

OPEN ACCESS

Repository of the Max Delbrück Center for Molecular Medicine (MDC) in the Helmholtz Association

<https://edoc.mdc-berlin.de/17193>

The Human Gut Microbiome: From Association to Modulation

Schmidt T.S.B., Raes J., Bork P.

This is the final version of the accepted manuscript. The original article has been published in final edited form in:

Cell
2018 MAR 08 ; 172(6): 1198-1215
2018 MAR 08 (first published online: final publication)
doi: [10.1016/j.cell.2018.02.044](https://doi.org/10.1016/j.cell.2018.02.044)

Publisher: [Cell Press](#) / [Elsevier](#)



Copyright © 2018. This manuscript version is made available under the [Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License](http://creativecommons.org/licenses/by-nc-nd/4.0/). To view a copy of this license, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/> or send a letter to Creative Commons, PO Box 1866, Mountain View, CA 94042, USA.

1 **The human gut microbiome: from association to modulation**

2 Thomas Sebastian Benedikt Schmidt¹, Jeroen Raes^{*2,3} and Peer Bork^{*1,4,5,6}

3

4

5 ¹ European Molecular Biology Laboratory, Structural and Computational Biology Unit, 69117
6 Heidelberg, Germany

7 ² KU Leuven – University of Leuven, Department of Microbiology and Immunology, Rega
8 Institute, Herestraat 49, B-3000 Leuven, Belgium

9 ³ VIB, Center for Microbiology, Heerestraat 49, B-3000 Leuven, Belgium

10 ⁴ Molecular Medicine Partnership Unit, University of Heidelberg and European Molecular Biology
11 Laboratory, 69120 Heidelberg, Germany

12 ⁵ Max-Delbrück Center for Molecular Medicine in the Helmholtz Association, 13125 Berlin,
13 Germany

14 ⁶ Department of Bioinformatics, Biocenter, University of Würzburg, 97074 Würzburg, Germany

15 *correspondence: jeroen.raes@kuleuven.vib.be; bork@embl.de

16

17 **Abstract**

18 Scientific progress on the human gut microbiome comes at an incredible pace and breadth.
19 Many prevalent gut species can now be represented by sequenced genomes and have been
20 linked to a wide range of factors in association studies, revealing that known co-variables of
21 microbiome composition only account for a small fraction of observed variation. Methodological
22 advances such as absolute quantification, increased taxonomic resolution to levels subordinate
23 to species, or refined, stratified study populations might improve this situation, but need to be
24 complemented by efforts towards better functional understanding of the microbiome as an
25 ecological system. Baseline longitudinal cohorts and perturbation experiments are essential in
26 this regard, combining insights from *in vitro*, *in vivo* and *in natura* approaches. Yet, the biggest
27 challenge ahead lies in transforming this knowledge into actionable items for targeted gut
28 microbiome modulation.

29

30 The human microbiota is the focus of one of the most dynamic research fields of our time, and
31 most efforts are directed at the gastrointestinal tract which harbors most of our microbes. In the
32 past decade, our understanding of the organisms inhabiting our gut, their functionality and their
33 roles in human health and disease has advanced greatly, facilitated by fast technological
34 development. Research on the gut microbiome is progressing through several steps that mirror
35 those of other fields on other biological systems: (i) compilation of parts lists, (ii) association of
36 the system or its components to external factors, (iii) establishment of functional knowledge, and
37 (iv) translation of that knowledge into applications. For the gut microbiome, this is reflected in
38 the following developments.

39 (i) The compilation of gut microbiome ‘parts lists’ has been in full swing for more than a decade
40 and is now almost complete, for the dominating prokaryotic domains, and at the resolution of
41 genera and species. Several studies established the baseline structure and function of the
42 microbiome – that is, lists of species and their genes – with major contributions from two large
43 collaborative efforts of the MetaHIT (MetaHIT Consortium et al., 2014; Qin et al., 2010) and
44 Human Microbiome Project (HMP, The Human Microbiome Jumpstart Reference Strains
45 Consortium et al., 2010; The Human Microbiome Project Consortium, 2012) consortia. Although
46 novel diversity continues to be discovered, in particular at subspecies and strain level, and
47 although a large fraction of microbial genes remains functionally uncharacterized, the census of
48 the most dominant lineages in industrialized populations is arguably approaching completion
49 (e.g., Zhou et al., 2018).

50 (ii) Using these parts list, a wealth of studies has probed for associations of the gut microbiome
51 to disease, host factors or the wider environment. As coverage and scope increase, these have
52 been collectively referred to as Metagenome-Wide Association Studies (MWAS) (Wang and Jia,
53 2016), in analogy to Genome-Wide Association Studies (GWAS). Recently, MWAS have
54 reached population level, as large-scale cross-sectional studies (Falony et al., 2016;
55 Zhernakova et al., 2016) started to provide an integrated view of the relative impact of various
56 host and environmental factors on microbiome composition (see Box 1).

57 (iii) Associations identified by MWAS are observational, can be indirect or confounded by
58 underlying factors, and do not easily translate into causal links. However, for a functional
59 understanding of a complex system such as the gut microbiome, it is necessary to connect parts
60 lists (1D) to networks (2D) in a spatial (3D) and temporal (4D) context (Raes and Bork, 2008),
61 and this requires adapted concepts (see below) and methodological approaches (see Box 2).
62 Although the study of the microbiome’s taxa interaction networks (2D), i.e. the interactions
63 between its parts (1D), is ongoing, the inference of species interactions from cross-sectional

64 data remains challenging (Weiss et al., 2016). This is in part because current readouts (fecal
65 samples) are still mostly non-quantitative (Vandeputte et al., 2017c) and poorly reflect the
66 spatial organization of the intestinal tract (3D). Moreover, interactions and microbiome function
67 are dynamic, and in consequence, individual gut microbes and entire communities need to be
68 studied in the context of time (4D), though longitudinal studies so far remain scarce.
69 Perturbation experiments, in particular, enable the study of a system's dynamics, both at the
70 level of individual parts and the entire system. An increasing number of intervention studies
71 adds to our functional understanding of the gut microbiome, but it remains unclear whether
72 observed responses are generic, stratified or indeed personal (see Box 3).

73 (iv) Finally, knowledge on the microbiome begins to be translated into applications, and this
74 entails a move from perturbation to modulation. Perturbations may trigger microbiome shifts, but
75 most of these are unforeseeable or not intended. Targeted microbiome modulation, preferably
76 with predictable outcome in terms of response and without side effects, will require a functional
77 understanding of the system, but also an accepted operational definition of desired "healthy"
78 endpoints, both intrinsically and in relation to the host. Given these, we expect microbiome
79 modulation to become a major translational asset in the near future, establishing the
80 microbiome as a versatile therapeutic target.

81 In this review, we focus on active and emerging areas in the context of the above (see Figure
82 1), and especially on studies of the human gut microbiome *in natura*, with less emphasis on *in*
83 *vivo* work in animal models. Specifically, we highlight recent findings on co-variables associated
84 to microbiome composition, discuss the strengths and limitations of MWAS, and argue that a
85 strong push towards longitudinal and perturbation-based study designs is essential for a deeper
86 functional understanding of the gut microbiome, as well as for the development of microbiome
87 modulation strategies towards improved health and well-being.

88

89 **Co-variates associated to human gut microbiome composition**

90 Taxonomic composition of the gut microbiome varies greatly between individuals, due to both
91 microbiome-intrinsic and microbiome-extrinsic factors (see Figure 2). The former depend on the
92 microbiome's state, e.g. following maturation during lifetime, which feeds back on itself, e.g. via
93 taxa interactions. The latter microbiome-extrinsic factors refer to the various environmental
94 layers that impact on or interact with the gut microbiome. These can explain part of the
95 observed variation within a population, and can be classified empirically into three overlapping
96 categories: *host-extrinsic* factors (i.e., factors influenced by host lifestyle to some extent, such
97 as dietary habits), *host-intrinsic* factors (e.g., host genetics), and *environmental* factors (e.g., the
98 vertical transmission of maternal strains to neonates, or neocolonization constraints by regional
99 strain pools; Figure 2).

100 Many small- to medium-scale MWAS have linked gut microbiome composition to such factors
101 (see e.g. Lynch and Pedersen, 2016; & Wang and Jia, 2016 for reviews). The majority of these
102 studies have probed associations of *taxonomic* composition, usually of genera or species,
103 whereas *functional* composition, i.e. gene and functional repertoire, has received less attention,
104 mostly due to technical and economical constraints. Moreover, only recently have increasing
105 cohort sizes and comprehensive phenotyping enabled the identification of associations to a
106 wide range of co-variates with sufficient statistical power (Falony et al., 2016; Goodrich et al.,
107 2016; Turpin et al., 2016; Wang et al., 2016a; Zhernakova et al., 2016). For the first time, such
108 studies have allowed to quantify the relative contributions of relevant co-variates to microbiome
109 composition. A key finding has been that even the strongest co-varying factors explain only a
110 surprisingly small fraction of inter-individual gut microbiome variation, at an estimated combined
111 effect size in the range of 10-15% (see Box 1). This is, nevertheless, considerably larger than
112 technical variation (Costea et al., 2017b) and known co-variates should therefore be taken into
113 account as potential confounders of MWAS (see below). Here, we summarize previous findings
114 on co-variates of human gut microbiome composition, with a focus on recent work.

115

116 *Microbiome state, including disease association and host age*

117 Microbiome compositional state is associated to microbiome-extrinsic factors and shaped by
118 stochastic or ecological effects (e.g., founder effects when re-seeding from the environment),
119 but also potentially self-reinforcing. Differences in microbiome state may underlie differential
120 associations to extrinsic factors, and it is necessary to stratify analyses accordingly (see Box 3).
121 One such intrinsic stratifying factor is probably the gut enterotype, although it is not clear
122 whether such community types follow external co-variates such as diet, transit time or

123 inflammation, or represent intrinsically different compositional optima with similar functionality,
124 or both (Costea et al., 2018). Importantly, microbiome associations are often complex and
125 seldom unidirectional: an external influence may trigger a compositional shift which then
126 becomes entrenched in an adapted microbiome state, but microbiome state also feeds back to
127 the host in various ways (e.g., via the production of certain metabolites).

128 An example of this are the complex associations between microbiome state and diseases from
129 various medical indication areas (Gilbert et al., 2016; Lynch and Pedersen, 2016; Wang and Jia,
130 2016). In some, e.g. in the case of colorectal cancer (Zeller et al., 2014) or arthritis (Scher et al.,
131 2013; Tito et al., 2016; Zhang et al., 2015b), individual marker taxa are associated to the
132 disease, whereas effects on overall composition are mild. Other disease states, in contrast, are
133 associated to marked shifts in overall compositional features, such as reduced diversity or
134 richness, as is e.g. the case for obesity (Le Chatelier et al., 2013; Turnbaugh et al., 2009) or
135 inflammatory bowel disease (IBD, Manichanh et al., 2006; Ott et al., 2004). However, for any
136 detected association, it is not clear *a priori* whether microbiome shifts cause the disease or vice
137 versa, or whether both the disease state and observed microbiome effects are caused by a third
138 factor. Indeed, a recent meta-study of 28 MWAS datasets found an overlap of microbiome
139 signatures between different diseases, implying that several reported disease-microbiome links
140 might be non-specific (Duvall et al., 2017) and possibly linked to other factors such as transit
141 time or inflammation (see also Falony et al., 2016). Hence, disease specificity of reported
142 microbiome markers needs to be established, and preferably tested *post hoc*, e.g. if
143 comorbidities or shared symptoms are known, as is the case for colorectal cancer and IBD
144 (Zeller et al., 2014).

145 Other well-established differences in microbiome state follow host age (reviewed recently by
146 (Kundu et al., 2017; Lynch and Pedersen, 2016)). Some age-related transitions are gradual,
147 while others are more clearly defined, e.g. between neonates and older infants, and can
148 correlate with lifestyle changes, such as the cessation of breastfeeding. After birth, infants are
149 colonized by species present in the environment and the mother (Tamburini et al., 2016). Strain-
150 level analyses have recently confirmed that a significant fraction of the developing microbiome
151 is indeed of maternal origin, but that seeding is selective, as strains from certain phyla are
152 acquired from the environment (Korpela et al., *in press*). Neonate and early life microbiome
153 composition has been linked to several childhood diseases, including atopy and asthma (e.g. by
154 Fujimura et al., 2016 & Stokholm et al., 2018). It has been suggested that this may be due to
155 early life disturbances of the microbiome, e.g. as a side effect of antibiotics treatment (reviewed
156 by Langdon et al., 2016). Other early life events such as birth mode (Caesarean section vs

157 vaginal birth) or feeding (breastfeeding vs formula) have been associated to developing or adult
158 microbiome composition (recently reviewed by Tamburini et al., 2016), but more recent
159 evidence with regard to longer-term effects is mixed (Chu et al., 2017; Falony et al., 2016).
160 Diversity increases after infancy and compositional shifts continue more gradually during late
161 childhood, adolescence and adulthood (Kundu et al., 2017; Odamaki et al., 2016). Elderly
162 people show signatures of diversity loss, decreased temporal compositional stability and
163 compositional shifts, all of which are associated to general health, but also to confounders like
164 diet and housing environment, a more constrained lifestyle (O'Toole and Jeffery, 2015) or
165 medication (Ticinesi et al., 2017).

166

167 *Extrinsic host factors including medication, diet, lifestyle, BMI & stool consistency*

168 A wealth of studies tested associations of the adult gut microbiome to factors that are host-
169 extrinsic (i.e., influenced by host lifestyle at least to some extent). For instance, medication is
170 emerging as a major co-variate. It is commonly accepted that broad-spectrum antibiotics –
171 administered to diminish pathogens – impact the gut microbiota as a side effect, both on
172 immediate and longer timescales (Becattini et al., 2016; Langdon et al., 2016). Perhaps more
173 surprisingly, an increasing number of reports also link non-antibiotic drugs to microbiome
174 modulation (reviewed by Le Bastard et al., 2017 and Maier and Typas, 2017). For example, the
175 type 2 diabetes drug metformin has been shown to have a stronger impact on microbiome
176 composition than the disease condition itself (Forsslund et al., 2015), an effect that has recently
177 been corroborated in a randomized crossover study (Wu et al., 2017). Similarly, proton pump
178 inhibitors (Freedberg et al., 2015; Imhann et al., 2016; Jackson et al., 2016), atypical
179 antipsychotics (Bahr et al., 2015; Flowers et al., 2017; Mäkivuokko et al., 2010) and non-
180 steroidal anti-inflammatory drugs (Rogers and Aronoff, 2016), among others, have been
181 reported to impact the gut microbiome. In the Flemish Gut Flora Project (FGFP) study,
182 medication (including antibiotics, but also e.g. anti-histamines and hormones) was found to be
183 the most important co-variate of microbiome composition (Falony et al., 2016). In a recent large-
184 scale *in vitro* screen testing 1200 marketed drugs, around half of non-bacterial anti-infectives
185 and a quarter of all human-targeted drugs were found to inhibit at least one gut commensal
186 (Maier et al., *in press*), implying that the effect of medication on the gut microbiome remains
187 massively underexplored.

188 Most drugs are defined chemical compounds, but the gut microbiome is regularly confronted
189 with a complex mix of millions of compounds of dietary origin. As gut commensals contribute to
190 food digestion, links between diet and the microbiome have been studied for years, at different

191 levels of resolution (reviewed e.g. by Flint et al., 2012; Sonnenburg and Bäckhed, 2016). These
192 include microbiome signatures of broad nutritional categories, such as plant- and animal-based
193 diets (David et al., 2014; Muegge et al., 2011), and longer-term dietary patterns (Smits et al.,
194 2017; Wu et al., 2011). However, although diet-microbiome associations were confirmed in
195 cross-sectional studies (Falony et al., 2016; Zeevi et al., 2015; Zhernakova et al., 2016), diet
196 explained only a low single digit percentage of observed microbiome variation after adjusting for
197 covariates. This range likely represents a lower limit, as most cross-sectional studies rely on
198 self-reported dietary data which has various issues (Ioannidis, 2013).

199 Several lifestyle factors such as cigarette smoking (Biedermann et al., 2013), alcohol usage
200 (Dubinkina et al., 2017) or physical exercise (Barton et al., 2017; Clarke et al., 2014; Petersen et
201 al., 2017) have been linked to microbiome composition, but were not among the top-ranking
202 covariates in recent population studies. Microbiome associations to Body Mass Index (BMI) and
203 obesity have received considerable attention, with links reported to decreased taxonomic
204 (Turnbaugh et al., 2009) and functional diversity (Le Chatelier et al., 2013). More recently, this
205 observation was extended to subspecies resolution (Costea et al., 2017a). A significant but mild
206 BMI-microbiome link was found in the FGFP (Falony et al., 2016), in line with recent meta-
207 analyses (Finucane et al., 2014; Sze and Schloss, 2016; Walters et al., 2014).

208 Stool consistency, as assessed by the Bristol Stool Scale, was the factor with the overall largest
209 effect size in the FGFP study, accounting for ~5% of observed compositional variation (Falony
210 et al., 2016). First quantified in a small-scale cohort (Vandeputte et al., 2015), this factor was
211 recently confirmed in independent cohorts (Tigchelaar et al., 2016; Vandeputte et al., 2017c;
212 Zhernakova et al., 2016), shown to be independent of water activity (Vandeputte et al., 2017a)
213 but driven by transit time (Roager et al., 2016).

214 Clearly, many of these host-extrinsic factors are not independent of each other (e.g., diet and
215 transit time, BMI and drug usage) and may moreover be linked to host-intrinsic or environmental
216 factors. It is therefore important to note that many observed microbiome signatures may be
217 driven by mixed effects.

218

219 *Intrinsic host factors such as genetics*

220 Some of the above factors (e.g. BMI) can be partially attributed to genetics. For other factors, a
221 host genetic component is more tangible: for example, the microbiome is intricately and
222 reciprocally linked to both the innate and adaptive immune system (reviewed by Belkaid and
223 Hand, 2014; Hooper et al., 2012; Thaïss et al., 2016), though it has remained challenging to
224 quantify the immune system's impact in shaping the gut microbiome independently of other

225 factors. Similarly, there is increasing evidence for a reciprocal brain-gut-microbiota axis
226 (reviewed e.g. by, Carabotti et al., 2015).

227 Several studies have probed for more direct associations of the microbiome with individual host
228 genetic loci (reviewed by Hall et al., 2017; Kurilshikov et al., 2017). In a large cross-sectional
229 study of British twins, relative abundances of several genera were found to be heritable
230 (Goodrich et al., 2016; 2014); this observation was later corroborated at species level and
231 extended to function (gene content) on a smaller sub-cohort (Xie et al., 2016). A study of 1,561
232 North Americans likewise reported taxa heritability, as well as an association of 6 human SNPs
233 to taxa abundance (Turpin et al., 2016), which has the same order of magnitude as the 9 and 33
234 loci associated with microbial taxa and pathways, respectively, reported in the Dutch LL-DEEP
235 cohort (Bonder et al., 2016). A study on a large Northern German cohort reported that 42
236 human SNPs accounted for ~10% of observed microbiome compositional variation (Wang et al.,
237 2016a). In contrast, a recent re-analysis of the above datasets, extended by 696 Israeli
238 individuals, estimated that host genetics account for less than 2% of microbiome variation
239 (Rothschild et al., *in press*). Overall, the impact of host genetics on the gut microbiota appears
240 significant, but with very low effect size. Potential discrepancies, such as with subject sex
241 (reported among the highest-ranking co-variables in the FGFP and LL-DEEP studies) may be
242 due to indirect effects, e.g. to culturally-influenced behavioral, dietary or proteotypic differences
243 that cannot be pinpointed to the genome, such as hormone levels.

244

245 *Environmental factors*

246 Environmental factors beyond the control of the human host have so far remained understudied,
247 although geographical patterns in community composition have been reported, possibly
248 connected to lifestyle (e.g., Suzuki and Worobey, 2014; Yatsunenko et al., 2012). When
249 extending the taxonomic resolution to subspecies level or to a loose operational definition of
250 strains, much more defined geographical patterns become obvious (Costea et al., 2017a;
251 Truong et al., 2017), implying the existence of regional strain pools that harbor different
252 functionality. Indeed, this can be further refined to the level of household and family where
253 replacement of gut strains can happen in adulthood (Korpela et al., *in press*), which may be part
254 of the reason why family members show a more similar taxonomic composition than non-family
255 members (Song et al., 2013). The study of effects of household in a broader context, the (built)
256 environment (Hoisington et al., 2015; Lax et al., 2014), and close contact with nature (Obregon-
257 Tito et al., 2015) will likely reveal further environmental factors influencing the individual gut
258 microbiome.

259 **Limitations to studying microbiome associations**

260 Increased cohort sizes, improved study designs and comprehensive metadata surveys have
261 greatly enhanced the statistical power of MWAS. However, they cannot overcome inherent
262 limitations to association studies, which are amplified by the complexity and variation of the
263 underlying data, and which need to be accounted for when interpreting and comparing MWAS
264 results.

265

266 *Technical variation*

267 Like other omics-driven research fields, MWAS are prone to within-study and between-study
268 batch effects. Two recent meta-analyses of microbiome-disease association studies found that
269 between-study variation required explicit or implicit batch effect correction (Duvall et al., 2017;
270 Pasolli et al., 2016). Almost every step in a typical microbiomics study, including sample
271 collection and storage (Hang et al., 2014; Song et al., 2016; Vandeputte et al., 2017d; Voigt et
272 al., 2015), DNA extraction and processing (Costea et al., 2017b; Sinha et al., 2017), and
273 bioinformatic analyses (Mallick et al., 2017), has been identified as an important source of
274 technical variation. Indeed, two recent large-scale studies on technical limits to reproducibility
275 have reported large variation between different workflows as well as between replications of the
276 same workflow in the same and in different laboratories (Costea et al., 2017b; Sinha et al.,
277 2017). This calls for refined standards, at least in comparison to reference standard operating
278 procedures (Costea et al., 2017b).

279

280 *Specificity and indirect associations*

281 Even if technical variation can be reduced, there are several limitations common to association
282 studies in general. First, the specificity of any link cannot be proven within such a study. For
283 instance, discovery of a disease association does not necessarily imply that observed
284 differences can serve as specific markers without independent replication and comparison with
285 other phenotypes. Second, any association can be indirect. A case in point are the repeatedly
286 reported microbiome associations to HIV that have recently been called into question, as most
287 of the observed signal comes from one of the risk groups, men having sex with men (Noguera-
288 Julian et al., 2016). Even this more direct association is probably confounded by further
289 untested factors, such as sexual practices, social status or life style. Similarly, confounders are
290 likely due to question several previously reported disease associations. For example, usage of
291 the drug metformin caused the majority of the signal underlying earlier reports on a strong
292 microbiome association with type 2 diabetes (Forslund et al., 2015). A comprehensive survey

293 indicated that indeed, a wide range of previously reported associations are at least in part
294 confounded by secondary factors (Falony et al., 2016).

295

296 *Taxonomic resolution and lack of functional characterization*

297 The majority of MWAS to date have relied on amplicon sequencing of the 16S rRNA gene. This
298 approach is comparatively cost effective and has enabled a dramatic scale-up in cohort sizes.
299 However, reliable taxonomic classification of current 16S amplicon sequences is generally
300 limited to genus level (Rodrigues et al., 2017), and several recent analyses indicate that many
301 taxonomic associations might only emerge at levels subordinate to species (e.g., Costea et al.,
302 2017a; Lloyd-Price et al., 2017). Moreover, amplicon approaches often limit the taxonomic
303 scope to bacteria and archaea, thereby missing potentially informative signals on eukaryal and
304 viral members of the gut flora. However, these limits to taxonomic resolution and scope may
305 soon be overcome as whole-genome shotgun metagenomic sequencing becomes more
306 affordable (see Box 2). This approach also provides readouts on the microbiome's gene and
307 functional repertoires, but this valuable information often remains untapped, partially due to a
308 blatant lack in functional annotation: a large fraction of gut microbial genes, both from cultured
309 isolates and metagenomes, is uncharacterized to date.

310

311 *Correlation does not imply causation*

312 It has become a scientific truism in microbiome research that *correlation does not imply*
313 *causation*: while causal directionality is trivial for some associations (e.g., antibiotics treatment
314 impacts the microbiome, and not vice versa), it is difficult or impossible to infer for others, based
315 on observational data only. Several mathematical approaches for causality inference that have
316 been applied successfully in other fields start to be adopted for microbiome data, such as
317 structural equation modeling or Bayesian network inference. However, their wider utilization has
318 been hampered by constraints on data size and complexity, and many inference frameworks
319 require repeated (longitudinal) observations (see below).

320 The gold standard for assessing causality of individual associations are classical, reductionist
321 approaches, often relying on mouse models. For example, a potentially protective role for
322 *Clostridium immunis* was recently discovered in a murine colitis model, using a framework
323 dubbed *microbe-phenotype triangulation* (Surana and Kasper, 2017) which satisfies a
324 "commensal" version of Koch's postulates (Neville et al., 2018). However, such workflows
325 require the successful isolation and cultivation of targeted taxa which often remains challenging
326 in practice. In some cases, MWAS findings are validated experimentally by transplanting human

327 fecal microbiota into mouse models (reviewed by Wang and Jia, 2016). However, while murine
328 models allow for controlled experimental setups, they suffer from several limitations, including
329 anatomical and physiological differences between the human and murine digestive tract, cage
330 effects due to coprophagy, fundamentally different microbiome composition with little species
331 overlap, and different host immune pressures affecting transplanted microbiotas (Hugenholtz
332 and de Vos, 2017; Nguyen et al., 2015). In consequence, the translation of *in vitro* or *in vivo*
333 findings to human context often remains difficult.

334

335

336 **Understanding microbiome dynamics using longitudinal studies**

337 Despite the discussed caveats, metagenome-wide association studies have identified important
338 microbiome-disease links that can be followed up for diagnostic purposes, and revealed major
339 co-variables of gut microbiome composition. However, most of these studies were cross-
340 sectional and hence mechanistic insights remain limited. Large-scale generation of longitudinal
341 data, covering (i) baseline dynamics of the unperturbed gut microbiome, and (ii) the response to
342 various perturbations (see next section), is crucial to understand the ‘wiring’ of the gut
343 ecosystem – temporal resolution of stimulus and response can help disentangle cause-effect
344 directionality of microbiome associations *in natura* (i.e., directly in the human host).

345 Many studies have concluded that the gut microbiome is remarkably stable over time at
346 baseline, in the absence of intervention, both in terms of taxonomic and functional composition.
347 For example, intra-individual genus and species-level compositional variation over time is lower
348 than inter-individual differences (see e.g., Faith et al., 2013; The Human Microbiome Project Consortium,
349 2012, among others), an observation that has since been extended to strain-level resolution
350 (Costea et al., 2017a; Lloyd-Price et al., 2017; Schloissnig et al., 2013). More recently, the fecal
351 microbiome has been reported to be transcriptomically stable over time as well, albeit to a
352 lesser extent (Abu-Ali et al., 2018). In contrast to this general temporal stability of the adult
353 unperturbed microflora, clear successional dynamics have been described for the developing
354 microbiome of infants (Bäckhed et al., 2015; Koenig et al., 2011; La Rosa et al., 2014), and
355 elderly people can show a marked loss of microbiome stability depending on further lifestyle
356 factors (Jeffery et al., 2016).

357 All in all, however, the temporal variation of the human gut microbiota remains understudied and
358 most of the currently published studies are statistically underpowered, either in number of
359 individuals, in number of time points or in temporal resolution. High resolution studies with
360 sufficient cohort sizes are essential to build predictive models of gut microbiome dynamics,
361 which can then be challenged to model perturbation response (Bucci and Xavier, 2014; Faust et
362 al., 2015). This will not be a trivial task: even the relatively defined community succession in
363 neonates has proven elusive to predictive modeling, probably due to the relative importance of
364 both maternally and environmentally contributed strains (Asnicar et al., 2017; Korpela et al., *in*
365 *press*).

366

367 **Disentangling the microbiome’s ‘wiring’ using perturbations**

368 Perturbation experiments have long been a framework of choice in both systems biology
369 (Jansen, 2003) and community ecology (Bender et al., 1984), as community-level responses to

370 a perturbation allow inferences about interactions between its members. Although the blind
371 application of classical ecological theory to the microbiome is not without risk (Koskella et al.,
372 2017), the value of perturbation designs in microbial ecology has been demonstrated repeatedly
373 (Faust et al., 2015; Shade et al., 2012). Indeed, perturbation experiments are much more
374 informative towards the development of (dynamic) predictive models for microbial community
375 ecology than cross-sectional studies, in particular when complemented with *in vitro* and *in vivo*
376 approaches (see Box 2). Such a *perturb-to-predict* paradigm can provide testable hypotheses
377 and will be essential towards a targeted modulation of the gut microbiome, which in turn is at the
378 heart of translational work (see next section).

379 Here, we review examples of how interventional studies can advance our understanding of the
380 gut microbiome and highlight emerging trends. We use a broad definition of *perturbation*,
381 including stimuli such as medication or dietary intervention.

382

383 *Perturbation response as a window into microbiome community structure and dynamics*

384 Whereas longitudinal analyses are essential to understand baseline microbiome dynamics,
385 perturbation of a microbial system allows much deeper insights into its ecological makeup
386 (Faust et al., 2015; Shade et al., 2012; Sommer et al., 2017). Arguably, the longest lasting
387 perturbation experiment on the human gut microbiome is diet intake, as this natural process has
388 evolved over millions of years. After adopting a more sedentary lifestyle, humans have adapted
389 to an omnivore diet with high variety, and the impact of moderate dietary shifts should therefore
390 be limited and transient. Indeed, several studies have shown that dietary interventions often
391 seem to elicit only specific effects (see Zmora et al., 2016 et al. for a recent review), although
392 more extreme shifts can show more pronounced signatures. For example, radical switches to
393 all-plant- or animal-based diets on the microbiome have a differential impact, and specific
394 groups of taxa respond similarly across individuals (David et al., 2014). Another study found a
395 consistent ecosystem-wide increase in gene richness in response to an energy-restricted high-
396 protein diet in obese patients (Cotillard et al., 2013). In general, most studies to date have
397 investigated rather broadly defined dietary shifts, e.g. to overall varying levels of non-specific
398 nutrient classes such as proteins or carbohydrates, but the effects of defined, specific dietary
399 interventions are only beginning to be explored.

400 In contrast to dietary shifts, clinical interventions can be expected to elicit more drastic
401 responses, as they can dramatically change environmental conditions in the intestine. Bowel
402 cleansing, often performed in preparation of other treatments, may be followed by a rapid
403 recovery of overall microbiome composition (Voigt et al., 2015), though it may trigger the

404 persistent loss of individual taxa (Jalanka et al., 2015). Other clinical interventions with long-
405 term microbiome effects include bariatric surgery (Tremaroli et al., 2015) or induced, iso-osmotic
406 diarrhea. The latter has been reported to induce marked but transient effects, with post-
407 perturbation recovery following a consistent succession across subjects (Fukuyama et al.,
408 2017). Treatment with broad-spectrum antibiotics can have pronounced, persistent and often
409 non-specific effects, and recovery of compositional state post perturbation is sometimes
410 incomplete, due to a loss of taxa from the community (Dethlefsen and Relman, 2011;
411 Dethlefsen et al., 2008; Jakobsson et al., 2010; Jernberg et al., 2007; Voigt et al., 2015).
412 Similarly, treatment with the narrow-spectrum antibiotic cefprozil triggered consistent responses
413 of individual taxa, while community-level response was stratified (Raymond et al., 2015).

414 In general, one must note that most controlled interventional studies focus on a putative role of
415 the microbiome in host response to perturbation, rather than on the microbiome's response
416 itself. Host and microbiota effects are often difficult to disentangle: while antibiotics treatment,
417 for example, clearly affects the microbiome (which may then mediate indirect effects on the
418 host), the independent host and microbiome responses to dietary intervention are more difficult
419 to unravel. In consequence, many perturbation studies have been conducted in mouse models
420 which allow to control for host effects to some extent, in spite of other limitations (Nguyen et al.,
421 2015). Moreover, *in vitro* approaches are gaining renewed attention (see Box 2), as these allow
422 fairly straightforward probing of the response of communities or individual strains to specific
423 perturbations, independently of the host (Maier and Typas, 2017). *In vitro* screens are scalable, can
424 go down to the resolution of individual genes in individual strains (e.g., Galardini et al., 2017), while at
425 the same time allowing for very broad designs, a recent example being a screen of 1,200 drugs
426 screened against 40 gut microbial strains (Maier et al., *in press*). Thus, *in vitro* screens can
427 serve as massive hypothesis generators to guide the study of microbiome perturbation
428 responses *in vivo*, either in animal models, or directly in humans, as shown in a recent study on
429 the impact of salt on the microbiome (Wilck et al., 2017).

430 Nevertheless, systematic perturbation studies in humans with the sole purpose of understanding
431 the microbial ecology of the gut microbiota will be needed as well. Larger and more controlled
432 prospective and interventional study designs are increasingly adopted, metadata acquisition
433 becomes more and more comprehensive and sophisticated, and data generation gets more
434 affordable. This will enable us to probe taxonomic and functional interactions among the
435 microbiome, and to understand the factors underlying differential perturbation response. Given
436 the complexity of the human-microbiome symbiosis, only 'real life' data will yield the necessary
437 information for building realistic predictive models.

438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463

From perturbation to prediction

So far, predictive modeling of perturbation responses has proven extremely challenging (Bucci and Xavier, 2014; Faust et al., 2015), both because of complexity and variation, but also because of our limited functional understanding of the wiring of the gut microbiome (see above). Moreover, it has been argued that the microbiome’s response to many perturbations is inherently stochastic (Zaneveld et al., 2017), and therefore not fully predictable.

Yet, a number of predictive models of microbiome dynamics at the level of individual taxa or taxa groups exist (Bucci and Xavier, 2014). For example, Lotka-Volterra models were used to predict community dynamics in response to *Clostridium difficile* infection in mice (Stein et al., 2013). The resulting models could subsequently predict the success of a *C. difficile*-protective probiotic treatment (Buffie et al., 2014). Moreover, using complex models trained on both microbiome composition and non-microbiome features, the impact of personalized dietary interventions on select microbiome features could be predicted to some extent (Shoaie et al., 2015; Zeevi et al., 2015).

Despite such progress, even higher-level perturbation responses are often difficult to predict, such as the gain or loss of taxonomic and functional diversity, or the overall strength (let alone direction) of compositional shifts. This is also true for microbiome *resilience* – the extent to which a perturbed system recovers to a pre-perturbation state (Shade et al., 2012). As discussed above, the microbiome has been reported to be generally resilient to smaller perturbations, though more pronounced disturbances can have lasting effects. It has been argued that the differential resilience between individuals could be indicative of health and disease (Lloyd-Price et al., 2016; Sommer et al., 2017), even though the factors and mechanisms underlying microbiome resilience remain poorly understood, and though it remains challenging to predict how resilient to perturbation a given microbiome will be.

464 **From perturbation towards modulation**

465 Empirical therapeutic modulation of the gut flora has been performed for thousands of years, for
466 example implicitly in the use of traditional herbal medication (Xu et al., 2015) or consciously by
467 fecal microbiota transplantation (de Groot et al., 2017). Despite a wealth of reports over the last
468 decade, links between the gut microbiota and diseases continue to be discovered (Lynch and
469 Pedersen, 2016), and in consequence the human gut microbiome continues to gain attention as
470 a therapeutic target (Langdon et al., 2016; Walsh et al., 2014).

471 Here, we review recent progress on attempts at both untargeted and targeted microbiome
472 modulation. In the context of this review, we broadly define *modulation* as an intervention with
473 the intent of pushing the gut microbiome towards a desired state. This includes, among others,
474 fecal microbiota transplantation, probiotic and prebiotic treatment, and directed dietary
475 interventions.

476

477 *Fecal microbiota transplantation (FMT)*

478 An FMT is the prime example of an untargeted microbiome modulation: stool from a (healthy)
479 donor is transferred into the gastrointestinal tract of a recipient, with the aim of improving their
480 health or an undesired microbiome state. FMTs have been shown to be highly efficient in the
481 treatment of recurrent *Clostridioides difficile* infection (RCDI), and indeed seem more suited
482 than antibiotics for this disease (van Nood et al., 2013). Although success is less pronounced in
483 other areas, such as e.g. for ulcerative colitis (Narula et al., 2017) or metabolic syndrome
484 (Vrieze et al., 2012), FMTs are explored as a treatment option for a growing list of indications,
485 with close to 200 registered clinical trials at the time of writing (clinicaltrials.gov, accessed
486 January 2018). An obvious long-term goal is the replacement of rather undefined donor stool
487 samples with formulated, recipient-tailored mixes of defined microbial strains.

488 FMTs are often preceded by preparatory antibiotics treatment or bowel cleansing in the clinical
489 practice, and effects can be difficult to disentangle. Several studies have investigated
490 microbiome-level effects of FMT, and reported that the treatment is followed by an increase of
491 alpha diversity in the recipient's microbiome, and a shift in community structure towards donor
492 composition in RCDI patients (Fuentes et al., 2014; Seekatz et al., 2014), a trend that was also
493 observed in inflammatory bowel disease (IBD, Vermeire et al., 2016). In contrast, post-FMT
494 community composition was only mildly associated to recipient pre-FMT composition in trials on
495 metabolic syndrome (Kootte et al., 2017) and ulcerative colitis (Fuentes et al., 2017), calling for
496 higher taxonomic resolution. Indeed, at the level of strain populations, engraftment of donor
497 strains could be demonstrated, although successful colonization was more likely if strains of the

498 same species were present in the recipient prior to the transplant (Li et al., 2016). Moreover,
499 donor and recipient strains were found to co-exist in the recipient for prolonged periods of at
500 least several months post FMT (Li et al., 2016), a finding that has since been corroborated on
501 independent cohorts for different indications (Kumar et al., 2017; Lee et al., 2017; Moss et al.,
502 2017).

503 While this is encouraging towards future adapted treatment options, our mechanistic
504 understanding of the microbiome's response to FMT remains so far insufficient. Indeed, from a
505 microbial ecology point of view, FMTs provide a unique setup to study microbiome colonization
506 resistance, succession and overall resilience.

507

508 *Probiotics*

509 Probiotics, defined as “live microorganisms which when administered in adequate amounts
510 confer a health benefit on the host” (Hill et al., 2014), have been shown to be clinically efficient
511 treatment options in some indications (Ford et al., 2014). In contrast to FMTs, probiotic
512 treatment is an attempt at targeted modulation of the gut microbiota, notably by adding the
513 probiotic to the community. However, microbiome-level effects of probiotics treatment may be
514 mild: a recent systematic review of seven randomized clinical trials found no effects of different
515 probiotics on microbiota composition, and no evidence for persistent probiotic engraftment
516 (Kristensen et al., 2016). This reaffirms the notion of gut microbiota colonization resistance, both
517 to probiotics and pathogens. Studies in mice, in contrast, have concluded that engraftment
518 success may depend on how complementary the probiotic is to the recipient's baseline
519 microbiome composition. For example, administration of *Clostridium scindens* was found to
520 metabolically complement the recipient's microbiota, and to enhance colonization resistance to
521 *Clostridioides difficile* (Buffie et al., 2014). This outcome was based on clinical data, mouse
522 models and mathematical modeling, and illustrates that an ecology-inspired approach can
523 enable successful microbiome modulation. The future of next-generation probiotics thus lies in
524 not only supplementing beneficial functionalities, but in also providing the necessary ecological
525 context to sustain them. Moreover, the shift of microbiome composition as a whole by
526 supplementation of more complex mixtures of organisms will arguably soon be within reach.

527

528 *Prebiotics and dietary intervention*

529 Prebiotics, defined as “substrate[s] that [are] selectively utilized by host microorganisms
530 conferring a health benefit” (Gibson et al., 2017), are another means of targeted microbiome
531 modulation. In contrast to the direct administration of probiotics, prebiotics treatment aims to

532 confer a selective advantage to beneficial members of the microbiota. While several studies
533 suggest a therapeutic potential of prebiotics for different indications (Beserra et al., 2015; Ford
534 et al., 2014), surprisingly little is known about their effect on whole microbiome composition.
535 Increased *Prevotella/Bacteroides* ratios and improved glucose metabolism have been reported
536 to follow a transient shift to a fiber-rich diet (Kovatcheva-Datchary et al., 2015). Similarly, a fiber-
537 rich diet, supplemented by other prebiotics, shifted gut microbiome functional composition and
538 contributed to weight loss in obese children (Zhang et al., 2015a). Treatment with inulin-type
539 fructans was reported to trigger an increase in *Bifidobacterium* and *Anaerostipes* with hardly any
540 community-level effects (Vandeputte et al., 2017b).

541 Beyond the supplementation of usually defined prebiotics, diet represents a vast pool of
542 chemical and biomolecular compounds, often implicitly amended with microbes. As such, it is an
543 important factor in shaping microbiome composition, as discussed above (reviewed by Flint et
544 al., 2017). In consequence, directed dietary interventions can not only provide informative
545 perturbation experiments, but are explored as mild, microbiome-mediated therapy options (Suez
546 and Elinav, 2017). Microbiome-wide metabolic models have been used to successfully predict
547 microbiome metabolic responses to a dietary intervention in obese and overweight individuals,
548 stratified by baseline microbial gene richness (Shoaie et al., 2015). Similarly, in using
549 microbiome, clinical and dietary data to train complex models, personalized dietary interventions
550 towards improved glycemic responses were suggested and validated in a blinded randomized
551 trial (Zeevi et al., 2015). Although both these studies optimized for host effects, the authors were
552 also able to predict microbiome responses to intervention, to some extent. Importantly, both
553 studies found that the microbiome stratified intervention effects and that the response to diet
554 might be truly individual (see Box 3). Moreover, it remains to be determined how much of these
555 inter-individual differences in response to intervention can be attributed to microbiome-intrinsic
556 or host factors (see Figure 2).

557

558 *Towards targeted and predictable modulation of the gut microbiome*

559 The potential of targeted microbiome modulation has been demonstrated in several recent
560 studies, albeit in mouse models. For example, it was found that *Clostridium sporogenes*
561 metabolizes aromatic amino acids into several compounds that accumulate in the host's blood
562 serum, that the replacement of wild type *C. sporogenes* with a genetically engineered strain in
563 gnotobiotic mice decreased serum levels of these metabolites, and affected gut permeability
564 and host immune response (Dodd et al., 2017). More recently, it was reported that tungstate
565 treatment selectively inhibited overgrowth of certain *Enterobacteriaceae* and ameliorated

566 symptoms in a murine colitis model (Zhu et al., 2018). The authors had previously found that
567 molybdenum-dependent enzymes (that are inhibited by tungsten) were implied in
568 *Enterobacteriaceae* blooms during induced colitis in mice (Hughes et al., 2017), and this
569 ecological and functional insight enabled a successful gut microbiome modulation.

570 Such studies reaffirm the notion that targeted, hypothesis-driven modulation requires an
571 understanding of the taxonomic and functional composition, the mutual interaction structure and
572 the relevant ecological dynamics of the microbiome. As this functional understanding is only
573 beginning to emerge, current models have limited power to predict the outcome of microbiome
574 modulations, and for many clinically effective interventions it is unclear how the microbiome
575 mediates host-level effects. There are numerous macro-ecological examples of unexpected or
576 catastrophic effects of human intervention on incompletely understood ecosystems. For
577 instance, the invasive toxic cane toad (*Bufo marinus*) in Australia, originally introduced as a
578 biological pest control in the 1930ies, has since developed into a major burden on the local
579 ecosystem (Phillips and Shine, 2004). In analogy, (rare) adverse effects have been reported for
580 microbiome modulatory interventions, most prominently for FMT (Wang et al., 2016b), and
581 microbiome-related causes of these remain poorly understood.

582 The majority of studies to investigate microbiome-level effects of modulation did so at genus or
583 species level. However, for several probiotics, only specific strains of a given species were
584 found to be clinically effective (Kristensen et al., 2016), and the efficacy of a given strain
585 probably depends on the recipient's microbiome. Indeed, some strains of *Escherichia coli* are
586 highly beneficial probiotics (Wassenaar, 2016), whereas others are potent pathogens (Kaper et
587 al., 2004). This illustrates the importance of an appropriate taxonomic resolution to successful
588 microbiome modulation (see Figure 3): precise intervention requires a precise understanding of
589 the target system.

590

591 *Defining a healthy microbiome in a healthy individual*

592 The definition of appropriate target endpoints remains a central challenge to microbiome
593 modulation, as a consensus on microbiome "health" so far remains elusive (see Lloyd-Price et
594 al., 2016 for a recent review). Recently, a microbiome "Global Positioning System" was
595 proposed, in which healthy and diseased states are distinguished based on multi'omic readouts
596 (Gilbert et al., 2016). However, while some disease states may be associated to specific
597 microbiome signatures, microbiome states that are unequivocally "healthy" across cohorts are
598 yet to be established (Lloyd-Price et al., 2016). Others have suggested distinctly time-resolved
599 definitions of microbiome health, e.g. with regard to distinct and characteristic patterns of

600 temporal variability to distinguish healthy and diseased states (Martí et al., 2017). Similarly, it
601 has been proposed that microbiome health manifests itself in the response to perturbations, and
602 that an “Anna Karenina” principle applies to the microbiome – that, in variation of Tolstoy,
603 “healthy microbiomes are all alike; each unhealthy microbiome is unhealthy in its own way”
604 (Zaneveld et al., 2017). Moreover, it has been repeatedly suggested that it is less the response
605 to perturbation, but rather post-perturbation *resilience* that is a hallmark of health (Sommer et
606 al., 2017).

607 Certainly, any definition of microbiome health will depend on the frame of reference. From a
608 clinical perspective, health is determined with a view of the human host – any microbiome state
609 associated to a healthy host state could be considered “healthy”. But such a host-centric
610 definition is arguably incomplete, and problematic for several reasons. As discussed above,
611 links between host and microbiome are multivariate and complex, so that many diseases of the
612 host do not necessarily carry clear and specific microbiome signatures, while even for well-
613 described associations, the direction of causality is usually unclear. And while disease-
614 associated microbiome imbalances are thus difficult to define, this has proven even more
615 challenging for unequivocally health-associated microbiome states. Although microbiome and
616 host health are clearly linked, multiple healthy microbiome states can probably exist within the
617 healthy host space.

618

619 **Conclusion & Perspective**

620 Our understanding of the human gut microbiome continues to evolve at a rapid pace. The
621 census of the microbiome – the establishment of its ‘parts lists’ – is arguably approaching
622 completion for the major prokaryotic lineages, although a surprising amount of novel diversity
623 continues to be discovered at sub-species and strain level, implying that the identification of
624 novel genes in the gut is ongoing. Although prokaryotic lineages contribute the vast majority of
625 the gut microbiome by abundance, important players may still be missed as the eukaryal and
626 viral microbiome remain incompletely charted. Metagenome-wide association studies have
627 identified major drivers of microbiome composition and linked individual microbial taxa and
628 genes to diseases, host lifestyle and physiology. However, they have also revealed that known
629 factors can only account for a surprisingly small fraction of total microbiome variation, at least
630 without stratification for microbiome state. Longitudinal studies have begun to establish a
631 baseline on the gut microbiome’s temporal dynamics and found it to be remarkably stable over
632 time. The study of perturbations has further advanced our functional understanding of the
633 microbiome, both with regard to its intrinsic interaction structure – the ‘wiring’ of its parts – and
634 to cause-effect relationships with external factors. Moreover, it is becoming increasingly clear
635 that the microbiome mediates, stratifies and possibly personalizes host-level responses to
636 intervention.

637 The increasing functional understanding of the microbiome begins to be translated into practice,
638 in form of targeted microbiome modulation. Most attempts at *in vivo* microbiome modulation are
639 of therapeutic intent: researchers aim to improve the wellbeing of patients, by proxy of the
640 microbiome. However, a consensus on desired microbial endpoints – on what a “healthy”
641 microbiome actually is – has yet to emerge.

642 Currently, understanding lags behind application: the underlying reasons why an untargeted
643 intervention like FMT is effective in some cases but not others are mostly unclear, and effective
644 informed, precise microbiome modulation is still in its infancy. This argues for a push towards
645 more and larger-scale longitudinal and interventional studies, with an updated methodological
646 toolkit, including multi’omic techniques and novel *in vitro* approaches, and with a focus less on
647 the host, but on the microbiome in its own right. Such studies will further advance our
648 understanding of the microbiome, have the power to elucidate missing links, and will enable us
649 to better predict responses to intervention. The integrated study of perturbations will thereby
650 allow us to truly advance research on the human gut microbiome, moving from association to
651 modulation.

652

653 **Acknowledgements**

654 This work was partially supported by EMBL and by the European Research Council MicrobioS
655 grant (ERC-AdG-669830, P.B.), the Fonds National de la Recherche Luxembourg microCancer
656 grant (T.S.B.S), and by KU Leuven/Rega, VIB and the FWO EOS programme (J.R.). We thank
657 Luis Pedro Coelho and Lisa Maier of EMBL for helpful comments on the manuscript, and all
658 members of the Bork and Raes labs for insights that led to this synthesis.

659

660 **Box 1: Why can we explain so little of observed microbiome variation?**

661 It has been a sobering observation that the combined effect size of different microbiome co-
662 variates (both technical and biological) appears to be intriguingly low: in the Flemish Gut Flora
663 Project and LifeLines-DEEP cohorts, the total non-redundant compositional variation explained
664 was in the single digit percent range (Falony et al., 2016; Zhernakova et al., 2016), the influence
665 of host genetics has been reported in a similar range (Bonder et al., 2016; Turpin et al., 2016;
666 Wang et al., 2016a) or below (Rothschild et al., 2017), as have disease associations (Duvall et
667 al., 2017). This could be due to the fact that (i) there are further important uncharacterized co-
668 variates or the current ones are not measured accurately enough, that (ii) associations of
669 individual taxa are more relevant than global compositional shifts, that (iii) intrinsic compositional
670 constellations or stable states are resilient, that (iv) true effects can only be detected at higher
671 taxonomic resolution (Costea et al., 2017a), or that (v) neutral or stochastic processes (drift)
672 have a stronger impact than previously appreciated. Moreover, (vi) the gut microbiome's
673 intrinsic ecological dynamics and interactions, ecological succession and ecosystem maturation
674 (Falony et al., *cond. acc.*) are possible factors that have so far remained understudied, in part
675 due to a lack of longitudinal data.

676 Nevertheless, the current total quantification of external factors to microbiome variation is
677 probably in the range of 10-15%, and thus of significant enough effect size to consider in clinical
678 studies, as even some individual factors can confound associations. This likely remains true
679 even if one extends the definition of MWAS to "Microbiome-Wide Association Studies" by also
680 taking into account other data types, such as metatranscriptomic or metabolomic readouts, as
681 recently suggested (Gilbert et al., 2016). Therefore, the proper consideration of and stratification
682 for known microbiome covariates as potential confounders will greatly improve the accuracy of
683 MWAS studies, but can also inform the interpretation of longitudinal and interventional datasets.

684

685 **Box 2: Methodological advances to boost microbiome research**

686 Microbiomics, as a research field, evolves at a breakneck pace, and this is certainly true with
687 regard to methodological advances (see Mallick et al., 2017 for a recent review). Here we
688 highlight recent developments that we expect to make a strong impact in the near future,
689 enabling us to tackle new questions, and further complementing the transition from
690 observational to interventional study designs.

691

692 *Multi'omics*

693 High-throughput 16S rRNA amplicon and whole genome shotgun (WGS) metagenomic
694 sequencing have boosted microbiome research for more than a decade, and these technologies
695 continue to dominate the field. More recently, however, the taxonomic and functional census
696 provided by metagenomics is increasingly complemented by readouts on *activity*, provided by
697 metatranscriptomics, metaproteomics and metabolomics (reviewed by Franzosa et al., 2015;
698 Mallick et al., 2017). Metabolomic analyses, in particular, have served as independent lines of
699 evidence to confirm hypotheses generated in MWAS, for example confirming a link of microbial
700 metabolism to cardiovascular disease (Wang et al., 2011), or the impact of gut microbiome
701 metabolism on insulin sensitivity (Pedersen et al., 2016).

702 Metatranscriptomic analyses provide a more direct readout on microbial gene expression
703 profiles, and relating this information to baseline microbiome functional potential can reveal
704 novel insights (see Abu-Ali et al., 2018; Schirmer et al., 2018 for recent examples). The gut
705 metaproteome, in contrast, has not been analyzed on a large scale, although a few pilot-sized
706 studies exist (Erickson et al., 2012; Heintz-Buschart et al., 2016; Kolmeder and de Vos, 2014).

707 An important challenge to multi'omic microbiome research is integration: the different data types
708 provide intermingled layers of evidence and need to be interpreted in light of each other, and
709 integrated analysis concepts (Heintz-Buschart et al., 2016; Mallick et al., 2017) start challenging
710 common conceptions on the microbiome, e.g. on the relative importance of functional plasticity
711 (Heintz-Buschart and Wilmes, 2017).

712

713 *Quantitative Microbiome Profiling (QMP)*

714 Most microbiome studies rely on compositional data – relative abundances of taxa or genes are
715 scaled by non-informative total library sizes, and compositionality effects may introduce false
716 positive taxa-taxa or taxa-covariate associations (Faust and Raes, 2012; Friedman and Alm,
717 2012; Weiss et al., 2017). The use of spiked-in standards (Satinsky et al., 2013), known cell
718 numbers (Stämmeler et al., 2016) or flow cytometry (Props et al., 2017; Vandeputte et al., 2017c)

719 can enable absolute microbial quantification. Indeed, total microbial load showed large inter-
720 individual variation, was linked to community composition, and was decreased in Crohn's
721 disease (Vandeputte et al., 2017c). Thus, QMP can increase sensitivity and specificity in MWAS
722 studies.

723

724 *In vitro* microbiomics & microfluidics

725 While *in vitro* approaches have long been used to probe the microbiome in classical reductionist
726 setups, they are currently experiencing a renaissance in high-throughput, explorative analyses.
727 Several microfluidics-based “gut on a chip” systems provide increasingly better approximations
728 of the human intestinal environment (Kim et al., 2012; Marzorati et al., 2014; Shah et al., 2016).
729 At the same time, high-throughput cultivation now encompasses fastidious, anaerobic
730 organisms (Rettedal et al., 2014), even in defined media (Tramontano et al., *in press*).

731

732 *Extended taxonomic breadth and resolution*

733 As bacteria account for the vast majority of gut flora biomass and are most accessible to
734 cultivation, microbiome research has mostly focused on the bacterial domain. Eukaryal (Parfrey
735 et al., 2011; Wlodarska et al., 2015), archaeal (Gaci et al., 2014), and viral (Hurwitz et al., 2016;
736 Lesley A Ogilvie, 2015; Yutin et al., 2018) members of the gut flora have been studied in the
737 past, but are receiving renewed attention (Conceição-Neto et al., 2017; Sokol et al., 2017). At
738 the same time, reference genomic representation of the archaeal and bacterial domain have
739 increased greatly, in part due to coordinated efforts to sequence type strains (Mukherjee et al.,
740 2017). This illustrates the dynamics of the field: just over a decade ago, early human fecal
741 metagenomes contained mostly unclassifiable reads (Eckburg et al., 2005), and even in 2013,
742 only around half the reads in a gut metagenome mapped to reference genomes (Sunagawa et
743 al., 2013). Only a few years later, this gap may soon be closed, at least for the major prokaryotic
744 lineages (e.g., Zhou et al., 2018).

745 This increase in taxonomic coverage is complemented by a similar increase in taxonomic
746 resolution. Following a first mapping of the landscape of microbial Single Nucleotide Variants
747 (SNVs) in the microbiome (Schloissnig et al., 2012), several tools to call microbial SNVs and to
748 profile subspecies to strain-level variation have been developed (Costea et al., 2017c; Nayfach
749 et al., 2016; Quince et al., 2017; Scholz et al., 2016; Truong et al., 2017) and applied to the
750 human gut microbiome. Several species-level observations of the Human Microbiome Project
751 were recently extended to strain level (Lloyd-Price et al., 2017), and associations of subspecies
752 to co-variables were reported that were not apparent at lower taxonomic resolution (Costea et al.,

753 2017a). This indicates that a resolution subordinate to species may help uncover novel and
754 previously overlooked microbiome features and links.
755

756 **Box 3: The microbiome stratifies and personalizes host response to perturbations**

757 It is becoming increasingly clear that inter-individual microbiome variation is associated to
758 differential response to perturbations. The human gut microbiome stratifies into distinct
759 compositional types, termed *enterotypes* (Arumugam et al., 2011; Costea et al., 2018). First
760 studies suggest that enterotypes are stable over time (Costea et al., 2018; Ding and Schloss,
761 2014), perhaps even upon short-term dietary intervention (Roager et al., 2014; Wu et al., 2011).
762 Enterotypes may contribute to several microbiome-disease associations, and have been linked
763 to differential pharmacokinetics and drug metabolism (see Costea et al., 2018 for a recent
764 review). For example, it was shown that *Prevotella copri* and *Bacteroides vulgatus*, two hallmark
765 species underlying enterotype splits, mediate insulin resistance (Pedersen et al., 2016). The
766 *Prevotella/Bacteroides* ratio was also found to predict improved glucose metabolism upon a
767 dietary intervention (Kovatcheva-Datchary et al., 2015), and enterotype was found to be
768 predictive of the response to treatment with the antibiotic cefprozil (Raymond et al., 2015),
769 reinforcing the idea that enterotypes may underlie stratified responses to perturbation.

770 Several studies have demonstrated stratification of drug responses by specific microbiome
771 features (recently reviewed by Vázquez-Baeza et al., 2018). For example, specific strains of
772 *Eggerthella lenta* have been shown to metabolize the cardiac drug digoxin, rendering it
773 inefficient in some patients (Haiser et al., 2013). The efficacy of anti-PD1 and anti-CTLA4
774 chemotherapy in melanoma patients has been shown to depend on the gut microbiome, with
775 predictive compositional differences between treatment responders and non-responders
776 (Gopalakrishnan et al., 2018; Matson et al., 2018; Routy et al., 2017; Sivan et al., 2015; Vetzou
777 et al., 2015). Similarly, recent work in *C. elegans* demonstrated how gut bacteria differentially
778 modulate the metabolism of fluoropyrimidine chemotherapeutics (García-González et al., 2017;
779 Scott et al., 2017).

780 The microbiome is also thought to mediate host response to dietary intervention (Sonnenburg
781 and Bäckhed, 2016), although in this case, even more complex and personalized patterns have
782 emerged (Zmora et al., 2016). It was reported that complex models (including lifestyle and blood
783 parameters beyond microbiome features) could successfully predict response to dietary
784 intervention, as validated in a randomized control study (Zeevi et al., 2015). Similarly,
785 microbiota-wide metabolic models could successfully predict differential effects of a dietary
786 intervention (Shoaie et al., 2015).

787 Such studies illustrate how the microbiome may mediate and therefore stratify and personalize
788 host-level response to intervention, and that microbiome stratification is a relevant factor to
789 account for in practice.

790 **Figure 1.**

791 The route towards targeted microbiome modulation entails three consecutive and mutually
792 dependent lines of investigation. A ‘parts list’ of the microbiome’s structure and function has now
793 been mostly established, and metagenome-wide association studies (MWAS) have identified
794 important co-variables of microbiome composition (see Figure 2). At the same time, longitudinal
795 studies have started to provide important insights into the microbiome’s intrinsic dynamics.
796 Taken together, these provide first cues towards a functional understanding of the gut
797 microbiome. Perturbation experiments can significantly extend this, while also providing insights
798 into the microbiome’s ecological dynamics – the ‘wiring’ of the system in terms of interactions
799 between its parts. An integrated functional understanding will be essential towards translating
800 microbiome research into targeted modulations, with dedicated benefits for the human host.

801

802 **Figure 2.**

803 Microbiome composition is associated to several known co-variables. Microbiome-extrinsic
804 factors can be empirically classified into three categories, *host-intrinsic*, *host-extrinsic* and
805 *environmental*. Moreover, microbiome state feeds back upon itself and thereby contributes to
806 compositional variation between individuals. Clearly, these categories overlap, and many factors
807 are also associated to each other. For example, diet contains microbes from environmental
808 strain pools which may colonize the gut or even, in the case of food poisoning, trigger a shift into
809 a diseased microbiome state that subsequently becomes entrenched intrinsically, but also
810 prompts medication. In practice, it is therefore challenging to disentangle the effect size of
811 individual factors, and it is often necessary to stratify for other co-variables, in particular also for
812 microbiome state (see Box 3). Indeed, the overall effect of known co-variables on human gut
813 microbiome variation is surprisingly small (Box 1).

814

815 **Figure 3.**

816 Microbiome research advances rapidly, but current approaches abstract the gut microbiome via
817 gradual approximations from different angles. A few of these access routes are depicted and
818 categorized here, and the required level of abstraction may vary between scientific questions or
819 study designs. A) Microbial composition is usually determined at genus level based on 16S
820 rRNA amplicon data, although many features in association studies emerge at higher resolution.
821 More recently, the focus shifts further to reach the level of strains, the preferred taxonomic unit
822 in microbiology. B) Functional associations are often determined for entire functional classes or
823 more fine-grained functional units, although even individual genes can be informative in some
824 contexts. C) Microbiome associations have been tested at the level of entire populations or of
825 certain cohorts, though it is becoming increasingly clear that stratification is often necessary to
826 increase observed signals. In some instances, associations are specific even at the level of
827 individuals. D) For experimental access, simpler systems allow for higher throughput, but they
828 are also less representative of the microbiome *in natura*, i.e. in humans with an individual
829 environment.

830

831

- 832
- 833 Abu-Ali, G.S., Mehta, R.S., Lloyd-Price, J., Mallick, H., Branck, T., Ivey, K.L., Drew, D.A.,
834 DuLong, C., Rimm, E., Izard, J., et al. (2018). Metatranscriptome of human faecal microbial
835 communities in a cohort of adult men. *Nature Microbiology* 106, 1.
- 836 Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D.R., Fernandes,
837 G.R., Tap, J., Bruls, T., Batto, J.-M., et al. (2011). Enterotypes of the human gut microbiome.
838 *Nature* 473, 174-180.
- 839 Asnicar, F., Manara, S., Zolfo, M., Truong, D.T., Scholz, M., Armanini, F., Ferretti, P., Gorfer, V.,
840 Pedrotti, A., Tett, A., et al. (2017). Studying Vertical Microbiome Transmission from Mothers to
841 Infants by Strain-Level Metagenomic Profiling. *mSystems* 2, e00164–16.
- 842 Bahr, S.M., Tyler, B.C., Wooldridge, N., Butcher, B.D., Burns, T.L., Teesch, L.M., Oltman, C.L.,
843 Azcarate-Peril, M.A., Kirby, J.R., and Calarge, C.A. (2015). Use of the second-generation
844 antipsychotic, risperidone, and secondary weight gain are associated with an altered gut
845 microbiota in children. *Translational Psychiatry* 2015 5:10 5, e652–e652.
- 846 Barton, W., Penney, N.C., Cronin, O., Garcia-Perez, I., Molloy, M.G., Holmes, E., Shanahan, F.,
847 Cotter, P.D., and O’Sullivan, O. (2017). The microbiome of professional athletes differs from that
848 of more sedentary subjects in composition and particularly at the functional metabolic level. *Gut*
849 *gutjnl–2016–313627*.
- 850 Bäckhed, F., Roswall, J., Peng, Y., Feng, Q., Jia, H., Kovatcheva-Datchary, P., Li, Y., Xia, Y.,
851 Xie, H., Zhong, H., et al. (2015). Dynamics and Stabilization of the Human Gut Microbiome
852 during the First Year of Life. *Cell Host & Microbe* 17, 690–703.
- 853 Becattini, S., Taur, Y., and Pamer, E.G. (2016). Antibiotic-Induced Changes in the Intestinal
854 Microbiota and Disease. *Trends in Molecular Medicine* 22, 458–478.
- 855 Belkaid, Y., and Hand, T.W. (2014). Role of the Microbiota in Immunity and Inflammation. *Cell*
856 157, 121–141.
- 857 Bender, E.A., Case, T.J., and Gilpin, M.E. (1984). Perturbation Experiments in Community
858 Ecology: Theory and Practice. *Ecology* 65, 1–13.
- 859 Beserra, B.T.S., Fernandes, R., do Rosario, V.A., Mocellin, M.C., Kuntz, M.G.F., and Trindade,
860 E.B.S.M. (2015). A systematic review and meta-analysis of the prebiotics and synbiotics effects
861 on glycaemia, insulin concentrations and lipid parameters in adult patients with overweight or
862 obesity. *Clinical Nutrition* 34, 845–858.
- 863 Biedermann, L., Zeitz, J., Mwinyi, J., Sutter-Minder, E., Rehman, A., Ott, S.J., Steurer-Stey, C.,
864 Frei, A., Frei, P., Scharl, M., et al. (2013). Smoking Cessation Induces Profound Changes in the
865 Composition of the Intestinal Microbiota in Humans. *Plos One* 8, e59260.
- 866 Bonder, M.J., Kurilshikov, A., Tigchelaar, E.F., Mujagic, Z., Imhann, F., Vila, A.V., Deelen, P.,
867 Vatanen, T., Schirmer, M., Smeekens, S.P., et al. (2016). The effect of host genetics on the gut
868 microbiome. *Nat Genet* 48, 1407–1412.
- 869 Bucci, V., and Xavier, J.B. (2014). Towards Predictive Models of the Human Gut Microbiome.
870 *Journal of Molecular Biology* 426, 3907–3916.

- 871 Buffie, C.G., Bucci, V., Stein, R.R., McKenney, P.T., Ling, L., Gobourne, A., No, D., Liu, H.,
872 Kinnebrew, M., Viale, A., et al. (2014). Precision microbiome reconstitution restores bile acid
873 mediated resistance to *Clostridium difficile*. *Nature* 517, 205–208.
- 874 Carabotti, M., Scirocco, A., Maselli, M.A., and Severi, C. (2015). The gut-brain axis: interactions
875 between enteric microbiota, central and enteric nervous systems. *Ann Gastroenterol* 28, 203–
876 209.
- 877 Chu, D.M., Ma, J., Prince, A.L., Antony, K.M., Seferovic, M.D., and Aagaard, K.M. (2017).
878 Maturation of the infant microbiome community structure and function across multiple body sites
879 and in relation to mode of delivery. *Nature Medicine* 23, 314–326.
- 880 Clarke, S.F., Murphy, E.F., O’Sullivan, O., Lucey, A.J., Humphreys, M., Hogan, A., Hayes, P.,
881 O’Reilly, M., Jeffery, I.B., Wood-Martin, R., et al. (2014). Exercise and associated dietary
882 extremes impact on gut microbial diversity. *Gut* 63, 1913–1920.
- 883 Conceição-Neto, N., Deboutte, W., Dierckx, T., Machiels, K., Wang, J., Yinda, K.C., Maes, P.,
884 Van Ranst, M., Joossens, M., Raes, J., et al. (2017). Low eukaryotic viral richness is associated
885 with faecal microbiota transplantation success in patients with UC. *Gut* gutjnl-2017-315281.
- 886 Costea, P.I., Coelho, L.P., Sunagawa, S., Munch, R., Huerta-Cepas, J., Forslund, K.,
887 Hildebrand, F., Kushugulova, A., Zeller, G., and Bork, P. (2017a). Subspecies in the global
888 human gut microbiome. *Mol Syst Biol* 13, 960.
- 889 Costea, P.I., Hildebrand, F., Manimozhiyan, A., Bäckhed, F., Blaser, M.J., Bushman, F.D., de
890 Vos, W.M., Ehrlich, S.D., Fraser, C.M., Hattori, M., et al. (2018). Enterotypes in the landscape of
891 gut microbial community composition. *Nature Microbiology* 3, 8–16.
- 892 Costea, P.I., Zeller, G., Sunagawa, S., Pelletier, E., Alberti, A., Levenez, F., Tramontano, M.,
893 Driessen, M., Hercog, R., Jung, F.-E., et al. (2017b). Towards standards for human fecal
894 sample processing in metagenomic studies. *Nat Biotech* 35, 1069.
- 895 Costea, P.I., Munch, R., Coelho, L.P., Paoli, L., Sunagawa, S., and Bork, P. (2017c). metaSNV:
896 A tool for metagenomic strain level analysis. *Plos One* 12, e0182392.
- 897 Cotillard, A., Kennedy, S.P., Kong, L.C., Prifti, E., Pons, N., Le Chatelier, E., Almeida, M.,
898 Quinquis, B., Levenez, F., Galleron, N., et al. (2013). Dietary intervention impact on gut
899 microbial gene richness. *Nature* 500, 585–588.
- 900 David, L.A., Maurice, C.F., Carmody, R.N., Gootenberg, D.B., Button, J.E., Wolfe, B.E., Ling,
901 A.V., Devlin, A.S., Varma, Y., Fischbach, M.A., et al. (2014). Diet rapidly and reproducibly alters
902 the human gut microbiome. *Nature* 505, 559–563.
- 903 de Groot, P.F., Frissen, M.N., de Clercq, N.C., and Nieuwdorp, M. (2017). Fecal microbiota
904 transplantation in metabolic syndrome: History, present and future. *Gut Microbes* 8, 253–267.
- 905 Dethlefsen, L., and Relman, D.A. (2011). Incomplete recovery and individualized responses of
906 the human distal gut microbiota to repeated antibiotic perturbation. *Proceedings of the National*
907 *Academy of Sciences* 108, 4554–4561.

- 908 Dethlefsen, L., Huse, S., Sogin, M.L., and Relman, D.A. (2008). The pervasive effects of an
909 antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *PLoS Biol*
910 *6*, e280.
- 911 Ding, T., and Schloss, P.D. (2014). Dynamics and associations of microbial community types
912 across the human body. *Nature* *509*, 357–360.
- 913 Dodd, D., Spitzer, M.H., Van Treuren, W., Merrill, B.D., Hryckowian, A.J., Higginbottom, S.K.,
914 Le, A., Cowan, T.M., Nolan, G.P., Fischbach, M.A., et al. (2017). A gut bacterial pathway
915 metabolizes aromatic amino acids into nine circulating metabolites. *Nature* *551*, 648.
- 916 Dubinkina, V.B., Tyakht, A.V., Odintsova, V.Y., Yarygin, K.S., Kovarsky, B.A., Pavlenko, A.V.,
917 Ischenko, D.S., Popenko, A.S., Alexeev, D.G., Taraskina, A.Y., et al. (2017). Links of gut
918 microbiota composition with alcohol dependence syndrome and alcoholic liver disease.
919 *Microbiome* *5*, 141.
- 920 Duvallet, C., Gibbons, S.M., Gurry, T., Irizarry, R.A., and Alm, E.J. (2017). Meta-analysis of gut
921 microbiome studies identifies disease-specific and shared responses. *Nat Comms* *8*, 1784.
- 922 Eckburg, P.B., Bik, E.M., Bernstein, C.N., Purdom, E., Dethlefsen, L., Sargent, M., Gill, S.R.,
923 Nelson, K.E., and Relman, D.A. (2005). Diversity of the Human Intestinal Microbial Flora.
924 *Science* *308*, 1635–1638.
- 925 Erickson, A.R., Cantarel, B.L., Lamendella, R., Darzi, Y., Mongodin, E.F., Pan, C., Shah, M.,
926 Halfvarson, J., Tysk, C., Henrissat, B., et al. (2012). Integrated Metagenomics/Metaproteomics
927 Reveals Human Host-Microbiota Signatures of Crohn's Disease. *Plos One* *7*, e49138.
- 928 Faith, J.J., Guruge, J.L., Charbonneau, M., Subramanian, S., Seedorf, H., Goodman, A.L.,
929 Clemente, J.C., Knight, R., Heath, A.C., Leibel, R.L., et al. (2013). The Long-Term Stability of
930 the Human Gut Microbiota. *Science* *341*, 1237439–1237439.
- 931 Falony, G., Joossens, M., Vieira-Silva, S., Wang, J., Darzi, Y., Faust, K., Kurilshikov, A.,
932 Bonder, M.J., Valles-Colomer, M., Vandeputte, D., et al. (2016). Population-level analysis of gut
933 microbiome variation. *Science* *352*, 560–564.
- 934 Faust, K., and Raes, J. (2012). Microbial interactions: from networks to models. *10*, 538–550.
- 935 Faust, K., Lahti, L., Gonze, D., de Vos, W.M., and Raes, J. (2015). Metagenomics meets time
936 series analysis: unraveling microbial community dynamics. *Current Opinion in Microbiology* *25*,
937 56–66.
- 938 Finucane, M.M., Sharpton, T.J., Laurent, T.J., and Pollard, K.S. (2014). A Taxonomic Signature
939 of Obesity in the Microbiome? Getting to the Guts of the Matter. *Plos One* *9*, e84689.
- 940 Flint, H.J., Duncan, S.H., and Louis, P. (2017). The impact of nutrition on intestinal bacterial
941 communities. *Current Opinion in Microbiology* *38*, 59–65.
- 942 Flint, H.J., Scott, K.P., Louis, P., and Duncan, S.H. (2012). The role of the gut microbiota in
943 nutrition and health. *Nature Reviews Gastroenterology and Hepatology* *9*, 577–589.

- 944 Flowers, S.A., Evans, S.J., Ward, K.M., McInnis, M.G., and Ellingrod, V.L. (2017). Interaction
945 Between Atypical Antipsychotics and the Gut Microbiome in a Bipolar Disease Cohort.
946 *Pharmacotherapy: the Journal of Human Pharmacology and Drug Therapy* 37, 261–267.
- 947 Ford, A.C., Quigley, E.M.M., Lacy, B.E., Lembo, A.J., Saito, Y.A., Schiller, L.R., Soffer, E.E.,
948 Spiegel, B.M.R., and Moayyedi, P. (2014). Efficacy of Prebiotics, Probiotics, and Synbiotics in
949 Irritable Bowel Syndrome and Chronic Idiopathic Constipation: Systematic Review and Meta-
950 analysis. *Am J Gastroenterol* 109, 1547–1561.
- 951 Forslund, K., Hildebrand, F., Nielsen, T., Falony, G., Le Chatelier, E., Sunagawa, S., Prifti, E.,
952 Vieira-Silva, S., Gudmundsdottir, V., Krogh Pedersen, H., et al. (2015). Disentangling type 2
953 diabetes and metformin treatment signatures in the human gut microbiota. *Nature* 528, 262–
954 266.
- 955 Franzosa, E.A., Hsu, T., Sirota-Madi, A., Shafquat, A., Abu-Ali, G., Morgan, X.C., and
956 Huttenhower, C. (2015). Sequencing and beyond: integrating molecular “omics” for microbial
957 community profiling. *13*, 360–372.
- 958 Freedberg, D.E., Toussaint, N.C., Chen, S.P., Ratner, A.J., Whittier, S., Wang, T.C., Wang,
959 H.H., and Abrams, J.A. (2015). Proton Pump Inhibitors Alter Specific Taxa in the Human
960 Gastrointestinal Microbiome: A Crossover Trial. *Gastroenterology* 149, 883–885.e889.
- 961 Friedman, J., and Alm, E.J. (2012). Inferring Correlation Networks from Genomic Survey Data.
962 *PLOS Computational Biology* 8, e1002687.
- 963 Fuentes, S., Rossen, N.G., van der Spek, M.J., Hartman, J.H., Huuskonen, L., Korpela, K.,
964 Salojärvi, J., Aalvink, S., de Vos, W.M., D'Haens, G.R., et al. (2017). Microbial shifts and
965 signatures of long-term remission in ulcerative colitis after faecal microbiota transplantation.
966 *Isme J* 11, 1877–1889.
- 967 Fuentes, S., van Nood, E., Tims, S., Heikamp-de Jong, I., Braak, ter, C.J., Keller, J.J.,
968 Zoetendal, E.G., and de Vos, W.M. (2014). Reset of a critically disturbed microbial ecosystem:
969 faecal transplant in recurrent *Clostridium difficile* infection. *Isme J* 8, 1621–1633.
- 970 Fujimura, K.E., Sitarik, A.R., Havstad, S., Lin, D.L., Levan, S., Fadrosch, D., Panzer, A.R.,
971 LaMere, B., Rackaitye, E., Lukacs, N.W., Wegienka, G., et al. (2016). Neonatal gut microbiota
972 associates with childhood multisensitized atopy and T cell differentiation. *Nature Medicine* 22,
973 1187-1191.
- 974 Fukuyama, J., Rumker, L., Sankaran, K., Jeganathan, P., Dethlefsen, Les, Relman, D.A., and
975 Holmes, S.P. (2017). Multidomain analyses of a longitudinal human microbiome intestinal
976 cleanout perturbation experiment. *PLOS Computational Biology* 13, e1005706.
- 977 Gaci, N., Borrel, G., Tottey, W., O'Toole, P.W., and Brugère, J.-F. (2014). Archaea and the
978 human gut: New beginning of an old story. *World Journal Gastroenterology* 20, 16062.
- 979 Galardini, M., Koumoutsi, A., Herrera-Dominguez, L., Cordero, J.V., Telzerow, A., Wagih, O.,
980 Wartel, M., Clermont, O., Denamur, E., Typas, A., et al. (2017). Phenotype inference in an
981 *Escherichia coli* strain panel. *eLife Sciences* 6, 68.

- 982 García-González, A.P., Ritter, A.D., Shrestha, S., Andersen, E.C., Yilmaz, L.S., and Walhout,
983 A.J.M. (2017). Bacterial Metabolism Affects the *C. elegans* Response to Cancer
984 Chemotherapeutics. *Cell* 169, 431–441.e438.
- 985 Gibson, G.R., Hutkins, R., Sanders, M.E., Prescott, S.L., Reimer, R.A., Salminen, S.J., Scott,
986 K., Stanton, C., Swanson, K.S., Cani, P.D., et al. (2017). Expert consensus document: The
987 International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement
988 on the definition and scope of prebiotics. *Nature Reviews Gastroenterology and Hepatology* 14,
989 491.
- 990 Gilbert, J.A., Quinn, R.A., Debelius, J., Xu, Z.Z., Morton, J., Garg, N., Jansson, J.K., Dorrestein,
991 P.C., and Knight, R. (2016). Microbiome-wide association studies link dynamic microbial
992 consortia to disease. *Nature* 535, 94–103.
- 993 Goodrich, J.K., Davenport, E.R., Beaumont, M., Jackson, M.A., Knight, R., Ober, C., Spector,
994 T.D., Bell, J.T., Clark, A.G., and Ley, R.E. (2016). Genetic Determinants of the Gut Microbiome
995 in UK Twins. *Cell Host & Microbe* 19, 731–743.
- 996 Goodrich, J.K., Waters, J.L., Poole, A.C., Sutter, J.L., Koren, O., Blekhman, R., Beaumont, M.,
997 Van Treuren, W., Knight, R., Bell, J.T., et al. (2014). Human Genetics Shape the Gut
998 Microbiome. *Cell* 159, 789–799.
- 999 Gopalakrishnan, V., Spencer, C.N., Nezi, L., Reuben, A., Andrews, M.C., Karpinets, T.V.,
1000 Prieto, P.A., Vicente, D., Hoffman, K., Wei, S.C., et al. (2018). Gut microbiome modulates
1001 response to anti-PD-1 immunotherapy in melanoma patients. *Science* 359, 97-103.
- 1002 Haiser, H.J., Gootenberg, D.B., Chatman, K., Sirasani, G., Balskus, E.P., and Turnbaugh, P.J.
1003 (2013). Predicting and manipulating cardiac drug inactivation by the human gut bacterium
1004 *Eggerthella lenta*. *Science* 341, 295–298.
- 1005 Hall, A.B., Tolonen, A.C., and Xavier, R.J. (2017). Human genetic variation and the gut
1006 microbiome in disease. *Nat Rev Genet* 14, e1002533.
- 1007 Hang, J., Desai, V., Zavaljevski, N., Yang, Y., Lin, X., Satya, R., Martinez, L.J., Blaylock, J.M.,
1008 Jarman, R.G., Thomas, S.J., et al. (2014). 16S rRNA gene pyrosequencing of reference and
1009 clinical samples and investigation of the temperature stability of microbiome profiles.
1010 *Microbiome* 2, 31.
- 1011 Heintz-Buschart, A., and Wilmes, P. (2017). Human Gut Microbiome: Function Matters. *Trends*
1012 *in Microbiology*, *in press*.
- 1013 Heintz-Buschart, A., May, P., Laczny, C.C., Lebrun, L.A., Bellora, C., Krishna, A., Wampach, L.,
1014 Schneider, J.G., Hogan, A., de Beaufort, C., et al. (2016). Integrated multi-omics of the human
1015 gut microbiome in a case study of familial type 1 diabetes. *Nature Microbiology* 2, 16180.
- 1016 Hill, C., Guarner, F., Reid, G., Gibson, G.R., Merenstein, D.J., Pot, B., Morelli, L., Canani, R.B.,
1017 Flint, H.J., Salminen, S., et al. (2014). Expert consensus document: The International Scientific
1018 Association for Probiotics and Prebiotics consensus statement on the scope and appropriate
1019 use of the term probiotic. *Nature Reviews Gastroenterology and Hepatology* 11, 506–514.

- 1020 Hoisington, A.J., Brenner, L.A., Kinney, K.A., Postolache, T.T., and Lowry, C.A. (2015). The
1021 microbiome of the built environment and mental health. *Microbiome* 3, 60.
- 1022 Hooper, L.V., Littman, D.R., and Macpherson, A.J. (2012). Interactions Between the Microbiota
1023 and the Immune System. *Science* 336, 1268–1273.
- 1024 Hugenholtz, F., and de Vos, W.M. (2017). Mouse models for human intestinal microbiota
1025 research: a critical evaluation. *Cell. Mol. Life Sci.* 75, 149–160.
- 1026 Hughes, E.R., Winter, M.G., Duerkop, B.A., Spiga, L., Furtado de Carvalho, T., Zhu, W., Gillis,
1027 C.C., Büttner, L., Smoot, M.P., Behrendt, C.L., et al. (2017). Microbial Respiration and Formate
1028 Oxidation as Metabolic Signatures of Inflammation-Associated Dysbiosis. *Cell Host & Microbe*
1029 21, 208–219.
- 1030 Hurwitz, B.L., U'Ren, J.M., and Youens-Clark, K. (2016). Computational prospecting the great
1031 viral unknown. *FEMS Microbiol Lett* 363, fnw077.
- 1032 Imhann, F., Bonder, M.J., Vila, A.V., Fu, J., Mujagic, Z., Vork, L., Tigchelaar, E.F.,
1033 Jankipersadsing, S.A., Cenit, M.C., Harmsen, H.J.M., et al. (2016). Proton pump inhibitors affect
1034 the gut microbiome. *Gut* 65, 740–748.
- 1035 Ioannidis, J.P.A. (2013). Implausible results in human nutrition research. *Bmj* 347, f6698–f6698.
- 1036 Jackson, M.A., Bell, J.T., Spector, T.D., and Steves, C.J. (2016). A heritability-based
1037 comparison of methods used to cluster 16S rRNA gene sequences into operational taxonomic
1038 units. *PeerJ* 4, e2341.
- 1039 Jakobsson, H.E., Jernberg, C., Andersson, A.F., Sjölund-Karlsson, M., Jansson, J.K., and
1040 Engstrand, L. (2010). Short-Term Antibiotic Treatment Has Differing Long-Term Impacts on the
1041 Human Throat and Gut Microbiome. *Plos One* 5, e9836.
- 1042 Jalanka, J., Salonen, A., Salojärvi, J., Ritari, J., Immonen, O., Marciani, L., Gowland, P., Hoad,
1043 C., Garsed, K., Lam, C., et al. (2015). Effects of bowel cleansing on the intestinal microbiota.
1044 *Gut* 64, 1562–1568.
- 1045 Jansen, R.C. (2003). Studying complex biological systems using multifactorial perturbation. *Nat*
1046 *Rev Genet* 4, 145–151.
- 1047 Jeffery, I.B., Lynch, D.B., and O'Toole, P.W. (2016). Composition and temporal stability of the
1048 gut microbiota in older persons. *Isme J* 10, 170–182.
- 1049 Jernberg, C., Löfmark, S., Edlund, C., and Jansson, J.K. (2007). Long-term ecological impacts
1050 of antibiotic administration on the human intestinal microbiota. *Isme J* 1, 56–66.
- 1051 Kaper, J.B., Nataro, J.P., and Mobley, H.L.T. (2004). Pathogenic *Escherichia coli*. *Nat Rev*
1052 *Micro* 2, 123–140.
- 1053 Kim, H.J., Huh, D., Hamilton, G., and Ingber, D.E. (2012). Human gut-on-a-chip inhabited by
1054 microbial flora that experiences intestinal peristalsis-like motions and flow. *Lab Chip* 12, 2165–
1055 2174.

- 1056 Koenig, J.E., Spor, A., Scalfone, N., Fricker, A.D., Stombaugh, J., Knight, R., Angenent, L.T.,
1057 and Ley, R.E. (2011). Succession of microbial consortia in the developing infant gut
1058 microbiome. *Pnas* 108 Suppl 1, 4578–4585.
- 1059 Kolmeder, C.A., and de Vos, W.M. (2014). Metaproteomics of our microbiome - developing
1060 insight in function and activity in man and model systems. *Journal of Proteomics* 97, 3–16.
- 1061 Kootte, R.S., Levin, E., Salojärvi, J., Smits, L.P., Hartstra, A.V., Udayappan, S.D., Hermes, G.,
1062 Bouter, K.E., Koopen, A.M., Holst, J.J., et al. (2017). Improvement of Insulin Sensitivity after
1063 Lean Donor Feces in Metabolic Syndrome Is Driven by Baseline Intestinal Microbiota
1064 Composition. *Cell Metab.* 26, 611–619.e616.
- 1065 Korpela, K., Costea, P.I., Coelho, L.P., Kandels-Lewis, S., Willemsen, G., Boomsma, D.I.,
1066 Segata, N., and Bork, P. (2018). Selective maternal seeding and environment shape the human
1067 gut microbiome. *Genome Research*, *in press*
- 1068 Koskella, B., Hall, L.J., and Metcalf, C.J.E. (2017). The microbiome beyond the horizon of
1069 ecological and evolutionary theory. *Nature Ecology & Evolution* 100, 1.
- 1070 Kovatcheva-Datchary, P., Nilsson, A., Akrami, R., Lee, Y.S., De Vadder, F., Arora, T., Hallen,
1071 A., Martens, E., Björck, I., and Bäckhed, F. (2015). Dietary Fiber-Induced Improvement in
1072 Glucose Metabolism Is Associated with Increased Abundance of *Prevotella*. *Cell Metab.* 22,
1073 971–982.
- 1074 Kristensen, N.B., Bryrup, T., Allin, K.H., Nielsen, T., Hansen, T.H., and Pedersen, O. (2016).
1075 Alterations in fecal microbiota composition by probiotic supplementation in healthy adults: a
1076 systematic review of randomized controlled trials. *Genome Medicine* 2016 8:1 8, 52.
- 1077 Kumar, R., Yi, N., Zhi, D., Eipers, P., Goldsmith, K.T., Dixon, P., Crossman, D.K., Crowley,
1078 M.R., Lefkowitz, E.J., Rodriguez, J.M., et al. (2017). Identification of donor microbe species that
1079 colonize and persist long term in the recipient after fecal transplant for recurrent *Clostridium*
1080 *difficile*. *Npj Biofilms and Microbiomes* 3:1 3, 12.
- 1081 Kundu, P., Blacher, E., Elinav, E., and Pettersson, S. (2017). Our Gut Microbiome: The Evolving
1082 Inner Self. *Cell* 171, 1481–1493.
- 1083 Kurilshikov, A., Wijmenga, C., Fu, J., and Zhernakova, A. (2017). Host Genetics and Gut
1084 Microbiome: Challenges and Perspectives. *Trends in Immunology* 38, 633–647.
- 1085 La Rosa, P.S., Warner, B.B., Zhou, Y., Weinstock, G.M., Sodergren, E., Hall-Moore, C.M.,
1086 Stevens, H.J., Bennett, W.E., Shaikh, N., Linneman, L.A., et al. (2014). Patterned progression of
1087 bacterial populations in the premature infant gut. *Pnas* 111, 12522–12527.
- 1088 Langdon, A., Crook, N., and Dantas, G. (2016). The effects of antibiotics on the microbiome
1089 throughout development and alternative approaches for therapeutic modulation. *Genome*
1090 *Medicine* 2016 8:1 8, 1283.
- 1091 Lax, S., Smith, D.P., Hampton-Marcell, J., Owens, S.M., Handley, K.M., Scott, N.M., Gibbons,
1092 S.M., Larsen, P., Shogan, B.D., Weiss, S., et al. (2014). Longitudinal analysis of microbial
1093 interaction between humans and the indoor environment. 345, 1048–1052.

- 1094 Le Bastard, Q., Al-Ghalith, G.A., Grégoire, M., Chapelet, G., Javaudin, F., Dailly, E., Batard, E.,
1095 Knights, D., and Montassier, E. (2017). Systematic review: human gut dysbiosis induced by
1096 non-antibiotic prescription medications. *Aliment Pharmacol Ther* 14, 508.
- 1097 Le Chatelier, E., Nielsen, T., Qin, J., Prifti, E., Hildebrand, F., Falony, G., Almeida, M.,
1098 Arumugam, M., Batto, J.-M., Kennedy, S., et al. (2013). Richness of human gut microbiome
1099 correlates with metabolic markers. *Nature* 500, 541–546.
- 1100 Lee, S.T.M., Kahn, S.A., Delmont, T.O., Shaiber, A., Esen, Ö.C., Hubert, N.A., Morrison, H.G.,
1101 Antonopoulos, D.A., Rubin, D.T., and Eren, A.M. (2017). Tracking microbial colonization in fecal
1102 microbiota transplantation experiments via genome-resolved metagenomics. *Microbiome* 5, 50.
- 1103 Lesley A Ogilvie, B.V.J. (2015). The human gut virome: a multifaceted majority. *Front. Microbiol.*
1104 6, 1753.
- 1105 Li, S.S., Zhu, A., Benes, V., Costea, P.I., Hercog, R., Hildebrand, F., Huerta-Cepas, J.,
1106 Nieuwdorp, M., Salojärvi, J., Voigt, A.Y., et al. (2016). Durable coexistence of donor and
1107 recipient strains after fecal microbiota transplantation. *Science* 352, 586–589.
- 1108 Lloyd-Price, J., Abu-Ali, G., and Huttenhower, C. (2016). The healthy human microbiome.
1109 *Genome Medicine* 2016 8:1 8, 51.
- 1110 Lloyd-Price, J., Mahurkar, A., Rahnavard, G., Crabtree, J., Orvis, J., Hall, A.B., Brady, A.,
1111 Creasy, H.H., McCracken, C., Giglio, M.G., et al. (2017). Strains, functions and dynamics in the
1112 expanded Human Microbiome Project. *Nature* 486, 207.
- 1113 Lynch, S.V., and Pedersen, O. (2016). The Human Intestinal Microbiome in Health and Disease.
1114 *N Engl J Med* 375, 2369–2379.
- 1115 Maier, L., and Typas, A. (2017). Systematically investigating the impact of medication on the gut
1116 microbiome. *Current Opinion in Microbiology* 39, 128–135.
- 1117 Maier, L., Pruteanu, M., Kuhn, M., Zeller, G., Telzerow, A., Anderson, E.E., Brochado, A.R.,
1118 Fernandez, K.C., Dose, H., Mori, H., Patil, K.R., Bork, P., and Typas, A. (2018). Extensive
1119 impact of non-antibiotic drugs on human gut microbiota. *Nature*, *in press*,
1120 doi:10.1038/nature25979
- 1121 Mallick, H., Ma, S., Franzosa, E.A., Vatanen, T., Morgan, X.C., and Huttenhower, C. (2017).
1122 Experimental design and quantitative analysis of microbial community multiomics. *Genome Biol*
1123 18, 260.
- 1124 Manichanh, C., Rigottier-Gois, L., Bonnaud, E., Gloux, K., Pelletier, E., Frangeul, L., Nalin, R.,
1125 Jarrin, C., Chardon, P., Marteau, P., et al. (2006). Reduced diversity of faecal microbiota in
1126 Crohn's disease revealed by a metagenomic approach. *Gut* 55, 205–211.
- 1127 Martí, J.M., Martínez-Martínez, D., Rubio, T., Gracia, C., Peña, M., Latorre, A., Moya, A., Garay,
1128 C.P., and Gilbert, J.A. (2017). Health and Disease Imprinted in the Time Variability of the
1129 Human Microbiome. *mSystems* 2, e00144–16.
- 1130 Marzorati, M., Vanhoecke, B., De Ryck, T., Sadaghian Sadabad, M., Pinheiro, I., Possemiers,
1131 S., Van den Abbeele, P., Derycke, L., Bracke, M., Pieters, J., et al. (2014). The HMI™ module:

- 1132 a new tool to study the Host-Microbiota Interaction in the human gastrointestinal tract in vitro.
1133 *Bmc Microbiol* 14, 133.
- 1134 Matson, V., Fessler, J., Bao, R., Chongsuwat, T., Zha, Y., Alegre, M.-L., Luke, J.J., and
1135 Gajewski, T.F. (2018). The commensal microbiome is associated with anti-PD-1 efficacy in
1136 metastatic melanoma patients. *Science* 359, 104–108.
- 1137 Mäkituokko, H., Tiihonen, K., Tynkkynen, S., Paulin, L., and Rautonen, N. (2010). The effect of
1138 age and non-steroidal anti-inflammatory drugs on human intestinal microbiota composition.
1139 *British Journal of Nutrition* 103, 227–234.
- 1140 MetaHIT Consortium, Li, J., Jia, H., Cai, X., Zhong, H., Feng, Q., Sunagawa, S., Arumugam, M.,
1141 Kultima, J.R., Prifti, E., et al. (2014). An integrated catalog of reference genes in the human gut
1142 microbiome. *Nat Biotech* 32, 834–841.
- 1143 Moss, E.L., Falconer, S.B., Tkachenko, E., Wang, M., Systrom, H., Mahabamunuge, J.,
1144 Relman, D.A., Hohmann, E.L., and Bhatt, A.S. (2017). Long-term taxonomic and functional
1145 divergence from donor bacterial strains following fecal microbiota transplantation in
1146 immunocompromised patients. *Plos One* 12, e0182585.
- 1147 Muegge, B.D., Kuczynski, J., Knights, D., Clemente, J.C., Gonzalez, A., Fontana, L., Henrissat,
1148 B., Knight, R., and Gordon, J.I. (2011). Diet Drives Convergence in Gut Microbiome Functions
1149 Across Mammalian Phylogeny and Within Humans. *Science* 332, 970–974.
- 1150 Mukherjee, S., Seshadri, R., Varghese, N.J., Elie-Fadrosh, E.A., Meier-Kolthoff, J.P., G ker, M.,
1151 Coates, R.C., Hadjithomas, M., Pavlopoulos, G.A., Paez-Espino, D., et al. (2017). 1,003
1152 reference genomes of bacterial and archaeal isolates expand coverage of the tree of life. *Nat*
1153 *Biotech* 38, 1094.
- 1154 Narula, N., Kassam, Z., Yuan, Y., Colombel, J.-F., Ponsioen, C., Reinisch, W., and Moayyedi,
1155 P. (2017). Systematic Review and Meta-analysis Fecal Microbiota Transplantation for Treatment
1156 of Active Ulcerative Colitis. *Inflamm Bowel Dis* 23, 1702–1709.
- 1157 Nayfach, S., Rodriguez-Mueller, B., Garud, N., and Pollard, K.S. (2016). An integrated
1158 metagenomics pipeline for strain profiling reveals novel patterns of bacterial transmission and
1159 biogeography. *Genome Res* 26, 1612–1625.
- 1160 Neville, B.A., Forster, S.C., and Lawley, T.D. (2018). Commensal Koch's postulates:
1161 establishing causation in human microbiota research. *Current Opinion in Microbiology* 42, 47–
1162 52.
- 1163 Nguyen, T.L.A., Vieira-Silva, S., Liston, A., and Raes, J. (2015). How informative is the mouse
1164 for human gut microbiota research? *Disease Models & Mechanisms* 8, 1–16.
- 1165 Noguera-Julian, M., Rocafort, M., Guillén, Y., Rivera, J., Casadellà, M., Nowak, P., Hildebrand,
1166 F., Zeller, G., Parera, M., Bellido, R., et al. (2016). Gut Microbiota Linked to Sexual Preference
1167 and HIV Infection. *EBioMedicine* 5, 135–146.
- 1168 Obregon-Tito, A.J., Tito, R.Y., Metcalf, J., Sankaranarayanan, K., Clemente, J.C., Ursell, L.K.,
1169 Xu, Z.Z., Van Treuren, W., Knight, R., Gaffney, P.M., et al. (2015). Subsistence strategies in
1170 traditional societies distinguish gut microbiomes. *Nat Comms* 6, 6505.

- 1171 Odamaki, T., Kato, K., Sugahara, H., Hashikura, N., Takahashi, S., Xiao, J.-Z., Abe, F., and
1172 Osawa, R. (2016). Age-related changes in gut microbiota composition from newborn to
1173 centenarian: a cross-sectional study. *Bmc Microbiol* 16, 90.
- 1174 Ott, S.J., Musfeldt, M., Wenderoth, D.F., Hampe, J., Brant, O., Fölsch, U.R., Timmis, K.N., and
1175 Schreiber, S. (2004). Reduction in diversity of the colonic mucosa associated bacterial
1176 microflora in patients with active inflammatory bowel disease. *Gut* 53, 685–693.
- 1177 O’Toole, P.W., and Jeffery, I.B. (2015). Gut microbiota and aging. *Science* 350, 1214–1215.
- 1178 Parfrey, L.W., Walters, W.A., and Knight, R. (2011). Microbial Eukaryotes in the Human
1179 Microbiome: Ecology, Evolution, and Future Directions. *Front. Microbiol.* 2, 153.
- 1180 Pasolli, E., Truong, D.T., Malik, F., Waldron, L., and Segata, N. (2016). Machine Learning Meta-
1181 analysis of Large Metagenomic Datasets: Tools and Biological Insights. *PLOS Computational*
1182 *Biology* 12, e1004977.
- 1183 Pedersen, H.K., Gudmundsdottir, V., Nielsen, H.B., Hyotylainen, T., Nielsen, T., Jensen, B.A.H.,
1184 Forslund, K., Hildebrand, F., Prifti, E., Falony, G., et al. (2016). Human gut microbes impact host
1185 serum metabolome and insulin sensitivity. *Nature* 535, 376–381.
- 1186 Petersen, L.M., Bautista, E.J., Nguyen, H., Hanson, B.M., Chen, L., Lek, S.H., Sodergren, E.,
1187 and Weinstock, G.M. (2017). Community characteristics of the gut microbiomes of competitive
1188 cyclists. *Microbiome* 5, 98.
- 1189 Phillips, B.L., and Shine, R. (2004). Adapting to an invasive species: toxic cane toads induce
1190 morphological change in Australian snakes. *Proc Natl Acad Sci USA* 101, 17150–17155.
- 1191 Props, R., Kerckhof, F.-M., Rubbens, P., De Vrieze, J., Sanabria, E.H., Waegeman, W.,
1192 Monsieus, P., Hammes, F., and Boon, N. (2017). Absolute quantification of microbial taxon
1193 abundances. *Isme J* 11, 584–587.
- 1194 Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K.S., Manichanh, C., Nielsen, T., Pons, N.,
1195 Levenez, F., Yamada, T., et al. (2010). A human gut microbial gene catalogue established by
1196 metagenomic sequencing. *Nature* 464, 59–65.
- 1197 Quince, C., Delmont, T.O., Raguideau, S., Alneberg, J., Darling, A.E., Collins, G., and Eren,
1198 A.M. (2017). DESMAN: a new tool for de novo extraction of strains from metagenomes.
1199 *Genome Biol* 18, 181.
- 1200 Raes, J., and Bork, P. (2008). Molecular eco-systems biology: towards an understanding of
1201 community function. *Nat Rev Micro* 6, 693–699.
- 1202 Raymond, F., Ouameur, A.A., Déraspe, M., Iqbal, N., Gingras, H., Dridi, B., Leprohon, P.,
1203 Plante, P.-L., Giroux, R., Bérubé, È., et al. (2015). The initial state of the human gut microbiome
1204 determines its reshaping by antibiotics. *Isme J* 10, 707–720.
- 1205 Rettedal, E.A., Gumpert, H., and Sommer, M.O.A. (2014). Cultivation-based multiplex
1206 phenotyping of human gut microbiota allows targeted recovery of previously uncultured bacteria.
1207 *Nat Comms* 5, 4714.

- 1208 Roager, H.M., Hansen, L.B.S., Bahl, M.I., Frandsen, H.L., Carvalho, V., Gøbel, R.J., Dalgaard,
1209 M.D., Plichta, D.R., Sparholt, M.H., Vestergaard, H., et al. (2016). Colonic transit time is related
1210 to bacterial metabolism and mucosal turnover in the gut. *Nature Microbiology* 1, 16093.
- 1211 Roager, H.M., Licht, T.R., Poulsen, S.K., Larsen, T.M., and Bahl, M.I. (2014). Microbial
1212 Enterotypes, Inferred by the Prevotella-to-Bacteroides Ratio, Remained Stable during a 6-Month
1213 Randomized Controlled Diet Intervention with the New Nordic Diet. *Appl Environ Microbiol* 80,
1214 1142–1149.
- 1215 Rodrigues, J.F.M., Schmidt, S.T., Tackmann, J., and Mering, von, C. (2017). MAPseq: highly
1216 efficient k-mer search with confidence estimates, for rRNA sequence analysis. *Bioinformatics*
1217 23, 3808-3810.
- 1218 Rogers, M.A.M., and Aronoff, D.M. (2016). The influence of non-steroidal anti-inflammatory
1219 drugs on the gut microbiome. *Clinical Microbiology and Infection* 22, 178.e1–178.e9.
- 1220 Rothschild, D., Weissbrod, O., Barkan, E., Korem, T., Zeevi, D., Costea, P.I., Godneva, A.,
1221 Kalka, I.N., Bar, N., Zmora, N., et al. (2018). Environmental factors dominate over host genetics
1222 in shaping human gut microbiota composition. *Nature*, *in press*.
- 1223 Routy, B., Le Chatelier, E., Derosa, L., Duong, C.P.M., Alou, M.T., Daillère, R., Fluckiger, A.,
1224 Messaoudene, M., Rauber, C., Roberti, M.P., et al. (2017). Gut microbiome influences efficacy
1225 of PD-1–based immunotherapy against epithelial tumors. *Science* 65, eaan3706.
- 1226 Satinsky, B.M., Gifford, S.M., Crump, B.C., and Moran, M.A. (2013). Use of internal standards
1227 for quantitative metatranscriptome and metagenome analysis. *Meth. Enzymol.* 531, 237–250.
- 1228 Scher, J.U., Sczesnak, A., Longman, R.S., Segata, N., Ubeda, C., Bielski, C., Rostron, T.,
1229 Cerundolo, V., Pamer, E.G., Abramson, S.B., et al. (2013). Expansion of intestinal Prevotella
1230 copri correlates with enhanced susceptibility to arthritis. *eLife Sciences* 2, e01202.
- 1231 Schirmer, M., Franzosa, E.A., Lloyd-Price, J., McIver, L.J., Schwager, R., Poon, T.W.,
1232 Ananthakrishnan, A.N., Andrews, E., Barron, G., Lake, K., et al. (2018). Dynamics of
1233 metatranscription in the inflammatory bowel disease gut microbiome. *Nature Microbiology* 7, 1.
- 1234 Schloissnig, S., Arumugam, M., Sunagawa, S., Mitreva, M., Tap, J., Zhu, A., Waller, A., Mende,
1235 D.R., Kultima, J.R., Martin, J., et al. (2013). Genomic variation landscape of the human gut
1236 microbiome. *Nature* 493, 45–50.
- 1237 Scholz, M., Ward, D.V., Pasolli, E., Tolio, T., Zolfo, M., Asnicar, F., Truong, D.T., Tett, A.,
1238 Morrow, A.L., and Segata, N. (2016). Strain-level microbial epidemiology and population
1239 genomics from shotgun metagenomics. *Nature Methods* 13, 435–438.
- 1240 Scott, T.A., Quintaneiro, L.M., Norvaisas, P., Lui, P.P., Wilson, M.P., Leung, K.-Y., Herrera-
1241 Dominguez, L., Sudiwala, S., Pessia, A., Clayton, P.T., et al. (2017). Host-Microbe Co-
1242 metabolism Dictates Cancer Drug Efficacy in *C. elegans*. *Cell* 169, 442–456.e18.
- 1243 Seekatz, A.M., Aas, J., Gessert, C.E., Rubin, T.A., Saman, D.M., Bakken, J.S., and Young, V.B.
1244 (2014). Recovery of the Gut Microbiome following Fecal Microbiota Transplantation. *mBio* 5,
1245 e00893–14.

- 1246 Shade, A., Peter, H., Allison, S.D., Baho, D.L., Berga, M., Bürgmann, H., Huber, D.H.,
1247 Langenheder, S., Lennon, J.T., Martiny, J.B.H., et al. (2012). Fundamentals of Microbial
1248 Community Resistance and Resilience. *Front. Microbiol.* 3, 417.
- 1249 Shah, P., Fritz, J.V., Glaab, E., Desai, M.S., Greenhalgh, K., Frachet, A., Niegowska, M., Estes,
1250 M., Jäger, C., Seguin-Devaux, C., et al. (2016). A microfluidics-based *in vitro* model of the
1251 gastrointestinal human–microbe interface. *Nat Comms* 7, 11535.
- 1252 Shoaie, S., Ghaffari, P., Kovatcheva-Datchary, P., Mardinoglu, A., Sen, P., Pujos-Guillot, E., de
1253 Wouters, T., Juste, C., Rizkalla, S., Chilloux, J., et al. (2015). Quantifying Diet-Induced
1254 Metabolic Changes of the Human Gut Microbiome. *Cell Metab.* 22, 320–331.
- 1255 Sinha, R., Abu-Ali, G., Vogtmann, E., Fodor, A.A., Ren, B., Amir, A., Schwager, E., Crabtree, J.,
1256 Ma, S., Consortium, T.M.Q.C.P., et al. (2017). Assessment of variation in microbial community
1257 amplicon sequencing by the Microbiome Quality Control (MBQC) project consortium. *Nat*
1258 *Biotech* 35, 1077.
- 1259 Sivan, A., Corrales, L., Hubert, N., Williams, J.B., Aquino-Michaels, K., Earley, Z.M., Benyamin,
1260 F.W., Lei, Y.M., Jabri, B., Alegre, M.-L., et al. (2015). Commensal Bifidobacterium promotes
1261 antitumor immunity and facilitates anti–PD-L1 efficacy. *Science* 350, 1084–1089.
- 1262 Smits, S.A., Leach, J., Sonnenburg, E.D., Gonzalez, C.G., Lichtman, J.S., Reid, G., Knight, R.,
1263 Manjurano, A., Changalucha, J., Elias, J.E., et al. (2017). Seasonal cycling in the gut
1264 microbiome of the Hadza hunter-gatherers of Tanzania. *Science* 357, 802–806.
- 1265 Sokol, H., Leducq, V., Aschard, H., Pham, H.-P., Jegou, S., Landman, C., Cohen, D., Liguori,
1266 G., Bourrier, A., Nion-Larmurier, I., et al. (2017). Fungal microbiota dysbiosis in IBD. *Gut* 66,
1267 1039–1048.
- 1268 Sommer, F., Anderson, J.M., Bharti, R., Raes, J., and Rosenstiel, P. (2017). The resilience of
1269 the intestinal microbiota influences health and disease. *Nat Rev Micro* 15, 630–638.
- 1270 Song, S.J., Amir, A., Metcalf, J.L., Amato, K.R., Xu, Z.Z., Humphrey, G., Knight, R., and
1271 Dearing, M.D. (2016). Preservation Methods Differ in Fecal Microbiome Stability, Affecting
1272 Suitability for Field Studies. *mSystems* 1, e00021–16.
- 1273 Song, S.J., Lauber, C., Costello, E.K., Lozupone, C.A., Humphrey, G., Berg-Lyons, D.,
1274 Caporaso, J.G., Knights, D., Clemente, J.C., Nakielny, S., et al. (2013). Cohabiting family
1275 members share microbiota with one another and with their dogs. *eLife Sciences* 2, 6378.
- 1276 Sonnenburg, J.L., and Bäckhed, F. (2016). Diet–microbiota interactions as moderators of
1277 human metabolism. *Nature* 535, 56–64.
- 1278 Stämmler, F., Gläsner, J., Hiergeist, A., Holler, E., Weber, D., Oefner, P.J., Gessner, A., and
1279 Spang, R. (2016). Adjusting microbiome profiles for differences in microbial load by spike-in
1280 bacteria. *Microbiome* 4, 28.
- 1281 Stein, R.R., Bucci, V., Toussaint, N.C., Buffie, C.G., Räscht, G., Pamer, E.G., Sander, C., and
1282 Xavier, J.B. (2013). Ecological Modeling from Time-Series Inference: Insight into Dynamics and
1283 Stability of Intestinal Microbiota. *PLOS Computational Biology* 9, e1003388.

- 1284 Stokholm, J., Blaser, M.J., Thorsen, J., Rasmussen, M.A., Waage, J., Vinding, R.K., Schoos, A.-
1285 M.M., Kunøe, A., Fink, N.R., Chawes, B.L., et al. (2018). Maturation of the gut microbiome and
1286 risk of asthma in childhood. *Nat Comms* 9, 141.
- 1287 Suez, J., and Elinav, E. (2017). The path towards microbiome-based metabolite treatment.
1288 *Nature Microbiology* 2, 17075.
- 1289 Sunagawa, S., Mende, D.R., Zeller, G., Izquierdo-Carrasco, F., Berger, S.A., Kultima, J.R.,
1290 Coelho, L.P., Arumugam, M., Tap, J., Nielsen, H.B., et al. (2013). Metagenomic species profiling
1291 using universal phylogenetic marker genes. *Nature Methods* 10, 1196–1199.
- 1292 Surana, N.K., and Kasper, D.L. (2017). Moving beyond microbiome-wide associations to causal
1293 microbe identification. *Nature* 375, 2369.
- 1294 Suzuki, T.A., and Worobey, M. (2014). Geographical variation of human gut microbial
1295 composition. *Biology Letters* 10, 20131037–20131037.
- 1296 Sze, M.A., and Schloss, P.D. (2016). Looking for a Signal in the Noise: Revisiting Obesity and
1297 the Microbiome. *mBio* 7, e01018–16.
- 1298 Tamburini, S., Shen, N., Wu, H.C., and Clemente, J.C. (2016). The microbiome in early life:
1299 implications for health outcomes. *Nature Medicine* 23:3 22, 713–722.
- 1300 Thaiss, C.A., Zmora, N., Levy, M., and Elinav, E. (2016). The microbiome and innate immunity.
1301 *Nature* 535, 65–74.
- 1302 The Human Microbiome Jumpstart Reference Strains Consortium, Nelson, K.E., Weinstock,
1303 G.M., Highlander, S.K., Worley, K.C., Creasy, H.H., Wortman, J.R., Rusch, D.B., Mitreva, M.,
1304 Sodergren, E., et al. (2010). A Catalog of Reference Genomes from the Human Microbiome.
1305 *PLoS* 3, 994–999.
- 1306 The Human Microbiome Project Consortium (2012). Structure, function and diversity of the
1307 healthy human microbiome. *Nature* 486, 207–214.
- 1308 Ticinesi, A., Milani, C., Lauretani, F., Nouvenne, A., Mancabelli, L., Lugli, G.A., Turroni, F.,
1309 Duranti, S., Mangifesta, M., Viappiani, A., et al. (2017). Gut microbiota composition is
1310 associated with polypharmacy in elderly hospitalized patients. *Sci. Rep.* 7, 11102.
- 1311 Tigchelaar, E.F., Bonder, M.J., Jankipersadsing, S.A., Fu, J., Wijmenga, C., and Zhernakova, A.
1312 (2016). Gut microbiota composition associated with stool consistency. *Gut* 65, 540–542.
- 1313 Tito, R.Y., Cypers, H., Joossens, M., Varkas, G., Van Praet, L., Glorieus, E., Van den Bosch, F.,
1314 De Vos, M., Raes, J., and Elewaut, D. (2016). Brief Report: Dialisteras a Microbial Marker of
1315 Disease Activity in Spondyloarthritis. *Arthritis & Rheumatology* 69, 114–121.
- 1316 Tramontano, M., Andrejew, S., Pruteanu, M., Klünemann, M., Kuhn, M., Galardini, M., Jouhten,
1317 P., Zelezniak, A., Zeller, G., Bork, P., Typas, A., and Patil, K. R. (2018). Nutritional preferences
1318 of human gut bacteria reveal their metabolic idiosyncrasies. *Nature Microbiology*, *in press*
- 1319 Tremaroli, V., Karlsson, F., Werling, M., Ståhlman, M., Kovatcheva-Datchary, P., Olbers, T.,
1320 Fändriks, L., le Roux, C.W., Nielsen, J., and Bäckhed, F. (2015). Roux-en-Y Gastric Bypass and

- 1321 Vertical Banded Gastroplasty Induce Long-Term Changes on the Human Gut Microbiome
1322 Contributing to Fat Mass Regulation. *Cell Metab.* 22, 228–238.
- 1323 Truong, D.T., Tett, A., Pasolli, E., Huttenhower, C., and Segata, N. (2017). Microbial strain-level
1324 population structure and genetic diversity from metagenomes. *Genome Res* 27, gr.216242.116–
1325 gr.216242.638.
- 1326 Turnbaugh, P.J., Hamady, M., Yatsunencko, T., Cantarel, B.L., Duncan, A., Ley, R.E., Sogin,
1327 M.L., Jones, W.J., Roe, B.A., Affourtit, J.P., et al. (2009). A core gut microbiome in obese and
1328 lean twins. *Nature* 457, 480–484.
- 1329 Turpin, W., Espin-Garcia, O., Xu, W., Silverberg, M.S., Kevans, D., Smith, M.I., Guttman, D.S.,
1330 Griffiths, A., Panaccione, R., Otley, A., et al. (2016). Association of host genome with intestinal
1331 microbial composition in a large healthy cohort. *Nat Genet* 48, 1413–1417.
- 1332 van Nood, E., Vrieze, A., Nieuwdorp, M., Fuentes, S., Zoetendal, E.G., de Vos, W.M., Visser,
1333 C.E., Kuijper, E.J., Bartelsman, J.F.W.M., Tijssen, J.G.P., et al. (2013). Duodenal Infusion of
1334 Donor Feces for Recurrent *Clostridium difficile*. *N Engl J Med* 368, 407–415.
- 1335 Vandeputte, D., Falony, G., D’hoë, K., Vieira-Silva, S., and Raes, J. (2017a). Water activity does
1336 not shape the microbiota in the human colon. *Gut* 66, gutjnl–2016–313530–1866.
- 1337 Vandeputte, D., Falony, G., Vieira-Silva, S., Tito, R.Y., Joossens, M., and Raes, J. (2015). Stool
1338 consistency is strongly associated with gut microbiota richness and composition, enterotypes
1339 and bacterial growth rates. *Gut* 65, gutjnl–2015–309618–62.
- 1340 Vandeputte, D., Falony, G., Vieira-Silva, S., Wang, J., Sailer, M., Theis, S., Verbeke, K., and
1341 Raes, J. (2017b). Prebiotic inulin-type fructans induce specific changes in the human gut
1342 microbiota. *Gut* 66, 1968–1974.
- 1343 Vandeputte, D., Kathagen, G., D’hoë, K., Vieira-Silva, S., Valles-Colomer, M., Sabino, J., Wang,
1344 J., Tito, R.Y., De Commer, L., Darzi, Y., et al. (2017c). Quantitative microbiome profiling links
1345 gut community variation to microbial load. *Nature* 551, 507–511.
- 1346 Vandeputte, D., Tito, R.Y., Vanleeuwen, R., Falony, G., and Raes, J. (2017d). Practical
1347 considerations for large-scale gut microbiome studies. *FEMS Microbiol Rev* 41, S154–S167.
- 1348 Vázquez-Baeza, Y., Callewaert, C., Debelius, J., Hyde, E., Marotz, C., Morton, J.T., Swafford,
1349 A., Vrbancac, A., Dorrestein, P.C., and Knight, R. (2018). Impacts of the Human Gut Microbiome
1350 on Therapeutics. *Annu. Rev. Pharmacol. Toxicol.* 58, 253–270.
- 1351 Vermeire, S., Joossens, M., Verbeke, K., Wang, J., Machiels, K., Sabino, J., Ferrante, M., Van
1352 Assche, G., Rutgeerts, P., and Raes, J. (2016). Donor Species Richness Determines Faecal
1353 Microbiota Transplantation Success in Inflammatory Bowel Disease. *Eccojc* 10, 387–394.
- 1354 Vetizou, M., Pitt, J.M., Daillere, R., Lepage, P., Waldschmitt, N., Flament, C., Rusakiewicz, S.,
1355 Routy, B., Roberti, M.P., Duong, C.P.M., et al. (2015). Anticancer immunotherapy by CTLA-4
1356 blockade relies on the gut microbiota. *Science* 350, 1079–1084.
- 1357 Voigt, A.Y., Costea, P.I., Kultima, J.R., Li, S.S., Zeller, G., Sunagawa, S., and Bork, P. (2015).
1358 Temporal and technical variability of human gut metagenomes. *Genome Biol* 16, 73.

- 1359 Vrieze, A., van Nood, E., Holleman, F., Salojärvi, J., Kootte, R.S., Bartelsman, J.F.W.M.,
1360 Dallinga-Thie, G.M., Ackermans, M.T., Serlie, M.J., Oozeer, R., et al. (2012). Transfer of
1361 intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic
1362 syndrome. *Gastroenterology* 143, 913–916.e917.
- 1363 Walsh, C.J., Guinane, C.M., O'Toole, P.W., and Cotter, P.D. (2014). Beneficial modulation of
1364 the gut microbiota. *FEBS Lett* 588, 4120–4130.
- 1365 Walters, W.A., Xu, Z., and Knight, R. (2014). Meta-analyses of human gut microbes associated
1366 with obesity and IBD. *FEBS Lett* 588, 4223–4233.
- 1367 Wang, J., and Jia, H. (2016). Metagenome-wide association studies: fine-mining the
1368 microbiome. *Nat Rev Micro* 14, 508–522.
- 1369 Wang, J., Thingholm, L.B., Skiecevičienė, J., Rausch, P., Kummen, M., Hov, J.R., Degenhardt,
1370 F., Heinsen, F.-A., Rühlemann, M.C., Szymczak, S., et al. (2016a). Genome-wide association
1371 analysis identifies variation in vitamin D receptor and other host factors influencing the gut
1372 microbiota. *Nat Genet* 48, 1396–1406.
- 1373 Wang, S., Xu, M., Wang, W., Cao, X., Piao, M., Khan, S., Yan, F., Cao, H., and Wang, B.
1374 (2016b). Systematic Review: Adverse Events of Fecal Microbiota Transplantation. *Plos One* 11,
1375 e0161174.
- 1376 Wang, Z., Klipfell, E., Bennett, B.J., Koeth, R., Levison, B.S., DuGar, B., Feldstein, A.E., Britt,
1377 E.B., Fu, X., Chung, Y.-M., et al. (2011). Gut flora metabolism of phosphatidylcholine promotes
1378 cardiovascular disease. *Nature* 472, 57–63.
- 1379 Wassenaar, T.M. (2016). Insights from 100 years of research with probiotic *E. coli*. *European*
1380 *Journal of Microbiology and Immunology* 6, 147–161.
- 1381 Weiss, S., Van Treuren, W., Lozupone, C., Faust, K., Friedman, J., Deng, Y., Xia, L.C., Xu, Z.Z.,
1382 Ursell, L., Alm, E.J., et al. (2016). Correlation detection strategies in microbial data sets vary
1383 widely in sensitivity and precision. *Isme J* 10, 1669–1681.
- 1384 Weiss, S., Xu, Z.Z., Peddada, S., Amir, A., Bittinger, K., Gonzalez, A., Lozupone, C., Zaneveld,
1385 J.R., Vázquez-Baeza, Y., Birmingham, A., et al. (2017). Normalization and microbial differential
1386 abundance strategies depend upon data characteristics. *Microbiome* 5, 59.
- 1387 Wilck, N., Matus, M.G., Kearney, S.M., Olesen, S.W., Forslund, K., Bartolomaeus, H., Haase,
1388 S., Mähler, A., Balogh, A., Markó, L., et al. (2017). Salt-responsive gut commensal modulates
1389 T_H17 axis and disease. *Nature* 551, 585.
- 1390 Wlodarska, M., Kostic, A.D., and Xavier, R.J. (2015). An Integrative View of Microbiome-Host
1391 Interactions in Inflammatory Bowel Diseases. *Cell Host & Microbe* 17, 577–591.
- 1392 Wu, G.D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y.Y., Keilbaugh, S.A., Bewtra, M.,
1393 Knights, D., Walters, W.A., Knight, R., et al. (2011). Linking Long-Term Dietary Patterns with
1394 Gut Microbial Enterotypes. *Science* 334, 105–108.
- 1395 Wu, H., Esteve, E., Tremaroli, V., Khan, M.T., Caesar, R., Mannerås-Holm, L., Ståhlman, M.,
1396 Olsson, L.M., Serino, M., Planas-Fèlix, M., et al. (2017). Metformin alters the gut microbiome of

- 1397 individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the
1398 drug. *Nature Medicine* 2017 23:3 23, 850–858.
- 1399 Xie, H., Guo, R., Zhong, H., Feng, Q., Lan, Z., Qin, B., Ward, K.J., Jackson, M.A., Xia, Y., Chen,
1400 X., et al. (2016). Shotgun Metagenomics of 250 Adult Twins Reveals Genetic and
1401 Environmental Impacts on the Gut Microbiome. *Cell Systems* 3, 572–584.e573.
- 1402 Xu, J., Lian, F., Zhao, L., Zhao, Y., Chen, X., Zhang, X., Guo, Y., Zhang, C., Zhou, Q., Xue, Z.,
1403 et al. (2015). Structural modulation of gut microbiota during alleviation of type 2 diabetes with a
1404 Chinese herbal formula. *Isme J* 9, 552–562.
- 1405 Yatsunenkov, T., Rey, F.E., Manary, M.J., Trehan, I., Dominguez-Bello, M.G., Contreras, M.,
1406 Magris, M., Hidalgo, G., Baldassano, R.N., Anokhin, A.P., et al. (2012). Human gut microbiome
1407 viewed across age and geography. *Nature* 486, 222–227.
- 1408 Yutin, N., Makarova, K.S., Gussow, A.B., Krupovic, M., Segall, A., Edwards, R.A., and Koonin,
1409 E.V. (2018). Discovery of an expansive bacteriophage family that includes the most abundant
1410 viruses from the human gut. *Nature Microbiology* 3, 38–46.
- 1411 Zaneveld, J.R., McMinds, R., and Thurber, R.V. (2017). Stress and stability: applying the Anna
1412 Karenina principle to animal microbiomes. *Nature Microbiology* 2, nmicrobiol2017121.
- 1413 Zeevi, D., Korem, T., Zmora, N., Israeli, D., Rothschild, D., Weinberger, A., Ben-Yacov, O.,
1414 Lador, D., Avnit-Sagi, T., Lotan-Pompan, M., et al. (2015). Personalized Nutrition by Prediction
1415 of Glycemic Responses. *Cell* 163, 1079–1094.
- 1416 Zeller, G., Tap, J., Voigt, A.Y., Sunagawa, S., Kultima, J.R., Costea, P.I., Amiot, A., Böhm, J.,
1417 Brunetti, F., Habermann, N., et al. (2014). Potential of fecal microbiota for early-stage detection
1418 of colorectal cancer. *Mol Syst Biol* 10, 766–766.
- 1419 Zhang, C., Yin, A., Li, H., Wang, R., Wu, G., Shen, J., Zhang, M., Wang, L., Hou, Y., Ouyang,
1420 H., et al. (2015a). Dietary Modulation of Gut Microbiota Contributes to Alleviation of Both
1421 Genetic and Simple Obesity in Children. *EBioMedicine* 2, 968–984.
- 1422 Zhang, X., Zhang, D., Jia, H., Feng, Q., Wang, D., Di Liang, Wu, X., Li, J., Tang, L., Li, Y., et al.
1423 (2015b). The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly
1424 normalized after treatment. *Nature Medicine* 2017 23:3 21, 895–905.
- 1425 Zhernakova, A., Kurilshikov, A., Bonder, M.J., Tigchelaar, E.F., Schirmer, M., Vatanen, T.,
1426 Mujagic, Z., Vila, A.V., Falony, G., Vieira-Silva, S., et al. (2016). Population-based
1427 metagenomics analysis reveals markers for gut microbiome composition and diversity. *Science*
1428 352, 565–569.
- 1429 Zhou, W., Gay, N., and Oh, J. (2018). ReprDB and panDB: minimalist databases with maximal
1430 microbial representation. *Microbiome* 6, 15.
- 1431 Zhu, W., Winter, M.G., Byndloss, M.X., Spiga, L., Duerkop, B.A., Hughes, E.R., Büttner, L., de
1432 Lima Romão, E., Behrendt, C.L., Lopez, C.A., et al. (2018). Precision editing of the gut
1433 microbiota ameliorates colitis. *Nature* 104, 13780.

1434 Zmora, N., Zeevi, D., Korem, T., Segal, E., and Elinav, E. (2016). Taking it Personally:
1435 Personalized Utilization of the Human Microbiome in Health and Disease. *Cell Host & Microbe*
1436 19, 12–20.

1437

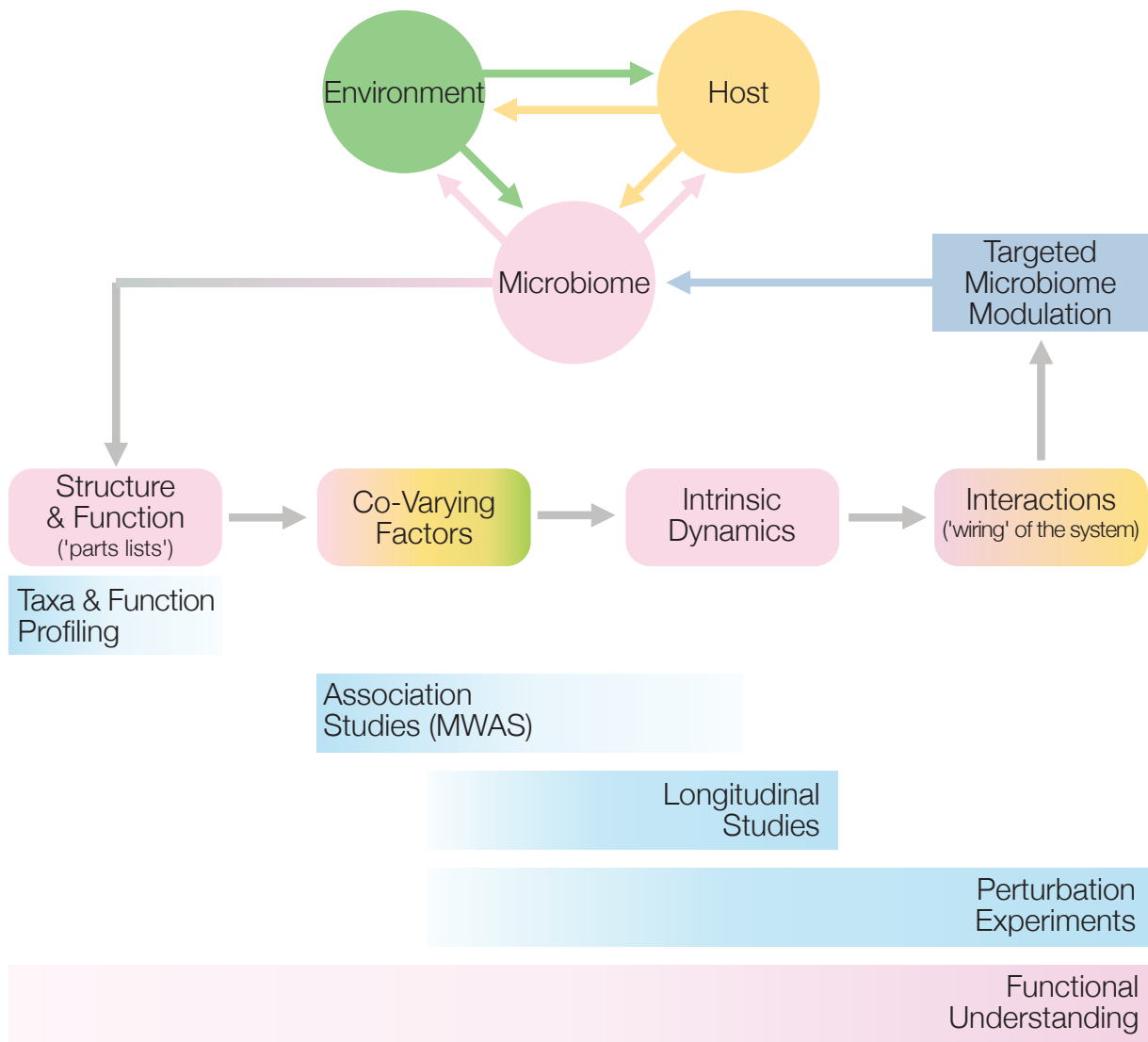


Figure 1

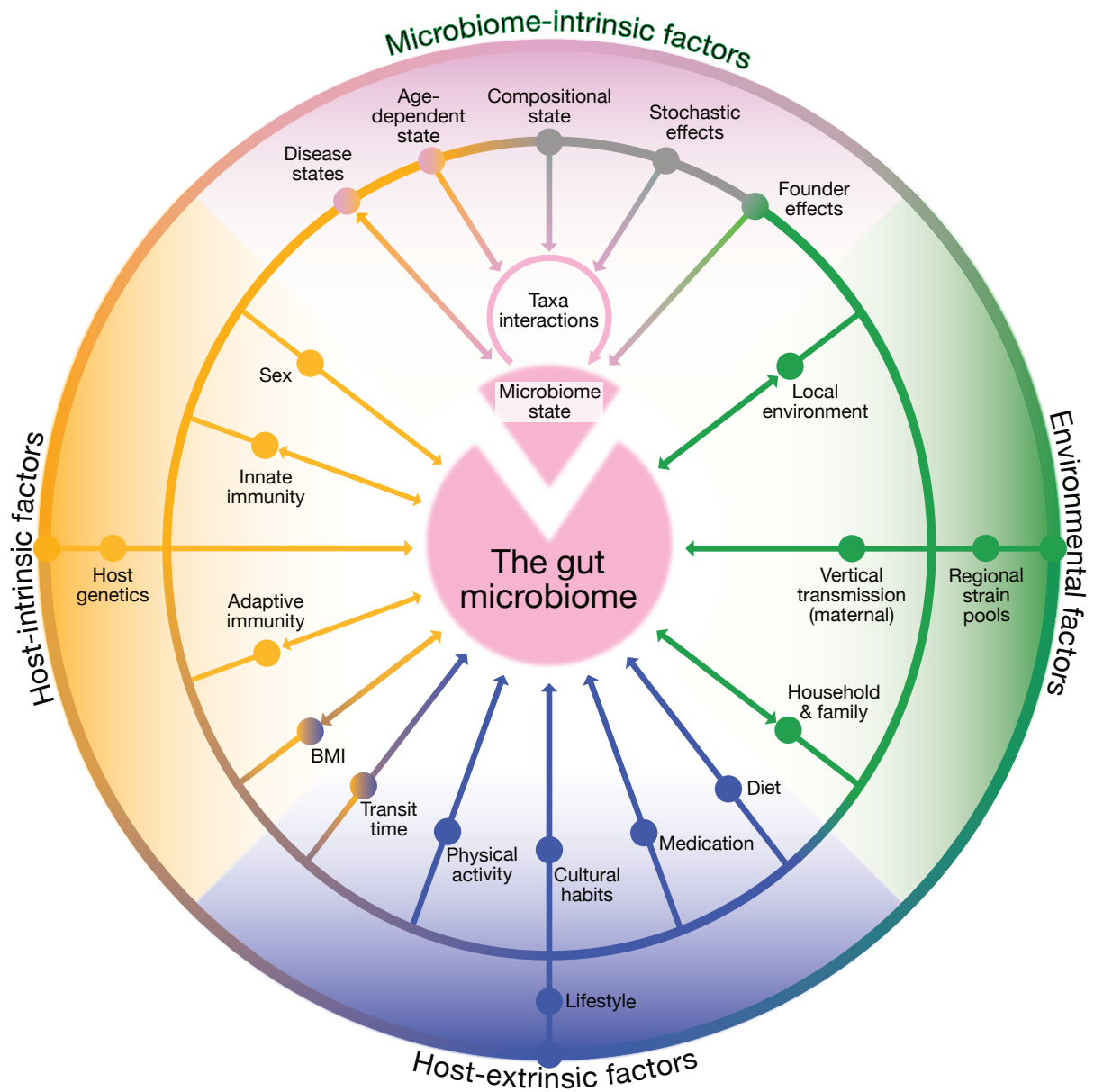


Figure 2

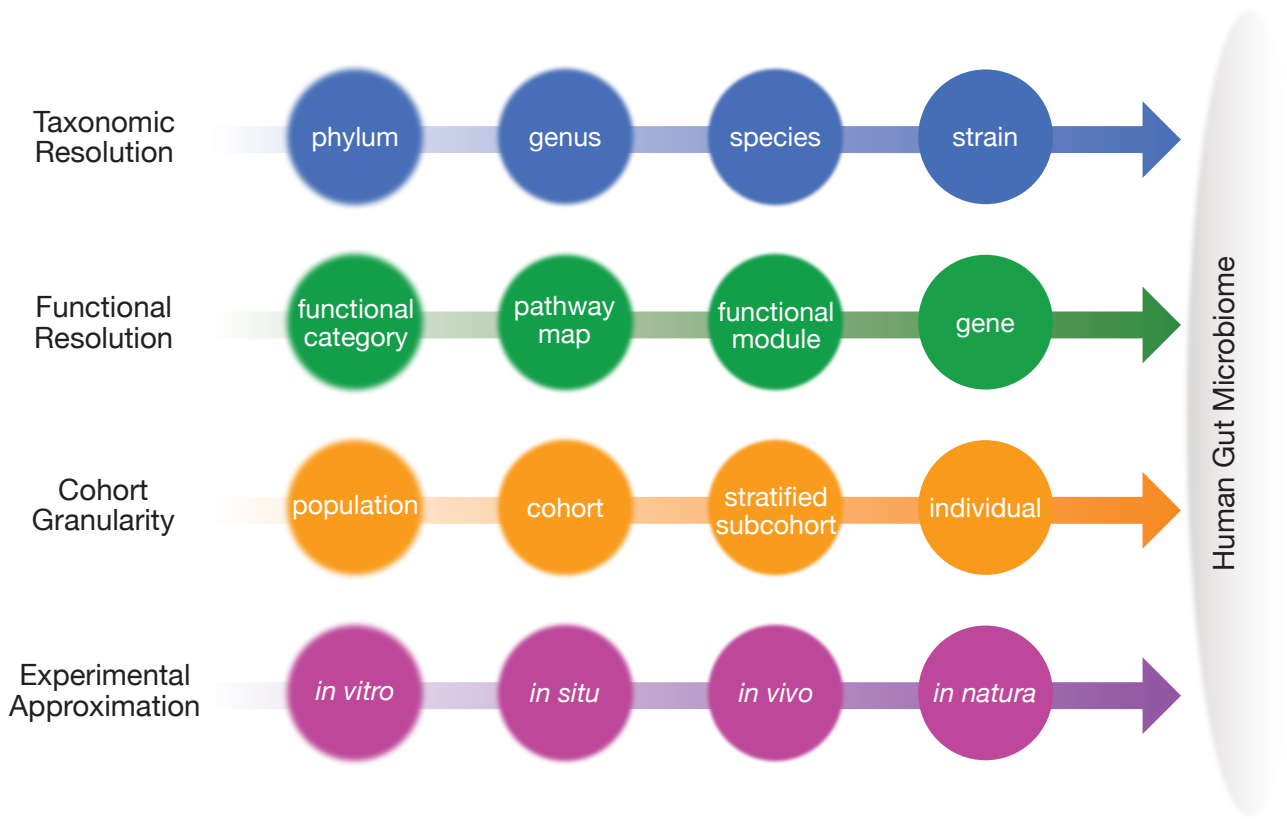


Figure 3