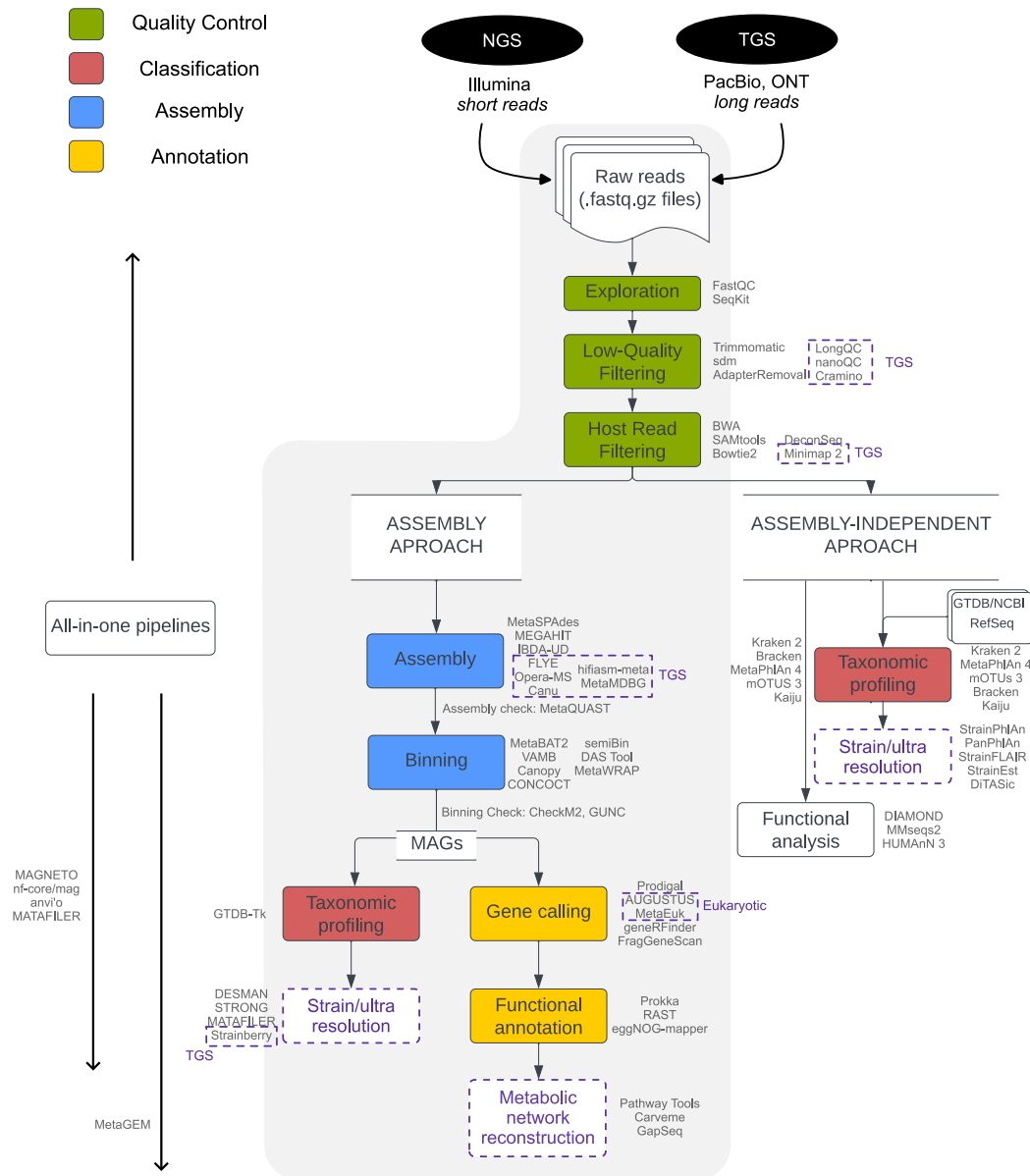
453 Amplicon sequencing and shotgun metagenomic sequencing have demonstrated the high diversity of  
454 microbial communities, for bacteria and archaea, but also eukaryotes such as fungi [Tedersoo et al., 2014].



**Figure 3. Dynamic models of metabolism: opportunities beyond GENRE-based models.** Different formalisms can be used to capture microbial population dynamics (upper panel). These models can be placed on a gradient, from phenomenological (left) to mechanistic models (right) of the metabolic capabilities of the community. Each formalism can be supplemented by additional spatio-temporal mechanisms (lower panel) that can be represented at the population (PDE) or individual scale (IBM). The addition of spatio-temporal features in the community dynamics induces simplifications in the metabolic models to keep the model computationally tractable.

**Table 3. Main short read and long read metagenomic sequencing technologies.** Abbrev. PacBio, Pacific Biosciences; ONT, Oxford Nanopore Technologies; bp, base pair; Q, quality score logarithmically related to the base calling error probability, Q20: 1%, Q30: 1‰ etc. Read length is subject to variation.

Method	sequencing platform	read lengths (bp)	throughput	specifications	applications advantages/pitfalls
short-read metagenomics	Illumina Miseq system	2x300	low	1-25M reads, up to 15 Gbp >70% bases higher than Q30	- Taxonomic profiling, - Relatively low data processing requirements
	Illumina Novaseq 6000	2x150	high	1.6B - 40B reads, up to 3 Tb, ≥ 85% of bases higher than Q30	- Taxonomic and functional profiling, - Genome assembly and binning; - High throughput, low cost for production-scale genomics
	Pacific Biosciences Onso	2x150	moderate	120-150 Gbp ≥90% bases Q40	- Taxonomic and functional profiling - Genome assembly and binning - Identification of variants - 15x higher accuracy than other short read platforms - High accuracy in characterisation of homopolymers - Higher costs/bp
long-read metagenomics	Pacific Biosciences Sequel II and IIE	typically 10k-30k	moderate	3M reads typically Q20-Q40 reads	- Taxonomic and functional profiling, - Genome assembly and binning - Can reconstruct circular MAGs - High sample input: ~2 µg - Full-length 16S rRNA seq
	Pacific Biosciences Revio system	~10k to ~30k	moderate	up to 90 Gbp Q30-Q40	- Higher HiFi read accuracy with DeepConsensus base calling, methylation calling - High sample input: ~2 µg - Full-length 16S rRNA seq
	Oxford Nanopore Technologies Promethion R10 series	on average ~10k to ~60k, strongly dependent on DNA extraction	moderate	~50 Gb > median Q20 most reads, ~Q30 (99.9%) for Duplex reads	- Very long reads - Transportable technology - Low sample input: ~1 µg - Less polishing needed than R9 series - Read quality still lower than PacBio



**Figure 4. Metagenomic sequencing and data processing.** After quality control, shotgun metagenomic reads can enter an assembly pipeline or an assembly-independent profiling pipeline. The assembly pipeline is highlighted as it permits the reference-free reconstruction of MAGs and subsequently GENRE reconstruction that directly survey the functions present in the ecosystem. Examples of tools associated to each step are indicated next to the box. Tools or steps related specifically to TGS, eukaryotic metagenomics and strain resolution are highlighted with dotted boxes.

## 455 6.1 Amplicon sequencing

456 A good *marker gene* is universally present in target populations and taxonomically robust because its in-  
457 frequent mutation is not biased by functional selection. Typical choices for marker genes are 16S ribosomal  
458 RNA genes in prokaryotes, and either 18S ribosomal RNA genes or different lineage-specific markers in eu-  
459 karyotes [Earl et al., 2018, Johnson et al., 2019, Bradley et al., 2016, Burki et al., 2020, Tekle et al., 2010].  
460 For a given environmental sample, the variable regions of the selected marker genes are amplified using  
461 PCR and later sequenced. Until recently, inexpensive short-read NGS sequencing has been used exclusively  
462 [Kim et al., 2021, Bukin et al., 2019], although this limits the size of the variable region that can be used,  
463 may be subject to amplification bias, and may limit taxonomic resolution to phylum and genus due to 16S  
464 polymorphism [Knight et al., 2018, Mitchell et al., 2020, Sieber et al., 2018a]. Long-read TGS sequencing  
465 of full-length 16S rRNA has recently been shown to reduce bias, broaden the choice of marker genes,  
466 and increase taxonomic resolution to species and subspecies [de Muinck et al., 2017, Johnson et al., 2019,  
467 de Oliveira Martins et al., 2019].

468 Amplicon sequences are clustered into *operational taxonomic units* (OTUs) or into *amplicon sequencing*  
469 *variants* (ASVs) that provide “species-like” delineation of organisms and estimations of relative abundance.  
470 Bioinformatics pipelines for amplicon sequencing analysis are reviewed in [Hakimzadeh et al., 2023]. While  
471 the functions present in a microbiome cannot be directly identified by amplicon sequencing [Almeida et al., 2021],  
472 approximations may be indirectly inferred using phylogenetic placement of markers [Douglas et al., 2020,  
473 Bowman and Ducklow, 2015], alignment [Wemheuer et al., 2020, Narayan et al., 2020], or incorporation of  
474 biological knowledge in the form of taxonomic and functional profiles [Mallick et al., 2019, Jing et al., 2021].  
475 Nonetheless, limitations resulting from the importance of the accessory genome in a species with respect  
476 to its core genome will impact the accuracy of the predictions.

477 Building mechanistic models of metabolism requires accurate taxonomic classification, both for identi-  
478 fying existing genomes and GENREs and characterising diversity. The KSGP database [Grant et al., 2023]  
479 combines the GTDB unified taxonomy [Parks et al., 2020] with a collection of environmentally sampled  
480 full-length sequences, to substantially increase taxonomic assignment of environmental samples and links  
481 to reference genomes. Resolution within reference genomes is typically limited to species and will miss  
482 most strain-specific variability.



**Table 4. Main sequence analysis methods.**

<b>OTU clustering §6.1</b>		<b>Long read assembly §7.2</b>	
QIIME 2	[Estaki et al., 2020]	MetaFlye	[Kolmogorov et al., 2020]
DADA2	[Callahan et al., 2016]	Canu	[Koren et al., 2017]
LotuS2	[Özkurt et al., 2022]	Opera-MS	[Bertrand et al., 2019]
<b>Function inference §6.1</b>		hifiasm-meta	[Feng et al., 2022]
PICRUSt2	[Douglas et al., 2020]	MetaMDBG	[Benoit et al., 2023]
paprica	[Bowman and Ducklow, 2015]	<b>Assembly evaluation §7.2</b>	
Tax4Fun2	[Wemheuer et al., 2020]	MetaQUAST	[Mikheenko et al., 2016]
Piphillin	[Narayan et al., 2020]	<b>Gene prediction §7.2.1</b>	
<b>Metabolic features §6.1</b>		Prodigal	[Hyatt et al., 2010]
MelonnPan	[Mallick et al., 2019]	MetaEuk	[Karin et al., 2020]
Meta-Apo	[Jing et al., 2021]	<b>Reference gene catalogues §7.2.1</b>	
<b>Taxonomic classification §6.1</b>		CD-HIT	[Fu et al., 2012]
KSGP	[Grant et al., 2023]	MMseqs2	[Steinegger and Söding, 2017]
GTDB	[Parks et al., 2020]	<b>Binning §7.2.2</b>	
<b>Adapter removal §7.1</b>		CONCOCT	[Alneberg et al., 2014]
Trimmomatic	[Bolger et al., 2014]	MaxBin 2	[Wu et al., 2016]
sdm	[Hildebrand et al., 2014]	MetaBAT 2	[Kang et al., 2019]
AdapterRemoval	[Schubert et al., 2016]	<b>Bin consolidation §7.2.2</b>	
<b>Quality control §7.1</b>		VAMB	[Nissen et al., 2021]
FastQC	[Andrews et al., 2010]	SemiBin2	[Pan et al., 2022]
SeqKit	[Shen et al., 2016]	MetaWRAP	[Uritskiy et al., 2018]
<b>Long read analysis §7.1</b>		DAS Tool	[Sieber et al., 2018b]
LongQC	[Fukasawa et al., 2020]	<b>Chimerism and contamination §7.2.2</b>	
nanoQC	[Coster et al., 2018]	CheckM	[Parks et al., 2015]
Cramino	[Coster and Rademakers, 2023]	GUNC	[Orakov et al., 2021]
Minimap2	[Li, 2018]	<b>MAG creation pipelines §7.2.2</b>	
<b>Taxonomic filtering §7.1</b>		MAGNETO	[Churchward et al., 2022]
MetaPhlan4	[Blanco-Miguez et al., 2022]	nf-core/mag	[Krakau et al., 2022]
mOTUs3	[Ruscheweyh et al., 2022]	anvi'o	[Eren et al., 2021]
<b>Functional profiling §7.1</b>		MATAFILER	[Hildebrand et al., 2019]
Kaiju	[Menzel et al., 2016]	MetaGEM	[Zorrilla et al., 2021]
DIAMOND	[Buchfink et al., 2014]	<b>Short read assembly §7.2</b>	
MMseqs2	[Steinegger and Söding, 2017]	metaSPAdes	[Nurk et al., 2017]
<b>Short read assembly §7.2</b>		IDBA-UD	[Peng et al., 2012]
metaSPAdes	[Nurk et al., 2017]	MEGAHIT	[Li et al., 2015]
IDBA-UD	[Peng et al., 2012]		
MEGAHIT	[Li et al., 2015]		

## 483 6.2 Shotgun metagenomics

484 Whole metagenomic sequencing uses total DNA extracted from the environmental sample to build a com-  
485 plete picture of the genomes of the organisms present in the sample. Also called *untargeted*, *shotgun*,  
486 *random*, or *WGS* sequencing, whole metagenomic sequencing does not target a specific amplified marker  
487 gene, and sequencing a larger amount of genetic material comes with increased expense. However, it avoids  
488 many of the shortcomings of amplicon sequencing: it can taxonomically resolve species, and even strains  
489 with sufficient data; it can identify the genes carried by species on a sample-specific basis, even for un-  
490 known taxa; and it can infer microbial functions including strain-specific variants in the accessory genome  
491 [Djemiel et al., 2022, Hildebrand, 2021, Hildebrand et al., 2019, Hildebrand et al., 2021].

492 Table 3 summarises the short-read and long-read sequencing technologies used for assembling genomes,  
493 and reconstructing metagenomes and MAGs [MetaHIT consortium et al., 2015, Nielsen et al., 2014, Pan et al., 2023].  
494 As above, long TGS reads can significantly improve results [Kim et al., 2022]. PacBio HiFi long read se-  
495 quencing has low error rates and provides near-finished microbial genomes from pure cultures or metagenomes,  
496 albeit at relatively high cost [Amarasinghe et al., 2020]. Oxford Nanopore (ONT) sequencing produces even  
497 longer reads at higher throughput on average, but with higher rates of homopolymer indels that may lead  
498 to gene calling errors [Watson and Warr, 2019]. Combining long-read ONT sequencing with short reads for  
499 post-assembly error correction is a common compromise [Delahaye and Nicolas, 2021], and more recently  
500 PacBio metagenomic assembly using metaMDBG [Benoit et al., 2023] has improved on that. Both PacBio  
501 and ONT are rapidly advancing their technological development, and due to improvements in sequence  
502 length, quality, and throughput will likely replace short read NGS technologies entirely in the near term.

## 503 6.3 Metagenomic strains

504 In order to fully understand microbial communities, it is necessary to resolve them into individual strains,  
505 the fundamental unit of microbiological diversity [Segata, 2018, Hildebrand, 2021]. *Escherichia coli*, which  
506 has a highly variable genome, is a well-known example of the importance of strains in functional char-  
507 acterisation: some are harmless commensals while others are harmful pathogens [Leimbach et al., 2013].  
508 Resolving metagenomic data at the strain level brings a new dimension to functional prediction and to the  
509 comparison of the metabolic potential of samples. A *metagenomic strain* is a set of clonal descendants of a  
510 single cell, with such low levels of recombination with other strains that it can be distinguished genetically  
511 from them. Members of a metagenome strain may differ substantially, particularly in the rapidly evolving

512 accessory genome [Maistrenko et al., 2020]. Reconstructing metagenomic strains is difficult since sequenc-  
513 ing depth may be insufficient for genome reconstruction and conspecific strains with overlapping genetic  
514 regions may be present. Strain-resolved genomes can be determined by sequencing cultured isolates or  
515 single cells, but the former are not representative of the community and the latter remain too expensive  
516 and low-throughput for many applications. For these reasons, there is a practical need for efficient methods  
517 that can profile microbial communities at high genomic resolution [Bickhart et al., 2022].

518 *Reference-based methods* for delineating microbial strains in metagenomes rely in general on aligning  
519 metagenomic reads to a reference database and inferring strain identity based on single nucleotide polymor-  
520 phisms (SNPs) [Scholz et al., 2016, Truong et al., 2017, Albanese and Donati, 2017, Fischer et al., 2017,  
521 Silva et al., 2021]. These methods identify gene family presence, strain identification for dominant strains,  
522 and abundance estimates.

523 *Strain-resolved metagenomics* compares *de novo* reconstructed MAGs from different metagenomes to  
524 each other, without use of a reference database. Gene catalogues can be used to trace core genes in MAGs  
525 across samples, which can be compared to create within-species strain-resolved phylogenies of metagenomes  
526 and avoid pan-genome-induced phylogenetic idiosyncrasies [Hildebrand et al., 2021].

527 When investigating conspecific strains in the same metagenomic sample, specialised tools model SNP  
528 frequencies across assemblies to infer bacterial haplotypes and identify conspecific strains, even to provid-  
529 ing nearly complete pan genome reconstructions, or delineate strains using TGS data of low-complexity  
530 metagenomes [Quince et al., 2017a, Quince et al., 2021, Vicedomini et al., 2021]. Metagenomic strains can  
531 be mapped to GENREs, as shown in [Basile et al., 2023a] where longitudinal community models were built  
532 to describe metabolic changes over time of a well sampled microbiome.

533 Single-cell technologies can also rise to the challenge of recovering genomes of low abundant strains  
534 and microbial dark matter (see [Lloréns-Rico et al., 2022] for review), especially through the genera-  
535 tion of *single amplified genomes* (SAGs) and the promise of modelling rare species [Bowers et al., 2022,  
536 Lyalina et al., 2022, Zheng et al., 2022, Arikawa et al., 2021]. Single-cell transcriptomics also promises to  
537 contribute to context-specific models [Hrovatin et al., 2021].

## 7. From metagenomic data to assembled genomes

### 7.1 First steps of data processing and assembly-free analysis

A standard metagenomic analysis workflow comprises: (1) sample collection, DNA extraction, and DNA sequencing; (2) data pre-processing and read curation, including quality control and filtering; (3) taxonomic profiling via either “read-based” or “assembly-based” approaches; and (4) further downstream analyses of genomic and functional characteristics, such as gene prediction, and functional or taxonomic annotation [Quince et al., 2017b] (Fig. 4). In this simplified workflow, (2), (3) and (4) are typically executed in a single pipeline, or can be adjusted to the analytical needs in a project. A crucial decision in the bioinformatics analysis is the choice between reliance on assemblies, or on analysing metagenomes independent of this. Assemblies will in most cases require further computational resources and user expertise, but offer in exchange a much richer datascape to explore in the follow up analysis. For example, to build models of metabolism and GENREs in particular, sequence assembly and MAG reconstruction are required to delineate the functional role of bacterial species in the community.

Once sequences for the chosen samples have been acquired, they are screened; trimmed to remove low-quality ends, bar codes, adaptors and technical artefacts; and filtered for quality (Table 4). Specific tools are available for quality filtering of long-read sequencing data [Fukasawa et al., 2020, Coster et al., 2018, Coster and Rademakers, 2023, Li, 2018]. Host-associated samples such as gut and oral metagenomes may be contaminated with reads originating in the host organism, that should be removed to avoid chimeric assemblies or false-positive assignment to bacterial taxa [Li et al., 2009, Li and Durbin, 2009, Schmieder and Edwards, 2011].

While assembly-based metagenomics is the approach of choice for building mechanistic models of metabolism, we briefly present here assembly-independent alternatives for taxonomic functional inference. Pre-processed metagenomic reads can also be used to infer the microbial taxonomic and functional potential composition in the sequenced samples, using DNA, marker gene, or protein-based approaches. Read-based taxonomic profilers estimate the relative abundance of microbes in an environmental sample by aligning the pre-processed sequencing reads to a reference sequence database such as GTDB [Parks et al., 2020] or NCBI RefSeq [O’Leary et al., 2016], using either mapping [Langmead and Salzberg, 2012] or k-mer matching [Lu et al., 2022]. The reference genome search space can be drastically reduced by including only conserved marker genes that are specific to certain taxonomic groups [Blanco-Miguez et al., 2022, Ruscheweyh et al., 2022]. Read-based profilers can also be used for functional inference [Beghini et al., 2021].

567 Protein-based functional profilers use 6-frame translations into amino acid sequences [Menzel et al., 2016,  
568 Buchfink et al., 2014, Steinegger and Söding, 2017], with the advantage of greater sensitivity because of  
569 the lower mutation rates in amino acids, but at the cost of significant increase in run time [Ye et al., 2019].

570 The choice of reference database plays an important role in read-based taxonomic profiling. Microbes  
571 lacking a reference genome will remain undetected, which is a problem in metagenomics as much of the  
572 microbial diversity is not yet represented in reference databases [Solden et al., 2016]. In addition, the read-  
573 based approach does not permit the direct association of microbial diversity to metabolic functions. While  
574 it can be used for building a community-wide model as a super-organism associated to a metagenome, a  
575 common protocol is instead to assemble the reads and reconstruct genomes.

## 576 7.2 Metagenomic assemblies

577 Reconstructing microbial genes or partial genomes by assembling sequences from environmental samples  
578 overcomes limitations of reference-based metagenomic approaches, but requires significant sequencing read  
579 depth and bioinformatics expertise. While shallow shotgun sequencing (*ca.* 0.5 million short reads per  
580 sample) can be enough to capture the taxonomic and functional diversity of microbiome samples at rel-  
581 atively low cost, it does not provide sufficient coverage for *de novo* assembly of novel genes and genomes  
582 [Lapidus and Korobeynikov, 2021].

583 Quality filtered reads can be directly assembled using metagenomic-specific assemblers, designed for  
584 short reads, long TGS reads, or in hybrid combinations (Table 4), see [Brown et al., 2021] for review.  
585 Assemblers that specifically target high-fidelity long reads perform well in general on middling-complex  
586 communities such as the gut microbiome, and have an acceptable computational efficiency for large scale  
587 metagenomes [Feng et al., 2022, Benoit et al., 2023]. When sampled metagenomes are expected to be  
588 highly similar to each other, as in *e.g.* time series data of the same ecosystem with reasonably stable  
589 strain persistence, co-assemblies of time series samples can be performed to increase assembly quality  
590 [Hildebrand et al., 2019, Hildebrand et al., 2021].

591 The drawback of assembly-based metagenomics is the high potential for errors in the assembly, and  
592 the inability to include a large part of the reads in the assembly, especially those from low-abundant  
593 genomes in highly diverse, complex ecosystems. The fraction of non-assembled reads depends on several  
594 factors, sequencing depth and microbiome diversity especially, and can range from <5% in gut, to >90%  
595 in soil metagenomes [Hildebrand et al., 2019, Bahram et al., 2018]. Even with deeper short-read shotgun

596 sequencing, *de novo* assembly remains a challenge due to the presence of repetitive DNA regions, shared  
597 genomic regions between strains, and the complexity of microbial communities with unknown and uneven  
598 representation of both diverse and closely-related strains [Somerville et al., 2019, Ayling et al., 2020]. TGS  
599 can overcome many of the difficulties in metagenomic assembly by spanning repetitive sequences and highly-  
600 conserved regions. Before the development of the latest low-error technologies, the need for short-read data  
601 to overcome high error rates and low coverage made this approach costly and complex [Kim et al., 2022].  
602 The low error rate of PacBio HiFi reads constitutes a valuable alternative with high quality, circular  
603 genomes frequently being reported in the data currently available (e.g. [Kim et al., 2022]), (Table 3).

### 604 7.2.1 Gene catalogue creation and gene prediction

605 Genes can be predicted on contigs, but having the complete sequence of a gene and its context makes its  
606 functional and taxonomic assignment and its annotation much easier and more reliable, facilitating the  
607 construction of community-wide GENREs [Tamames and Puente-Sánchez, 2018].

608 To capture the set of proteins represented in a set of metagenomes, one can generate reference gene  
609 catalogues. These typically start with the genes predicted on contigs from metagenomic assemblies, that  
610 are subsequently clustered e.g. at 95% identity, in order to remove trivial differences between sequences  
611 due to fragmentary data (e.g. genes that miss the start or stop codons), sequencing errors or small, strain-  
612 level variations [Qin et al., 2010]. The clustering can be performed either at the nucleotide or amino acid  
613 level [Fu et al., 2012, Steinegger and Söding, 2017]; the former provides greater resolution for taxonomic  
614 classification, whereas the latter is better suited for identifying functionally-relevant similarities between  
615 distant orthologues [Commichaux et al., 2021, Wang and Jia, 2016].

616 The underlying goal of the clustering process is to reproducibly group together sequences that have the  
617 same function or the same taxonomic origin, thereby identifying the genes from which the sequences are  
618 derived in a way that is consistent across samples. Each cluster is typically represented by one sequence,  
619 either a representative selected from the sequences clustered together, or a sequence that represents the  
620 consensus of the clustered sequences. Cluster representatives can be used in a broad range of sequence-  
621 based analyses (*e.g.* database searches, function or structure predictions), and also to estimate the rel-  
622 ative abundance of a single gene across metagenomes using a gene catalogue [Commichaux et al., 2021,  
623 Plaza Oñate et al., 2019]. As gene catalogues reduce the redundancy of the data, they can be used as a  
624 basis of exhaustive surveys that compare the metabolic potential of metagenomic samples by associating

625 metabolic functions to the genes.

### 626 7.2.2 Creation of metagenome-assembled genomes (MAGs)

627 Most contigs obtained from assemblies are only fragments of pro- or eukaryotic genomes. TGS permits the  
628 assembly of near-complete genomes for abundant species. For less abundant organisms and when using  
629 short read sequencing data, it is necessary to add a *binning step* that sort contigs into “bins” according to  
630 the genome they likely originate from, based on assumptions of shared sequence composition, orthologous  
631 linkage data, and shared co-abundance within and between samples [Alneberg et al., 2014, Wu et al., 2016,  
632 Kang et al., 2019, Nissen et al., 2021, Pan et al., 2022]. VAMB [Nissen et al., 2021] in particular allows  
633 splitting clusters into sample-specific bins according to their sample of origin, an approach termed “multi-  
634 split,” which is useful for comparing pan genomes of conspecific strains. *Bin consolidation* assimilates bins  
635 created by different tools into a set of refined bins [Uritskiy et al., 2018, Sieber et al., 2018b].

636 Genome bins of sufficient quality to approximate a genome are commonly called “MAGs,” for *metagenome-*  
637 *assembled genome*. Popular measures of MAG quality are the genome *completeness*—a desirable measure  
638 of the proportion of genome recovered for the species of interest—and genome *contamination*, the undesir-  
639 able amount of genome fragments that are of different species origin and been mistakenly binned together  
640 [Chen et al., 2020]. Tools exist for quality assessment using single-copy marker genes as well as detection  
641 of contamination and chimeric assemblies (Table 4). A MAG is said to be of high quality when it has  
642 over 90% of the expected count of SCGs with less than 5% redundancy of their prevalence, and containing  
643 assemblies of ribosomal RNA (rRNA) and transfer RNA (tRNA) genes [Bowers et al., 2017]. Abundance  
644 of MAGs in a sample can be estimated using (1) mapping raw metagenomic reads on the newly created  
645 MAG and (2) extracting informative marker genes for each MAG to which then either reads are mapped,  
646 or their abundance is derived from a gene catalogue (as used in [Hildebrand et al., 2021]).

647 Even though building MAGs is data- and computation-intensive, they provide significant insights into  
648 the genomic and functional diversity found in different habitats [Almeida et al., 2019, Kashaf et al., 2022].  
649 MAGs are contig-based clusters and thus preserve the relative order of genes along contigs, which is of  
650 importance as genes found in close physical proximity may encode biosynthetic pathways for the synthesis  
651 of specialised metabolites [Almeida et al., 2021]. Dozens of high quality MAGs can be reconstructed from  
652 short-read data of a single gut metagenome, but rarely as continuous, circular genomes. Here, TGS tech-  
653 nologies can potentially yield dozens or hundreds of closed genomes without binning, markedly increasing

654 the quality of culture-independent genome reconstructions [Kim et al., 2022].

655 All-in-one pipelines combine tools to reconstruct MAGs directly from sequencing reads [Churchward et al., 2022,  
656 Krakau et al., 2022, Eren et al., 2021, Hildebrand et al., 2019]. MAGs can be used for GENRE recon-  
657 struction and thus enable metabolic modelling, as in [Bernardini et al., 2022] where the authors per-  
658 formed FBA simulations to examine interspecies metabolic interactions in anaerobic digestion. MetaGEM  
659 [Zorrilla et al., 2021] is a MAG reconstruction pipeline that directly bridges the gap between metagenomics  
660 and metabolic modelling, as it proposes add additional pipeline steps for the automatic reconstruction of  
661 GENREs using Carveme and metabolic interaction inference using SMETANA. While all-in-one pipelines  
662 are valuable, the difficulty of metagenome assembly combined with the complexity of microbial ecosystems  
663 maintain the need for curation and additional analyses, especially to capture certain taxonomic clades such  
664 as eukaryotes (see subsection 7.2.3).

### 665 7.2.3 Extending metagenomic assembly to eukaryotes

666 Along with prokaryotes, eukaryotes are significant drivers of microbiomes. For instance, autotrophic and  
667 mixotrophic protists fix carbon in aquatic environments, and heterotrophic protists catalyse nutrient cycling  
668 in aquatic and terrestrial ecosystems [Worden et al., 2015, Massana and López-Escardó, 2022]. Fungi are  
669 much more prevalent soil and aquatic ecosystems [Bahram et al., 2020], and play essential roles in global  
670 nutrient cycles and the plant rhizosphere [Tedersoo et al., 2014]. Much can be learned about the diversity  
671 and role of eukaryotes in our environment from eukaryotic MAG retrieval, but several technical hurdles pre-  
672 vent this currently: (1) eukaryotic genomic complexity, due to larger genomes, multiple chromosomes, and  
673 the many non-coding and repetitive regions, complicates both metagenome assembly and MAG retrieval;  
674 (2) commonly studied environments, such as the human gut, contain only very few microeukaryotes, at  
675 typically low abundance [Bahram et al., 2020, Tito et al., 2019]; and (3) there is a bias in the currently  
676 available metagenomic computational tools towards the study of bacterial and archaeal members of the  
677 community [Massana and López-Escardó, 2022, Pronk and Medema, 2022].

678 The differences in gene structure between prokaryotes and eukaryotes is a major obstacle to the iden-  
679 tification and annotation of eukaryotic genes. Prokaryotic tools applied to eukaryotic contigs tend to  
680 predict incomplete, erroneous and discontinuous genes and thus protein sequences. Consequently, eu-  
681 karyotic proteins are neglected in microbiome data, with a risk of being truncated and assigned an un-  
682 reliable annotations [Pronk and Medema, 2022, Karlicki et al., 2022]. Most public metagenomic repos-



683 itories use annotation pipelines tailored for prokaryotes, regardless of the taxonomic origin of contigs  
684 [Lind and Pollard, 2021].

685 Recent methodological developments tackle this challenge, through the identification of eukaryotic con-  
686 tigs and genes [Pronk and Medema, 2022, Karlicki et al., 2022, Stanke et al., 2004] and their subsequent  
687 annotation, for instance with MetaEuk [Karin et al., 2020]. A few examples of eukaryotic MAGs have  
688 been reported, but these relied either on metagenomes with a higher fraction of eukaryotes present (such  
689 as e.g. [Duncan et al., 2022]), or on human gut samples that were clearly dysbiotic and had an overgrowth  
690 of a single fungal species [Olm et al., 2019]. These examples demonstrate some of the difficulties associated  
691 to investigating eukaryotic metagenomes and that methodological challenges remain. Nonetheless, the re-  
692 covery of eukaryotic genomes in microbiomes has a major ecological interest and it will be facilitated by  
693 TGS.

## 694 **8. Importance of data reuse and repurposing in metagenomics and** 695 **metabolic modelling**

696 The data pipeline from the reads to the model encompasses a long list of steps, tools and parameters that  
697 scientists may use and combine in the process. This raises important questions related to the reproducibility  
698 of the analysis outcomes, and the traceability of the data and processes involved. We provide below an  
699 overview of the importance of these aspects in the context of the topics treated in the review.

700 Characterising microbial communities requires data, data are acquired in context, and context provides  
701 key clues for deciphering the function of a gene or an organism. *Metadata* are descriptions of, and facts  
702 about, data and other research outputs<sup>1</sup>. Metadata for genomic and metagenomic data from environ-  
703 mental samples can describe the context of the source material providing the data, including space, time,  
704 habitat, characteristics of the environment, biotic relationships; as well as sequencing and assembly meth-  
705 ods [Yilmaz et al., 2011]. For metabolic modelling, metadata can include references to genomes, traces of  
706 curation and tools used for reconstruction [Aite et al., 2018]. Indeed, the computational processes that  
707 produce research outputs are also performed in a context, which provides key information for reproduc-  
708 ing, evaluating, and reusing those research outputs. Metadata can therefore also describe computational  
709 processes across an analytic stack consisting of input data, tools, reports, pipelines, and publications

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<sup>1</sup><https://schema.datacite.org>

710 [Leipzig et al., 2021].

711 Data and reporting standards must be used in order to guarantee interoperability, reuse, and repurpos-  
712 ing. Reuse of publicly available raw sequences of metagenomes, or metagenomic assemblies is very common  
713 thanks to dedicated resources such as MGnify [Richardson et al., 2022], and MAGs as well are made avail-  
714 able in databases. The Genomic Standards Consortium (GSC) has formalised minimum reporting standards  
715 for genomes, metagenomes—Minimum Information about Metagenomic Sequence (MIMS)—and marker  
716 gene sequences with these purposes in mind [Yilmaz et al., 2011, ten Hoopen et al., 2017]. In particular,  
717 the Minimum Information about a Metagenome-Assembled Genome (MIMAG) [Bowers et al., 2017] is a  
718 community-developed standard for reporting bacterial and archaeal genome MAGs.

719 Metabolic modelling is not exempt from considerations related to traceability. Given the variety of tools  
720 suitable for GENRE generation, it is important to promote their sharing and standardisation. The use  
721 of standardised nomenclatures for GENREs reconstructed in different labs, such as in the BiGG database  
722 [Norsigian et al., 2019], is crucial for GENRE comparison and construction of multi-species GEMs. In-  
723 deed, a recurring problem lies in the reconciliation of identifiers across databases, for which MetaNetX  
724 provides identifier mapping resources [Moretti et al., 2016]. A standard format for the representation and  
725 exchange of GENREs but also cell signaling pathways and other systems biology models is the Systems Bi-  
726 ology Markup Language [Hucka et al., 2018]. This declarative format based on XML (eXtensible Markup  
727 Language) not only permits the representation of the metabolic network, but also includes mathematical  
728 parameters used for its simulation. However, the use of this format does not guarantee by itself the quality  
729 of the model contents, its reusability with other tools, or the compatibility of the identifiers with different  
730 databases. MEMOTE is a community-based test suite for the assessment of GENRE quality, representing a  
731 valuable effort in the direction of standardisation, reproducibility and interoperability [Lieven et al., 2020].

732 Widespread adoption of *FAIR* guidelines [Wilkinson et al., 2016] is a further driver of the formal-  
733 isation of data and metadata, since the latter are essential for finding and reusing the former. Going  
734 beyond this basic requirement, a key opportunity is to promote *reuse* and *repurposing* of research objects  
735 [Bechhofer et al., 2013]. Capturing the computational processes that produce research objects, is critical for  
736 reproducibility; [Cohen-Boulakia et al., 2017, Leipzig et al., 2021] identify the needs—and challenges—of  
737 improved reproducibility and define criteria for “reproducibility-friendly” scientific workflow systems.

## 738 9. Conclusion and future prospects

739 Improvements in genome reconstruction from metagenomic data have made it possible to build data-  
740 informed metabolic models for microbial communities. These mechanistic models describe the phenomemo-  
741 logical behaviour of the community, but furthermore capture the metabolic functions of under-represented  
742 species and strain-specific metabolic variations that play an important role in the community dispropor-  
743 tionate to their abundance. Third-generation sequencing has proved to be a key technology for answering  
744 challenges in metagenomics, providing better recovery of eukaryotic genomes, allowing strain-level analy-  
745 sis and pangenomics, and meeting algorithmic challenges in genome delineation. Improved metagenomics  
746 leads to improved metabolic models that more fully describe communal functions as well as the added  
747 value of individual species and strains. The translation of the latest technologies into routine experimental  
748 design will likely take years, however, so algorithmic developments addressing these same questions for  
749 NGS short read metagenomics are still needed.

750 Metagenomics is the -omics that contributes the most to community-wide metabolic models, in the  
751 same way that genomics contributes to models of individual species. Integrating transcriptomics and  
752 metabolomics to build *integrative models*, following a systems biology and systems ecology approach, is  
753 necessary to fully comprehend the (eco)system. Even if these data are not obtained systematically, they  
754 can be valuable for calibrating metabolic models.

755 Depending on the complexity of the ecosystem and despite the use of TGS, the number of MAGs  
756 retrieved from environmental samples is today subpar, and the proportion of raw reads they make use of  
757 can remain limited [Pessi et al., 2022]. Those limitations may prevent MAG-derived metabolic models from  
758 accurately reflecting the complete functional diversity of the microbiome. Continuing efforts in developing  
759 efficient algorithms for MAG reconstruction must be synergetic with sequencing technique improvements.

760 Throughout this review we contrasted phenomenological and mechanistic models as complementary  
761 ways of describing the complex behaviour of microbial communities. As alluded to in section 5, hybrid  
762 models are a promising avenue for reconciling both approaches. They could combine coarse-grained but  
763 highly-scalable phenomenological modelling with limited fine-grained modelling of selected systems, such as  
764 the often-overlooked secondary metabolism. Developing a hybrid paradigm would combine the advantages  
765 of the two approaches and greatly contribute to building complete understanding of natural microbial  
766 communities.

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## 11. Conflicts of interest

The authors declare no conflict of interest.

## 12. Copyright

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