1 # MMinte: An application for predicting metabolic interactions

2 # among the microbial species in a community

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31 **Abstract**

32 **Background.** The explosive growth of microbiome research has yielded great quantities of data. These

33 data provide us with many answers, but raise just as many questions. 16S rDNA—the backbone of

34 microbiome analyses—allows us to assess α-diversity, β-diversity, and microbe-microbe associations,

35 which characterize the overall properties of an ecosystem. However, we are still unable to use 16S rDNA

36 data to directly assess the microbe-microbe and microbe-environment interactions that determine that

37 system's broader ecology. Thus, properties such as competition, cooperation, and nutrient conditions

38 remain insufficiently analyzed. Here, we apply predictive community metabolic models of microbes

39 identified with 16S rDNA data to probe the ecology of microbial communities.

40 **Results.** We developed a methodology for the large-scale assessment of microbial metabolic

41 interactions (MMinte) from 16S rDNA data. MMinte assesses the relative growth rates of interacting pairs

42 of organisms within a community metabolic network and whether that interaction has a positive or

43 negative effect. Moreover, MMinte's simulations take into account the nutritional environment, which

44 play a strong role in determining the metabolism of individual microbes. We present two case studies

45 that demonstrate this software's utility. In the first, we show how diet influences the nature of the

46 microbe-microbe interactions. In the second, we use MMinte's modular feature set to better understand

47 how the growth of *Desulfovibrio piger* is affected by, and affects the growth of, other members in a

48 simplified gut community under metabolic conditions suggested to be determinant for their dynamics.

49 **Conclusion.** By applying metabolic models to commonly available sequence data, MMinte grants the

50 user insight into the metabolic relationships between microbes, highlighting important features that may

51 relate to ecological stability, susceptibility, and cross-feeding. These relationships are at the foundation

52 of a wide range of ecological questions that impact our ability to understand problems such as

53 microbially-derived toxicity in colon cancer.

54

55 **Keywords**: metabolic network reconstruction, network, microbiome, 16S rDNA, predictive community

56 modeling

57 **Background**

58   Advances in sequencing technology have culminated in an explosion of 16S rDNA–based microbiome

59 projects, both small [1] [2] [3] and large [4, 5] [6]. The microbial ecosystems characterized in these

60 projects are the basis for many critical life processes, from global nutrient cycles [7, 8] to homeostasis in

61 the human body [9-11]. The importance of microbiome research is embodied in recent calls for the

62 formation of a worldwide microbiome consortium [12]. The gut microbiome exemplifies a complex

63 system and contains trillions of interacting bacterial cells [13]. It is not sufficient to treat bacterial taxa as

64 independent entities in a statistical framework of association and diversity. Instead, ecological

65 investigation requires examining the biological interactions underlying the complexities of our microbial

66 communities [14].

67   Efforts to understand complex microbial communities range from inference based on 16S rDNA

68 sequences [15] to the use of 'omics technologies across multiple time points [16]. A variety of software

69 and tools for analyzing 16S rDNA data exist, and range from identifying taxa [17, 18] and calculating

70 diversity [19] to producing microbe-microbe association networks [20] However, none of these utilize

71 16S rDNA to understand the mechanistic basis of microbe-microbe interactions. Each measure captures

72 part of a complex picture, but none captures the functional basis [21] for the microbial interactions that

73 make up a community—i.e., the building blocks of the microbiome.

74   Bridging the gap between association and mechanism in microbe-microbe interactions requires an

75 approach centered on mechanistic principles. One avenue to deciphering the role of a microbe in a

76 community is through the use of a predictive modeling approach [22, 23]. Metabolic models recapitulate

77 the biological processes of nutrient uptake and metabolite secretion [24], which are at the basis of most

78 microbial interactions. Computationally, the reconstruction of genome-scale metabolic models [25, 26]

79 has been automated through large-scale computing efforts such as RAST [27] and ModelSEED [28].

80 Tools such as COBRA Toolbox [29, 30] provide an interface for manipulating and investigating metabolic

81 network models. Recently, community metabolic models have been generated to explore the gut

82 microbiome in health and disease [31-34], but these efforts have been driven largely by manual

83 curation—a time consuming and laborious practice [26]. Building on these past research efforts, we

4

84   explore an alternative path to generating predictive community metabolic models for large-scale

85   microbial communities.

86   MMinte (pronounced /'minti/) is an integrated pipeline that allows users to explore the pairwise

87   interactions (positive or negative) that occur in a microbial network. From an association network and

88   16S rDNA sequence data, MMinte identifies corresponding genomes, reconstructs metabolic models,

89   estimates growth under specific metabolic conditions, analyzes pairwise interactions, assigns interaction

90   types [35] to network links, and generates the corresponding network of interactions. Our application is

91   composed of a set of seven individual functionalities, known as widgets, that run sequentially, and each

92   widget may also be run as an independent module. Below, we present two case studies from the gut

93   microbiome that illustrate how MMinte can be used to predict ecological features of a microbial

94   community based on metabolic maps of bacterial species. In doing so, MMinte provides a valuable tool

95   for generating well-defined mechanistic hypotheses for further exploration.

96

97   **Implementation**

98   MMinte consists of minimally overlapping functions that come together to perform a single task. In

99   designing it, our goal was to facilitate code re-use by focusing on modularity, allowing the user to

100   streamline the parts presented here for other purposes. Indeed, we do not view MMinte as a single-

101   purpose code, but as a set of widgets that can be repurposed for multiple queries, ranging from testing

102   interactions between a set pair of species to reconstructing a community metabolic network.

103   The web browser interface creates a point-and-click experience that allows the user to perform

104   complex analysis on large data sets without programming expertise. For those seeking more control or to

105   implement their own pipelines using MMinte widgets, MMinte functions can also be run in a command-

106   line environment. Because all of the code is provided to the user, it can be changed to fit a particular

107   need. Finally, MMinte is under continuous development, it is publicly available on Github

108   (www.github.com/mendessoares/MMinte) for use by the community, and the authors welcome

109   contributions to further its development.

110   A full run of MMinte generates a predicted network of microbe-microbe interactions for a microbial

111   community using a sequence of seven widgets that progressively analyze 16S rDNA sequences, then

112   genomes, metabolic models, and finally community metabolic networks. The analysis can be run

113   uninterrupted, and all intermediate files are stored. The seven widgets that constitute MMinte are

114   depicted in **Figure 1**.

115   <u>Widget 1</u>: *Reduces data for the downstream analysis*. The purpose of this step is to remove operational

116   taxonomic units (OTUs) that will not be used in future analyses, based on an existing list of OTU

117   associations.

118   *Inputs*: (1) 16S rDNA sequences of all representative OTUs (as one might obtain from, for instance,

119   QIIME [36] or mothur [37]) and (2) an association table between pairs of OTUs.

120   *Output*: 16S rDNA sequences of OTUs.

121   <u>Widget 2</u>: *Matches 16S rDNA signatures with corresponding genomes*. Using BLAST, the user's 16S

122   rDNA sequences are matched with 16S rDNA sequences from publicly available, complete genomes in

123   NCBI [38]. Importantly, we output a table of percent similarities between OTU- and genome-derived 16S

124   rDNA sequences. This information is used to limit potential sources of error from imperfect OTU-genome

125   pairings and to color code nodes in the final network (*Widget 7*).

126   *Input*: 16S rDNA sequences of OTUs.

127   *Output*: (1) Genome IDs and (2) percent similarity table.

128   <u>Widget 3</u>: *Obtains metabolic models*. This widget takes advantage of the ModelSEED [28] framework to

129   reconstruct and gap-fill metabolic models for a list of genomes.

130   *Input*: Genome IDs.

131   *Output*: Single-species metabolic models.

132   <u>Widget 4</u>: *Merges models*. Using COBRApy [39], this function creates metabolic models of two-species

133   communities from a list of pairs of species [33, 40]. The list can be provided by the user or created by

134   MMinte.

135   *Input*: (1) Species-species associations (optional) and (2) single-species metabolic models in the

136   Systems Biology Markup Language (SBML) format.

6

137   *Output*: Two-species metabolic models.

138   <u>Widget 5</u>: *Runs flux balance analysis.* This step estimates the growth rates for each species under

139   defined nutrient conditions, in isolation and in the presence of another species, by running a flux balance

140   analysis in COBRApy [39, 41]. The user has the option to specify the nutrient conditions to reflect the

141   specific conditions of the environment being studied.

142   *Inputs*: (1) Two-species metabolic models and (2) choice of metabolic conditions to be used from media

143   file (provided in the supportFiles folder, default choice = "complete").

144   *Output*: Growth-rate predictions.

145   <u>Widget 6</u>: *Evaluates metabolic interactions.* Using previously calculated growth rates, this function

146   quantifies the effect of pairwise interactions and assigns an interaction type to each pair, following

147   Heinken and Thiele [33]. The possible types are positive (commensalism, mutualism), negative

148   (parasitism, amensalism, competition), or no (neutralism) interaction.

149   *Input*: Growth-rate predictions.

150   *Output*: Quantitative effect of interaction and interaction type predicted.

151   <u>Widget 7</u>: *Draws community metabolic network.* This function generates a color-coded interaction

152   network using the D3.js [42] visualization platform, based on the associations provided to Widget 1. Links

153   are colored according to the type of interaction predicted by MMinte (*Widget 6*). A node's shading

154   reflects the percent similarity between OTUs and genomes (*Widget 2*)

155   *Input*: (1) Association table between pairs of OTUs, (2) percent similarity table, and (3) quantitative

156   effect of interaction and interaction type predicted.

157   *Output*: Metabolic interaction network (see **Fig. 2**)

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159

160   **Results and Discussion**

161   **Usage**

162   Below we present two case studies that exemplify how MMinte can be used to predict microbial

163   interactions under user defined metabolic conditions. All files used in these examples, and a full tutorial

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164   on how to perform these analyses, can be found in the Supplementary Materials and in the project folder

165   at github.com/mendessoares/MMinte.

166

167   *Case Study 1*

168   With just two user-provided files, one containing correlations between pairs of OTUs and the other

169   representative sequences from a microbiome study, MMinte creates an association network in which the

170   color of the links represents the types of predicted interactions between pairs of OTUs in a microbial

171   community.

172   In Case Study 1, we use data from the Human Microbiome Project to demonstrate MMinte's potential

173   for exploratory research studies that focus on the dynamics of host-associated microbial communities.

174   The Human Microbiome Project is a multi-institutional collaboration and the study was reviewed by each

175   participating institution's Institutional Review Boards. Full information can be found in [5]. The data used

176   in this example are a subset of what is available on the Human Microbiome Project page

177   (http://hmpdacc.org/HMQCP/, uncompressed files from rep_set_v13.fna.gz and otu_table_v13.txt.gz) [4,

178   5], allowing users to rerun the analysis in a straightforward and fast way, while taking advantage of

179   publicly available data. The reduced dataset contains 659 associations for 308 OTUs representing 176

180   species.

181

182   **The problem**. The number of positive and negative interactions in a community influences its level of

183   stability and consequently its resistance to invasion by pathogens [43]. With MMinte, we can run our

184   analysis in a variety of metabolic conditions and quantify the number of positive and negative

185   interactions predicted for each. This will generate hypothesis regarding the metabolic conditions likely to

186   favor stability of the system.

187

188   **The results**. We ran the full MMinte pipeline by clicking the "run all" tab on MMinte's introductory page

189   and providing two files, corrs.txt (which contains the associations between OTUs) and seqs.txt (which

190   contains representative sequences). Using the default setting of "complete" for the metabolite

8

191 availability condition, which represents a condition with 380 metabolites available in large amounts.

192 MMinte predicted 252 positive and 323 negative interactions between pairs of OTUs in addition to 179

193 pairs lacking any type of of interaction (**Figure 2, *left panel***). We then reran Widget 5 with different

194 metabolite availability conditions. **Figure 2 (*right panel*)** shows the same correlation network with a

195 metabolite availability that is 10 times lower, resulting in different predicted interactions (58 positive and

196 651 negative interactions between OTUs and 45 pairs lacking any interaction). This result is consistent

197 with the prediction that lower nutrient availability will favor more competition between organisms.

198 The results of this analysis highlight some possible characteristics of the community that could not be

199 inferred solely from association data. For instance, assuming stability, and thus protection against

200 pathogen invasion, is greater in communities with more competitive interactions [43], then the

201 metabolic conditions that lead to the predicted network shown in **Figure 2 (*right panel*)** are likely to

202 promote more stability. In addition, if we assume that stronger positive correlation values between pairs

203 of species indicate positive interactions [44], the network of interactions observed under metabolic

204 conditions equivalent to the ones listed under "complete" are more reflective of the real system than the

205 alternative metabolic conditions tested. These are just two examples of the window microbe-microbe

206 interactions—the building blocks of community networks—provide for understanding their ecology.

207

208 *Case Study 2*

209 In the following example, we use data from Rey et al [45], who investigated the growth of the

210 sulfate-reducing bacterium *Desulfovibrio piger* in the guts of gnotobiotic mice, in the presence of eight

211 other bacterial species and under different nutritional conditions. *D. piger* is the most commonly found

212 sulfate-reducing bacterium in healthy adults, and is thought to shape the responses of the gut microbiota

213 to dietary changes [45]. However, relatively little is still known about the niche this species occupies and

214 how it may influence the metabolism of the other microbial species found in the gut [45].

215

216 **The problem.** The interactions between *D. piger* and other members of the gut microbial community

217 have been shown to influence the level of H_2S in the gut. However, *D. piger* has a variety of potential

9

218   metabolic pathways, only some of which will lead to the production of H_2S. The role of interactions in

219   determining the metabolic niche of *D. piger* in the gut is both important and not fully understood. Using,

220   MMinte, we explored the types of interactions that are predicted to occur between nine different species

221   of microbes that co-occur in the human gut and whose interactions are believed to be metabolically

222   based [45, 46]. We created a set of metabolic conditions where we varied the availability of oxygen,

223   chondroitin sulfate, and fructose. These represent some of the metabolites that were manipulated in the

224   experiments of Rey et al. [45].

225

226   **Results**. We started by providing a list of species IDs to Widget 3 (*D. piger*: model 411464.8, *Bacteroides*

227   *thetaiotaomicron*: model 226186.12, *Bacteroides caccae*: model 411901.7, *Bacteroides ovatus*: model

228   28116.7, *Eubacterium rectale*: model 657318.4, *Marvinbryantia formatexigens*: model 478749.5, *Collinsella*

229   *aerofaciens*: model 411903.6, *Escherichia coli*: model 83333.113, and *Clostridium symbiosium*: model

230   742740.3). After reconstructing the individual species metabolic models and creating two-species

231   communities (*Widget 4*), we predicted species growth rates in the presence and absence of another

232   species in the community by running Widget 5 under 17 different metabolic conditions, listed in Supp.

233   Table 1. To parallel our analysis in Case Study 1, we also calculated the number of positive and negative

234   interactions under metabolic conditions containing 380 metabolites with different availabilities.

235   A look at the predicted growth rate of *D. piger* in the presence and absence of other species in the

236   community shows that this species is likely to benefit from the presence of each of the other species in

237   the community under "Complete" metabolic conditions. *D. piger* is consistently predicted to grow under

238   aerobic conditions, but under anaerobic conditions, growth is only predicted to occur if either *B. ovatus*,

239   *B. thetatiotaomicron*, *B. caccae*, *C. symbiosium*, or *E. coli* are present. Thus, using the models

240   reconstructed using ModelSEED, MMinte predicts an obligate association between *D. piger* and these

241   species in anaerobic environments (**Supp. Table 1**). Interestingly, *D. piger* impaired the growth of most

242   species it was paired with under all conditions except "complete" (**Supp. Table 1**). Exceptions were *E. coli*

243   and *E. rectale*; the magnitude of the effect of *D. piger* on their growth depended on the flux conditions for

244   oxygen, chondroitin sulfate, and sulfate (**Supp. Table 1**). Even though our analysis only focused on

245   variations in three metabolites, the results provide some insight into the niches that these species occupy

246   and they are predicted to interact under a variety of metabolic conditions

247   Overall, the number of each type of interaction changed depending on metabolite availability, but not

248   linearly (**Figure** 3). For instance, with a 10-fold decrease in metabolite availability, the number of

249   predicted parasitic interactions increased—but with a further 10-fold decrease in metabolite availability,

250   the number of predicted parasitic interactions then decreased. This suggests that alternative metabolic

251   pathways may be invoked depending on the amount of particular metabolites and not necessarily on

252   their presence or absence, affecting how different organisms interact with each other. These results are

253   in concordance with the observation that the nutrient conditions that organisms experience are

254   predicted to have marked effects on the kinds of interactions they have (**Figure** 3).

255

256   **General Discussion.** MMinte bridges the gap between association and mechanism in microbe-microbe

257   interactions by assessing the metabolic influence that two microbes have on each other. Our predictive

258   modeling approach involves reconstructing the pairwise metabolic community models that make up the

259   basic unit of interaction within a community. More specifically, MMinte advances microbiome research

260   by assigning functional interactions instead of simply calculating associations or correlations based on

261   abundance [44]. This allows us to capture the effect of metabolite exchange on the interactions of an

262   entire microbial community across different nutrient conditions, thus providing an important link to the

263   overall drivers of environmental dynamics.

264   The metabolic interactions that MMinte identifies can be used to understand the broader ecological

265   features of a biological system. Dynamic ecological features such as stability and robustness are linked to

266   competitive-cooperative interactions and the nature of the positive-negative feedback loops they

267   engender [43]. For example, it has been widely posited that negative interactions "self-regulate" and

268   stabilize fluctuations within a community [47, 48]. In Case Study 1, MMinte showed that out of 754 total

269   associations detected among a subset of human microbiome species, 33.4% were predicted to be

270   positive and 42.8% negative under "complete" metabolic conditions. The rest (23.7%) were predicted to

271   not represent significant metabolic interactions between the species. When fasting conditions are

272   modeled by decreasing the availability of metabolites by an order of magnitude, MMinte predicts that

273   7.7% of interactions will be positive, 86.3% will be negative, and in 6% of the cases, no interactions will

274   occur. This finding intuitively matches the expectation that competition increases in a community with

275   limited nutrient availability [49]. MMinte enables users to grasp these important ecological interactions

276   and better understand the role of competition and cooperation in community stability [50, 51].

277   Case Study 2 highlights the modular nature of MMinte and the user's ability to explore the effect that

278   changes in the availability of a particular metabolite may have on the interactions between organisms.

279   The results give us an important window into the role of the environment, specifically the presence or

280   absence of oxygen, chondroitin sulfate, and sulfate, on the interactions between *D. piger* and other

281   organisms commonly found in the gut. *In vivo* experiments have shown that mice colonized solely with *D.*

282   *piger* have significantly increased levels of H_2S, which is a genotoxic metabolite that may be involved in

283   the development of colorectal cancer [52, 53], compared to mice colonized with a consortium containing

284   the other eight species analyzed here. Understanding the metabolite conditions favoring the dominance

285   of the other species over *D. piger* can help inform dietary interventions aimed at reducing the abundance

286   of this species in the gastrointestinal tract.

287   Like all algorithms, MMinte has potential limitations. For example, MMinte's predictions are only as

288   accurate as the metabolic models used. These metabolic models are linked from 16S in a multi-step

289   process that involves identification of genomes and metabolic network reconstruction using ModelSEED

290   [28]. Missing data in the genome database or in the biochemical database are both potential sources of

291   error. Conversely, as databases rapidly grow, so will the accuracy of MMinte's predictions. MMinte helps

292   the user minimize the potential for over-interpretation by visually displaying the percent similarity

293   between 16S rDNA provided by the user and the genomic data,

294

295   **Conclusions**

296   MMinte is the first tool that predicts the type of interactions occurring between organisms in a

297   complex microbial community under defined metabolite conditions based on the metabolic models of

298   each species. A full run takes data of an association network and 16S rDNA sequences, identifies the

299 genomes, reconstructs metabolic models, and estimates the effect of being in a two-species community

300 for each species under user defined metabolic conditions. The predicted interactions are then plotted in

301 an interaction network. Additionally, the widgets that make up MMinte can be run independently

302 allowing the user to run specific tasks and bypass some of the steps of the analysis. We have

303 incorporated the design principles of clear modularity, usability, and open access into the development

304 of MMinte. In our view, part of the value of MMinte to the development of predictive community

305 metabolic modeling is the potential for integration into other analytical platforms. We view the ability to

306 build on existing development efforts as critical to expanding systems biology tools to wider and broader

307 scales of ecology and data [14]. MMinte is thus a fundamental tool for exploring a large number of

308 interactions, allowing researchers to move beyond the use of statistical measures of association into

309 biologically relevant analysis of interactions between the species in a microbiome.

310

311 **Availability of supporting data**

312 All files required to reproduce the results are provided in the project folder at

313 www.github.com/mendessoares/MMinte .

314 Additionally, results and supplementary files can be found in the following project folder

315 **MMinte/supportFiles/ResultsAndSupplMaterial**. The folder **ResultsCaseStudy1** contains output files

316 from the first example workflow. The folder **ResultsCaseStudy2** contains the file with the

317 Supplementary Table 1, listing the growth rates of the 9 species analyzed in the second example in the

318 presence and absence of another species in the two-species communities under a variety of metabolic

319 conditions.

320

321 **Ethics statement**

322 In Case Study 1, we use data from the Human Microbiome Project to demonstrate MMinte's potential

323 for exploratory research studies that focus on the dynamics of host-associated microbial communities.

324 The Human Microbiome Project is a multi-institutional collaboration and the study was reviewed by each

325 participating institution's Institutional Review Boards. Full information can be found in [5].

326

Abbreviations

MMinte - Microbial Metabolic interactions

FBA – Flux Balance Analysis

COBRA – Constraint-Based Reconstruction and Analysis

Competing interests

The authors declare that they have no competing interests.

Author contributions

HMS, NC and MM designed the algorithm. HMS, MM and LMS implemented the algorithm. HMS performed the case studies' analyses. HMS and NC wrote the manuscript. All authors read and approved the final manuscript.

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345

346 **Figure Legends**

347 **Figure 1. Schematic of the MMinte pipeline.** Each green rectangle represents one widget. **Widget 1**

348 takes two files, a network of associations between operational taxonomic units (OTUs) and a FASTA file

349 containing the 16S rDNA sequences from a microbiome study, and reduces the latter data set to include

350 only the sequences for OTUs present in the network. **Widget 2** identifies the sequences provided and

351 assigns them a genome ID. The percent similarity between the query OTU and the 16S sequence of the

352 genome to which it was matched is stored in a file to be used by **Widget 7**. **Widget 3** Calls the

353 ModelSEED service [28] with the list of genome IDs produced by Widget 2, which reconstructs species

354 metabolic models that are exported to the user's local machine. **Widget 4** then uses these species

355 models to create metabolic models for two-species communities. **Widget 5** estimates the growth rate of

356 each species in the community under defined metabolic conditions, which can be changed by the user.

357 **Widget 6** assesses the types of interaction (mutualism, parasitism, commensalism, competition,

358 amensalism, or neutralism) occurring between the pairs of species in a community based on the effect

359 that each species has on the growth of another. **Widget 7** takes the initial information about the

360 topology of the network, the information about the percent similarity between OTUs and the closest

361 genomes, and the types of interactions and plots an interaction network in which the color of the links

362 represents the type of interaction (positive, green; negative, red; no interaction, grey).

363

364 **Figure 2.** Network and number of the different types of interaction for operational taxonomic units in

365 Case Study 1 under "Complete" (*left*) and "Complete/10" (r*ight*) metabolic conditions. **There are 380**

366 **metabolites in the "Complete" metabolic conditions and they exist as highly available. The**

367 **metabolic condition "Complete/10" contains the same metabolites as "Complete" but at 10 times**

368 **lower availability. Please see file Diet.txt for a complete list of the metabolites, and their**

369 **availabilities represented as uptake metabolic fluxes. It can be seen from the figure that a 10 fold**

370 **reduction in metabolite availability resulted in a significant decrease in the number of positive**

371 **interactions predicted to occur between the members of this community, with parallel increase in**

372 **the number of negative interactions.**

373

374   **Figure** 3. Number of each type of interaction predicted to occur between pairs of the nine bacterial

375   species (*D. piger*, *Bacteroides thetaiotaomicron*, *Bacteroides caccae*, *Bacteroides ovatus*, *Eubacterium*

376   *rectale*, *Marvinbryantia formatexigens*, *Collinsella aerofaciens*, *Escherichia coli*, and *Clostridium*

377   *symbiosium*) inoculated into the guts of gnotobiotic mice under different metabolic conditions in [45] and

378   used in Case Study 2. The metabolic conditions simulated in MMinte were "Complete", "Complete/10"

379   and "Complete/100". There are 380 metabolites in the "Complete" metabolic conditions and they exist as

380   highly available. The metabolic condition "Complete/10" contains the same metabolites as "Complete"

381   but at 10 times lower availability and "Complete/100" contains the same metabolites as "Complete" but

382   at 100 times lower availability. Please see file Diet.txt for a complete list of the metabolites, and their

383   availabilities represented as uptake metabolic fluxes.
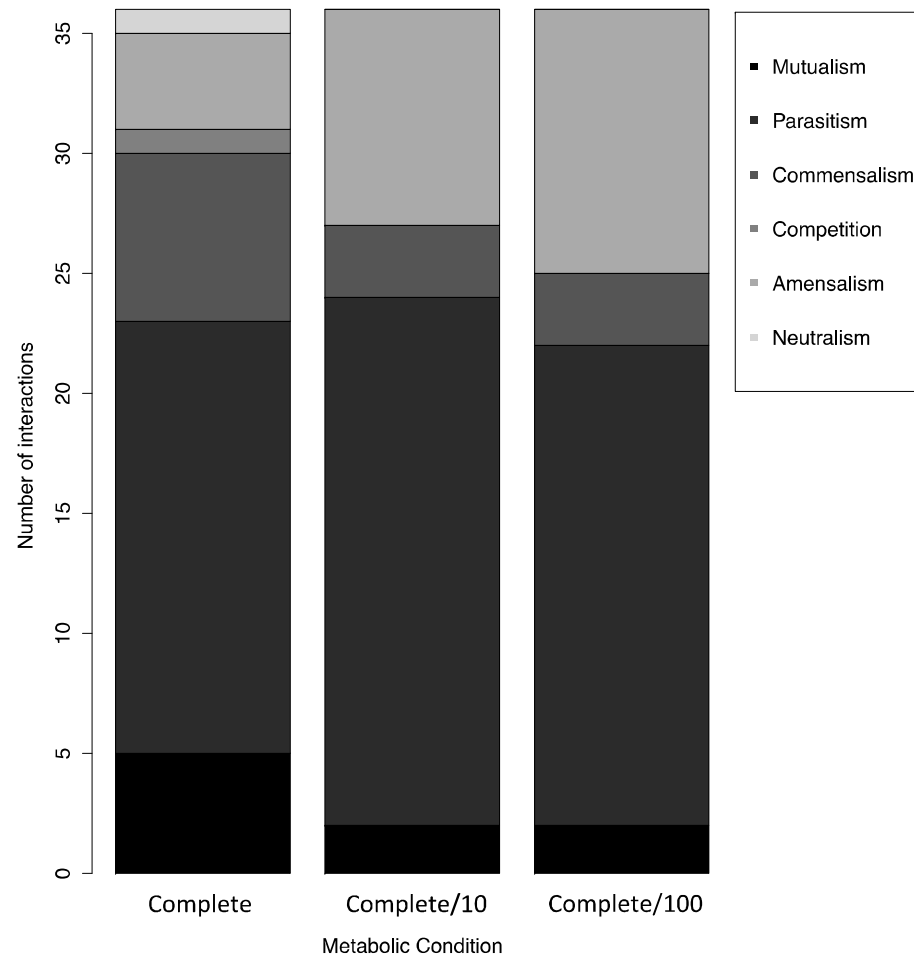
384

385 **Figure 1.**
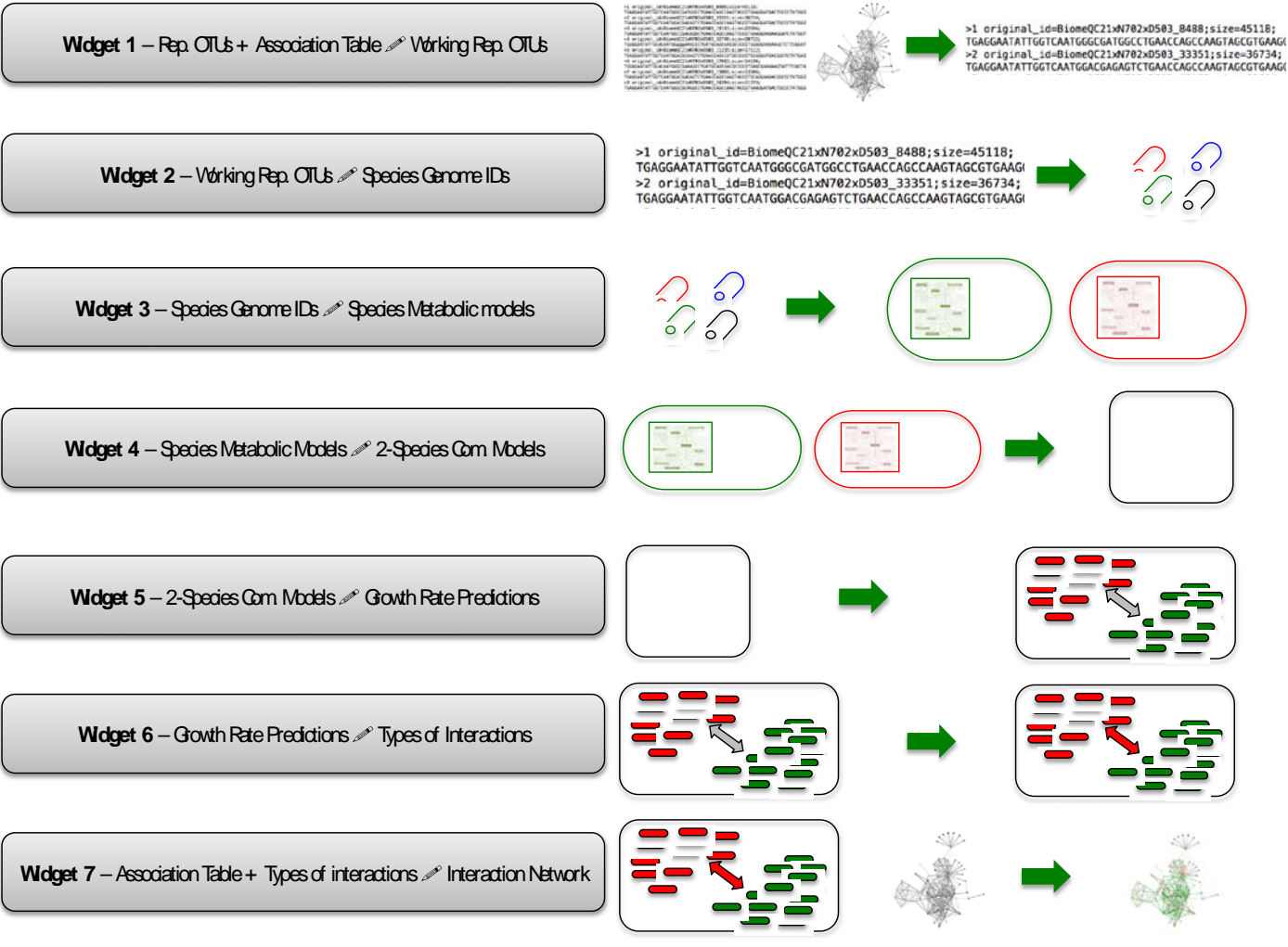
386

387   Figure 2.

Interactions		Complete	Complete/10
Positive	———	252	58
Negative	———	323	651
No Interaction	———	179	45

388

389   <u>Figure 3.</u>

391
392
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