

1 **Maternal effects on early-life gut microbiome maturation in** 2 **a wild nonhuman primate**

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23 **ABSTRACT**

24 Early-life gut microbial colonization is an important process shaping host physiology, immunity
25 and long-term health outcomes in humans and other animals. However, our understanding of this
26 dynamic process remains poorly investigated in wild animals, where developmental mechanisms
27 can be better understood within ecological and evolutionary relevant contexts. Using 16s rRNA
28 amplicon sequencing on 525 fecal samples from a large cohort of infant and juvenile geladas
29 (*Theropithecus gelada*), we characterized gut microbiome maturation during the first three years
30 of life and assessed the role of maternal effects in shaping offspring microbiome assembly.
31 Microbial diversity increased rapidly in the first months of life, followed by more gradual changes
32 until weaning. As expected, changes in gut microbiome composition and function with increasing
33 age reflected progressive dietary transitions: in early infancy when infants rely heavily on their
34 mother's milk, microbes that facilitate milk glycans and lactose utilization dominated, while later
35 in development as graminoids are progressively introduced into the diet, microbes that metabolize
36 plant complex polysaccharides became dominant. Furthermore, the microbial community of
37 nursing infants born to first-time (primiparous) mothers was more "milk-oriented" compared to
38 similarly-aged infants born to experienced (multiparous) mothers. Comparisons of matched
39 mother-offspring fecal samples to random dyads did not support vertical transmission as a conduit
40 for these maternal effects, which instead could be explained by slower phenotypic development
41 (and associated slower gut microbiome maturation) in infants born to first-time mothers. Together,
42 our findings highlight the dynamic nature of gut colonization in early life and the role of maternal
43 effects in modulating this trajectory in a wild primate.

44 INTRODUCTION

45 The colonization of the gastrointestinal tract begins at birth and develops into a trajectory that can
46 be highly variable between individuals [1–8]. Variation in the source and timing of postnatal
47 microbial colonization influences somatic growth [9–12], neuroendocrine [13,14] and immune
48 physiology [15–17], with health and fitness consequences that can extend across the life course
49 [16,18,19]. In humans, for instance, infants that take antibiotics during the first year of life are
50 more likely to develop allergies, asthma, and inflammatory bowel disease during childhood [20–
51 23]. Germ-free rodent models demonstrate that at least some of these effects are causally related
52 to the microbiome and are long-lasting. For example, germ-free rodents develop structural
53 abnormalities of the gastrointestinal tract [15,24] that translate into immune system dysfunction
54 later in life [25,26], an outcome that can only be partly reversed by introducing microbes during
55 critical periods of development [27,28]. Despite the critical role that early-life gut microbial
56 colonization plays in host development, research thus far has mainly focused on clinical studies in
57 humans [14,22,29–31], complemented by experimental studies on laboratory rodents [32–35] and
58 domestic animals [9,36–39]. Studies of wild animals are needed if we want to understand host-
59 microbiome coevolution within a broader ecological and evolutionary context and without the
60 confounding factors associated with medical interventions (e.g., Cesarean section, antibiotic use,
61 formula feeding) [17,40,41].

62 The maternal microbiota drives gut microbiome assembly in offspring via vertical
63 transmission of a large number of microbial lineages [42–46]. Vertical transmission is thought to
64 be particularly strong in mammals due to viviparity and extended periods of lactation and post-
65 weaning maternal care [47]. The first important exposure to microbes occurs during birth, when
66 infants are inoculated with maternal vaginal, fecal, and skin microbiota [3,5,8,29,44,48].

67 Postnatally, vertical transmission is primarily accomplished through nursing, with numerous
68 microbes and milk glycans (i.e., oligosaccharides) transmitted through milk that, together,
69 determine the microbial composition of the infant's gut [42,49,50]. While milk microbes directly
70 seed the offspring's gut, milk glycans promote the growth of beneficial microbes, such as
71 *Bifidobacterium* and *Bacteroides*, that in turn break the glycans down into forms usable by the host
72 [51–53]. Although breastmilk is the most obvious route by which vertical transmission takes place
73 [10,49,50,54], studies on humans suggest that the maternal gut microbiome is also a major source
74 of transmitted strains [42,44,55,56]. Maternal gut microbes might be transmitted to offspring via
75 milk, as the gastrointestinal tract is hypothesized to be the major reservoir of microbes colonizing
76 the mammary gland (the enteromammary pathway) [49,57]. Alternatively or additionally, mothers
77 may transmit gut microbes to offspring via preferential body contact [58], a mechanism that
78 suggests vertical transmission can continue in some capacity past weaning [47]. Because maternal
79 microbial taxa are the first to colonize and tend to be better adapted to the gut ecological niche
80 compared to other environmental microbes, they often persist longer in offspring than those
81 acquired from other sources [42,56,59].

82 Recent studies suggest that microbiome-mediated maternal effects are indeed possible. In
83 several mammals, maternal traits, such as parity (i.e., the number of times a mother has given
84 birth), have been associated with differences in the composition of both maternal [39,60] and
85 offspring microbial communities [10,39]. In nonhuman primates (vervet monkeys: *Chlorocebus*
86 *pygerythrus*), infants born to low-parity mothers harbored reduced microbial diversity and a
87 greater abundance of *Bacteroides fragilis* [10], a bacterium derived from the milk microbiota that
88 is specialized in digesting milk glycans [61,62]. In turn, infants from low-parity females grew
89 faster, suggesting that low-parity mothers may compensate for poor milk production by vertically

90 transmitting milk microbes that could help infants extract more energy from lower milk volumes
91 [10]. Such strategies may be broadly beneficial to dyads in which mothers cannot provide adequate
92 nutritional resources to offspring (e.g., low-ranking mothers) [63,64]. Thus, maternal vertical
93 transmission of microbes may be an important mechanism of phenotypic plasticity during lactation
94 [64,65].

95 Primates are particularly relevant models for understanding postnatal microbiome
96 development and maternal effects because they are closely related to humans, display prolonged
97 lactation periods, and engage in high maternal investment [66,67]. Furthermore, maternal
98 condition (e.g., energetic status) and maternal traits (e.g., dominance rank, social integration
99 parity) are known to influence offspring developmental and long-term fitness outcomes [68–71].
100 Although studies on host-associated microbial communities in wild primates are emerging, many
101 remain limited in scope, hampered by cross-sectional samples and small sample sizes of unweaned
102 infants (particularly in the first few weeks of life), which together prevent longitudinal
103 characterization of gut microbial colonization processes [72–75]. Here, we used dense cross-
104 sectional and longitudinal monitoring to characterize gut microbial colonization during the first
105 three years of life and assess the role of maternal effects in shaping offspring maturation
106 trajectories in wild gelada monkeys (*Theropithecus gelada*). Geladas live in the high-altitude
107 plateaus of Ethiopia and have a specialized graminivorous diet (at times, comprising 90% grass)
108 [76,77], which strongly shapes the composition and function of the adult gut microbiome [78,79].
109 Because geladas live in polygynous reproductive units that range together in larger bands
110 (comprised of 200 or more individuals) [80], we are able to monitor over 50 immatures at any
111 given time, offering an unprecedented sample size to examine gut microbial characteristics during
112 early life in a wild primate. We used 16s rRNA amplicon sequencing on 525 fecal samples from

113 89 immatures to profile changes in gut microbiome diversity, composition, and function during
114 the first three years of life ($N=5.9\pm 5.5$ samples per individual, range:1-18, **Figure S1**). In our
115 population, geladas reach weaning at approximately 1.5 years of age and become sexually mature
116 around 4.6 years [81]; and maternal characteristics, such as parity and dominance rank, are known
117 to influence inter-individual variation at both of these developmental milestones [Feder et al., in
118 revision; Lu et al., unpublished]. We predicted that early life microbial changes would reflect
119 dietary transitions associated with weaning, as infants transition from milk to a plant-based diet
120 [5,48,82,83]. We also predicted that maternal traits, such as dominance rank and parity would be
121 associated with inter-individual differences in gut microbiome diversity, composition, and
122 function in offspring. More specifically, we predicted that infants born to primiparous and low-
123 ranking mothers would have a microbiome more functionally adapted to digest milk to compensate
124 for poorer maternal energetic allocation during lactation. Lastly, we tested if we could detect
125 evidence of vertical transmission between mother and offspring using fecal-fecal comparisons of
126 mother-infant dyads (with 398 matched fecal samples between mother and offspring collected on
127 the same day throughout development) and if greater vertical transmission in certain females (e.g.,
128 low rank, first-time mothers) could be the conduit for putative maternal effects on offspring's
129 microbiome composition. We expected a stronger signal of vertical transmission in early life
130 [10,42,44,55,56], likely driven by a combination of greater microbial transfer via milk when
131 infants are nursing and are also in more frequent body contact with their mother.

132

133 **RESULTS**

134 *General pattern of gut microbiome maturation in geladas*

135 We characterized the gut microbiome across 525 immature gut microbiome samples, and detected
136 3,784 Amplicon Sequence Variants (ASVs) (mean \pm SD per sample: 728 ± 261 , min-max: 65-1,498)

137 belonging to 19 phyla and 76 families. The gut microbiome composition of immature geladas
138 changed quickly following birth, with an initial phase of taxonomic succession and diversification
139 during the first few months of life, followed by a progressive stabilization of the overall community
140 (**Figures 1A,B**).

141 To characterize broad changes in gut microbial community composition across
142 development, we first focused on patterns of alpha diversity (i.e., the microbial diversity within a
143 sample) and beta diversity (i.e., the overall difference of composition between samples). The
144 Shannon Index of alpha diversity was initially low in early life and increased rapidly with age
145 (GAMM: edf=7.2, $P < 2.0 \times 10^{-16}$) (**Figure 1C, Table S1**), converging to adult-like values at 7.3
146 months (nonlinear quadratic plateau model: $R^2=0.62$) (see **Figure S2, Table S1** for similar results
147 on alternative alpha diversity metrics). Furthermore, age was one of the strongest predictors of the
148 difference in microbial composition between samples (PERMANOVA based on Aitchison
149 dissimilarity metric of beta diversity: $R^2=0.75$, $P < 9.9 \times 10^{-05}$, **Table S2, Figures 1D,E**) and samples
150 clustered tightly by age on the first axis (PC1) of a Principal component analysis of beta diversity
151 (Pearson correlation coefficient between age and PC1=0.62, $P < 2.2 \times 10^{-16}$). Compared to alpha
152 diversity, beta diversity reached an adult-like composition later in development, at 17.2 months
153 (nonlinear quadratic plateau model between PC1 and age: $R^2=0.55$; **Figure 1D**), which is
154 approximately the age at which gelada mothers return from lactational amenorrhea and resume
155 reproductive cycles [81]. Other important structuring factors of the immature gut microbiome
156 included infant identity ($R^2=0.24$) and group membership ($R^2=0.05$) (**Table S2**).

157 To assess the compositional maturation of the gut microbiome of immature geladas relative
158 to the maternal gut microbiome across age, we calculated the number of shared ASVs and beta
159 diversity dissimilarity (unweighted and weighted UniFrac) between 398 matched immature-

160 mother pairs of fecal samples collected the same day. As offspring got older, they shared an
161 increasing number of bacteria with their mother (GAMM: effective degree of freedom, $\text{edf}=4.7$,
162 $P<2.0\times 10^{-16}$; **Table S3, Figure 1F**) and became more similar to maternal (i.e., adult-like) gut
163 microbiome composition (unweighted UniFrac: $\text{edf}=4.7$, $P<2.0\times 10^{-16}$; weighted UniFrac: $\text{edf}=3.5$,
164 $P<2.0\times 10^{-16}$; **Table S3, Figure S3**). Convergence with maternal gut occurred at 14.5 months for
165 the number of shared ASVs (nonlinear quadratic plateau model: $R^2=0.44$; **Figure 1F**) and 14.8-
166 15.5 months for beta diversity dissimilarity ($R^2=0.48$ for unweighted UniFrac and $R^2=0.17$ for
167 weighted UniFrac; **Figure S3**).

168 Despite the strong age-related patterns noted above, inter-individual variability in
169 composition (as measured by the minimal pairwise beta diversity dissimilarity value among
170 immature samples, see Methods) was higher among younger infants compared to older juveniles
171 (**Figure 1G**). Some young infants (~3-6 months) in particular had a gut microbiome that were
172 relatively mature (i.e. adult-like) for their age (**Figure 1D**). Such “individuality” in the gut
173 microbiome in early life was likely driven by the presence of rare taxa, since the pattern was
174 stronger using unweighted UniFrac (which does not take into account taxa abundance) as opposed
175 to weighted UniFrac measures of beta diversity (**Figure 1G**).

176

177 *Taxonomic and functional changes during development*

178 To characterize age-associated changes in microbial composition and function, we used
179 autoregressive integrated moving average (ARIMA) models to identify significant developmental
180 changes in the abundance of each microbial taxa (at the family and genus levels) and each predicted
181 functional pathway (at the metabolic level: KEGG Orthologs, KOs and enzymatic level: Enzyme
182 Commission numbers, EC [84]). We then used hierarchical clustering to group microbial taxa and

183 functional pathways based on similar age-related abundance trajectories. Maturation trajectories
184 fell into one of four distinct clusters at both the taxonomic (**Figure 2, S4; Table S4**) and functional
185 (KOs: **Figure S5-S6, Table S5**; EC: **Figure S7, Table S6**) levels.

186

187 **Cluster 1: The early-life microbiome is adapted to process milk**

188 Cluster 1 contained microbes that were abundant during the earliest months of infancy (18
189 families: **Figure 2A, Table S4** and 39 genera: **Figure S4, Table S4**) and are broadly involved in
190 using and fermenting milk sugars (see supplemental results 1 for additional details on cluster 1).
191 These early colonizers comprised bacteria that break down milk glycans (Bacteroidaceae,
192 Bifidobacteriaceae) and lactose (Streptococcaceae, [*Ruminococcus*] *gnavus* group) and other
193 groups that ferment glycans and lactose into butyrate (Lachnospiraceae: *Lachnoclostridium*,
194 *Blautia*, *Anaerostipes*, and Ruminococcaceae: *Faecalibacterium*, *Butyricoccus*, *Butyricimonas*)
195 or propionate (Veillonellaceae) (**Figures 2B and 3A**). *Bacteroides* appeared to be the main
196 degrader of milk glycans in geladas, representing the most abundant genus in early life (~30% of
197 the gut microbes at 1 month) (**Figure 3A**). One *Bacteroides* ASV – *B. fragilis*, a proficient
198 degrader of milk glycans [61] – was particularly abundant in early life (i.e., with a high loading
199 score on PC1, **Table S7**). By contrast, *Bifidobacterium* – an important milk glycan degrader in
200 humans – was present at extremely low abundance across development (<0.01% at 1 month in
201 geladas vs ~40% in humans [85]) (**Figure 3A**).

202 Functional cluster 1 also reflected the involvement of the gut microbiome in milk
203 utilization and immunity pathways (metabolic cluster 1: **Figures S5-S6, Tables S5** and enzymatic
204 cluster 1: **Figure S7, Table S6**). Young infant gut microbiomes contained high levels of bacterial
205 genes involved in carbohydrate metabolism, notably in the catabolism of fructose, mannose, and
206 galactose (3 abundant milk sugars [86]), and in the conversion of sugars to energy (e.g., via

207 glycolysis/gluconeogenesis, pyruvate metabolism, pentose phosphate pathway) (**Figure 4A**).
208 Similar functional signatures of cluster 1 were also apparent at the enzymatic level, as the gut
209 microbes encoded a specialized enzymatic toolkit (alpha and beta glucosidase, alpha and beta
210 galactosidase, fucosidase, sialidase, beta-hexosaminidase) necessary to cleave complex milk
211 glycans (**Figure S8, Table S6**). *Bacteroides* was the main microbial group encoding those
212 enzymes (**Figure S8**), confirming its central role in milk glycan degradation in geladas.

213 Interestingly, cluster 1 also included several putatively pathogenic genera (**Figure 3B**),
214 including some bacterial species most responsible for enteric infections and diarrheal diseases in
215 human newborns and captive animals (e.g., *Clostridioides difficile*, *Helicobacter macacae*,
216 *Clostridium butyricum*, *C. perfringens* [87–91]) (**Table S7**). It also included 3 major groups of
217 mucin-degrading bacteria (*Akkermansia*, [*Ruminococcus*] *gnavus* group and [*Ruminococcus*]
218 *torques*) (**Figure 3C**) that are involved in the development of the intestinal mucosa, a primary line
219 of immune defense [92]. These taxa reflect the importance of the developing immune system at
220 this stage in life. In line with this interpretation, the early life microbial metabolic pathways tended
221 to be more involved in processes related to the host immune system (e.g. NOD-like receptor) and
222 nervous system (e.g., glutamatergic synapse pathway) (**Figure 4A, Tables S5**).

223

224 **Clusters 2 & 3: The weaning transition is accompanied by important gut microbial**
225 **rearrangements**

226 Around 10 months of age, a small number of microbial taxa (**Figures 2A and S4, Tables S4**) and
227 metabolic pathways (**Figures S5-S6, Tables S5**) peak (cluster 2) or decrease (cluster 3) in
228 abundance. Of these changes, the most notable included peaks in Lactobacillaceae (genus
229 *Lactobacillus*), Prevotellaceae, and Lachnospiraceae (cluster 2, **Figure 2C**). While
230 Lactobacillaceae is a keystone lactic acid bacterial group producing large amounts of lactate from

231 milk sugars, Prevotellaceae and Lachnospiraceae (**Figure 2C**) are fiber-degrading genera. These
232 transient shifts highlight the role of the gut microbiome in digesting both milk and plant items at
233 this age.

234 Taxonomic changes at ten months translated at the functional level into a remodeling of
235 the metabolism of amino acids, with an increase in microbial genes involved in alanine, aspartate,
236 glutamate, cysteine, and methionine metabolism (cluster 2), and a decrease in microbial genes
237 involved in phenylalanine (found in breast milk), glutathione (antioxidant typically enriched in the
238 first weeks of life in humans), and tyrosine metabolism (cluster 3) (**Figure 4B, Tables S5**).
239 Microbial genes involved in sporulation and germination were also more highly expressed (**Figure**
240 **4B, Table S5**), suggesting some changes in persistence strategy from the spore-forming microbes
241 in the gut.

242

243 **Cluster 4: The later-life gut microbiome is adapted to a plant-based diet**

244 Cluster 4 was characterized by 22 families (**Figure 2A, Table S4**) and 63 genera (**Figure S4,**
245 **Table S4**) that increased sharply with age and plateaued in older immatures (from 10 months of
246 age onward), including cellulolytic (Spirochaetaceae, Fibrobacteraceae, *Cellulosilyticum*) and
247 fermentative taxa (Lachnospiraceae, Clostridiales Family XIII, several genera from Prevotellaceae
248 and Ruminococcaceae), as well as RFP12 (**Figure 2D**), which are all commonly found in adult
249 geladas [79]. These taxa are involved in metabolizing complex plant polysaccharides found in
250 graminoid leaves and roots, which comprise the majority of the adult gelada diet.

251 At the functional level (cluster 4, **Figures S5-S6, Tables S5**), the gut of old immatures
252 harbored more bacterial genes involved in energy, amino acid, and lipid metabolism and in the
253 regulation of genetic expression and bacteria growth (nucleotide metabolism, replication and

254 repair, genetic information processing and translation) (**Figure 4C**), a functional profile that is
255 typical of the adult gelada gut [79].

256

257 *Maternal effects on offspring gut microbiome composition and function*

258 We next examined whether inter-individual variability in gut microbiome composition early in life
259 (**Figure 1D,G**) could be explained, in part, by maternal traits, including maternal dominance rank
260 and parity. We ran these analyses using (i) all samples (0-3 years, N=525), but since we predicted
261 that maternal effects on the offspring microbiome would be strongest in early life (when infants
262 are still nursing), we also ran separate analyses only focusing on (ii) young infants (<12 months of
263 age, still relying largely on milk, N=184) and (iii) old immatures (>18 months, relying largely on
264 plants, N=259). Note that we ran separate analyses for each age group because it is not possible to
265 fit an interaction between a smooth term (i.e., age) and covariates (i.e., maternal attributes) in
266 GAMMs.

267 Maternal dominance rank did not influence the alpha or beta diversity (**Tables S1-S2,**
268 **Figure S9**) of immature gut microbiomes, nor did it predict differences in microbial families,
269 genera, or functional pathways (**Tables S8-S10** for (ii) young infants, results not shown for (i) all
270 immatures or for (iii) old immatures). Maternal parity also did not exert a significant influence on
271 the diversity, composition, or relative abundance of taxa in the immature gut microbiome (**Tables**
272 **S1, S2, S8**). However, parity was significantly associated with the relative abundance of several
273 microbial metabolic pathways (**Table S9**) and enzymes (**Table S10**) during the first 12 months of
274 life (results non-significant for (i) all immatures or (iii) old immatures). Namely, infants born to
275 primiparous females had functional profiles more typical of early life (<12 months) and related to
276 milk digestion, both at the metabolic and enzymatic levels. Their gut microbes were more involved

277 in carbohydrate metabolism (e.g., galactose, fructose and mannose metabolism), cellular processes
278 and signaling, and nervous system function (**Figure 5A**); and they harbored a higher abundance
279 of key enzymes that cleave milk glycans (**Figure S10**). By contrast, young infants (<12 months)
280 born to multiparous females had a more functionally “mature” gut microbiome for their age, with
281 higher abundance of later-life microbial pathways such as amino acid metabolism and nucleotide
282 metabolism (**Figure 5B**). To determine why maternal parity had an effect at the functional level
283 but not at the taxonomic level, we examined the bacterial taxa that showed a statistical trend to be
284 more abundant in young infants (<12 months) born to primiparous females (i.e., with p-values<0.1
285 before FDR correction, **Table S9**). Offspring of primiparous mothers indeed tended to harbor a
286 higher abundance of microbial taxa involved in milk digestion (e.g., Lachnospiraceae,
287 Bacteroidaceae, Clostridiaceae 1) (**Figure S11**, see supplemental results 2), which suggests that
288 individual taxa exert small additive effects that were only detected at the functional level.

289

290 *Mother-to-infant vertical transmission*

291 Previous work on captive primates suggests that the effect of maternal parity on microbial function
292 could be mediated by differences in vertical transmission between multi- and primiparous females,
293 with primiparous females transferring more milk-oriented microbes to their offspring (via the
294 milk) [10]. We tested if we could statistically detect evidence of vertical transmission between
295 mother and offspring using fecal-fecal microbiome comparisons. We used a nonparametric
296 resampling approach to test if mother-offspring pairs of fecal samples were more similar than
297 expected by chance (i.e compared to when we match the immature sample with random adult
298 female samples), as measured by the number of shared ASVs or beta dissimilarity. We predicted
299 that vertical transmission would be strongest in early life (when infants are still nursing), thus we
300 ran analyses using either (i) all samples (0-3 years, N=398 pairs) or focusing on (ii) young infants

301 (<12 months of age, N=136 pairs) and (iii) old immatures (>18 months of age, N=201 pairs)
302 separately. Using all pairs, we found that immatures shared 3.4% more ASVs (observed
303 value=355, random value=343, $P<1.0\times 10^{-3}$) and were 1.8% more similar compositionally
304 (unweighted UniFrac dissimilarity: observed=0.55, random=0.56, $P=1.0\times 10^{-3}$) to their own
305 mother than with random adult females of the population (**Table S11**), potentially indicative of
306 vertical transmission. However, unexpectedly, this signal was weaker and non-significant in the
307 youngest infants (0-12 months: number of shared ASVs: observed=251, random=245, $P=0.09$ and
308 unweighted UniFrac dissimilarity: observed=0.67, random=0.67, $P=0.26$; **Figures 5C and S12**,
309 **Table S11**), and was strongest and significant in older juveniles (>18 months: number of shared
310 ASVs: observed=412, random=398, $P=2.0\times 10^{-3}$ and unweighted UniFrac dissimilarity: observed
311 value=0.49, random value=0.50, $P=1.0\times 10^{-3}$; **Figures 5C and S12, Table S11**). The finding of
312 greater vertical transmission after, rather than before, nursing cessation suggests that these mother-
313 offspring similarities were mostly mediated by non-nursing interactions and that milk vertical
314 transmission may not be adequately captured by comparing infant and maternal fecal microbiomes.

315 Moreover, the ASVs shared between mother-infant pairs in the first 12 months of life were
316 not the same ASVs found abundant in early-life (i.e., ASVs with a negative score on PC1) and
317 therefore not related to nursing (**Figure 5D, Table S12**). For example, *Bacteroides fragilis* is found
318 in 49% of infants <12 months but is only shared in 9% of mother-infant pairs. Instead, the most
319 commonly shared ASVs among mother-infant pairs between 0-12 months tended to be ASVs
320 characterizing later life (i.e., with positive scores on PC1), characteristic of older offspring and of
321 adult females (**Figure 5D, Table S12**). Thus, mother-infant pairs share more bacteria and have
322 more similar gut microbial community than expected by chance, but this shared community

323 belongs to the typical adult microbiome of geladas, and is not specific to microbes functionally
324 beneficial to processing milk during the early developmental period.

325 Since infants of primiparous females possessed more milk-oriented microbes (i.e., far from
326 adult-like microbes), we also found that they shared fewer ASVs ($\beta=-74.5$, $P=0.01$) and were more
327 dissimilar to their mother (unweighted UniFrac: $\beta=0.07$, $P=0.03$) in the first 12 months of life than
328 infants born to multiparous females (**Figure 5E, Table S3**). However, this effect of greater
329 dissimilarity in primiparous-infant dyads disappeared later in life (>18 months of age) when the
330 effect of maternal parity was no longer detected (number of shared ASVs: $\beta=11.0$, $P=0.46$,
331 unweighted UniFrac: $\beta=-3.5 \times 10^{-3}$, $P=0.81$) (**Figure 5E, Table S3**). This result shows that the
332 effect of maternal parity on the offspring gut microbiome in the first 12 months of life is not
333 mediated by stronger vertical transmission of milk-oriented microbes when using fecal-fecal
334 comparisons.

335

336 **DISCUSSION**

337 We provide a detailed description of the compositional assemblage and functional development of
338 the infant gut microbiome in a nonhuman primate during the first three years of life. As expected,
339 age was the strongest structuring factor of the diversity, composition, and function of the gut
340 microbiome. Most microbial taxa had clear age-related trajectories and could be grouped into four
341 main clusters that reflected progressive dietary transitions associated with weaning. In addition,
342 our data show that maternal effects were an important factor modulating offspring gut microbiome
343 both during nursing and after weaning.

344 The broad dynamic of microbial colonization in geladas presents many similarities with
345 previous reports on humans [2,5,8] and other mammals ([10,37,73], but see[74]). We observed a

346 low initial number of microbes and a rapid increase in microbial diversity in the first seven months
347 of life, followed by more gradual changes in microbial composition until weaning (~17 months).
348 The fact that maximal microbial diversity was attained by the time infants reached 7 months, while
349 the microbial community continued to evolve until weaning, suggests that numerous events of
350 lineage extinction and *de novo* colonization continue to take place in the gelada gut until weaning.
351 Similar to humans [5,42,93], it is the cessation of nursing rather than just the introduction of solid
352 foods (which usually starts as early as the first few weeks after birth in geladas) that really drives
353 the maturation of the developing gut microbiome to an adult-like composition. Indeed, weaning
354 marks two important transitions that can have dramatic effects on the maturing gut microbiome.
355 First, as milk is replaced by solid foods, the nutrient sources for host and microbes both change,
356 altering the types of microbes that are likely to flourish. Second, weaning is accompanied by the
357 loss of maternal-origin immunologic factors and milk-derived microbes [94], both of which can
358 further alter the microbiome through processes of selective seeding. Shifts in gut microbiome
359 composition and function closely followed progressive dietary transitions: gut bacteria that
360 facilitate milk glycans and lactose utilization were dominant in the gelada microbiome during early
361 infancy, while cellulolytic and fibrolytic bacteria that metabolize plant complex polysaccharides
362 were dominant later in development as graminoids were progressively introduced in the diet [76].
363 Many of the early life colonizers were similar to those found in humans, such as *Bacteroides*,
364 *Streptococcus*, *Faecalibacterium*, Lachnospiraceae, *Blautia*, *Clostridium*, *Veillonea*, *Escherichia*-
365 *Shigella*, and Pasteurellaceae [48,82,95] which perhaps suggest a set of universal mammalian or
366 primate infant microbial taxa. These early-life microbes work as a metabolic network that relies
367 on cross-feeding between primary degraders (e.g., lactose-degraders such as *Streptococcus*) and
368 secondary fermenters (e.g., lactate-utilizers such as *Veillonea*) to convert milk sugars into energy

369 [96]. The functional enrichment in carbohydrate metabolism and fermentative pathways found in
370 gelada infants is also typically observed in human newborns [2,5,55,97].

371 *Bacteroides*, and in particular *B. fragilis* [61,98], appear to be the primary microbial taxa
372 involved in milk glycan degradation in geladas, as evidenced by their high abundance in early-life
373 and the fact that they encode the enzymatic toolkits necessary to cleave complex milk glycans
374 (e.g., fucosidase, sialidase, beta-galactosidase). These bacterial enzymes are critical for host
375 nutrition, as mammalian hosts are unable to produce them and therefore cannot utilize milk glycans
376 independently of gut bacteria [82]. In humans, this function is largely met by *Bifidobacterium*, a
377 taxa commonly found in high abundance in breastfed humans that also breaks down milk glycans
378 [8,48,99,100]; however this taxon was almost entirely absent in young geladas. In fact, variation
379 in the dominance of *Bifidobacterium* and *Bacteroides* appears the norm at both the species and
380 population level: several studies in mammals [10,37,72,101] and in some human populations
381 [3,85,95,97,102] have noticed the absence of *Bifidobacterium* but abundance of *Bacteroides* in
382 most or at least some nursing infants. *Bifidobacterium* and *Bacteroides* have different glycan-use
383 profiles [61,62,97] linked to species and population differences in milk composition, particularly
384 the structure and the relative abundance of different milk glycans [103–105].

385 The early-life microbiome of geladas was also characterized by a high number of
386 potentially pathogenic bacteria known to cause enteric infection in human newborns and captive
387 animals (*Clostridioides*, *Helicobacter*, *Clostridium*) [87–91] and several bacterial groups involved
388 in the activation of the host immune system such as butyrate-producing (*Blautia*,
389 *Faecalibacterium*, *Butyricoccus*, *Butyricimonas*) and mucin-degrading bacteria (*Akkermansia*,
390 *Ruminococcus gnavus* and *R. torques*). Collectively, this microbial profile suggests that immune
391 function is a priority for gelada infants. Butyrate plays a key role in the maintenance of gut integrity

392 [106,107] and protection against enteric infection [108]. This microbial metabolite is also an
393 important immunoregulator via its action on intestinal macrophages [109,110]. Mucolytic bacteria
394 play an essential role in mucus turnover [111] and contribute to an essential immune barrier
395 protecting the underlying epithelium from luminal pathogens [111] and are thus strongly involved
396 in immunity in early life. *Bacteroides* are also likely involved in regulation of intestinal immunity
397 in early life [112,113]. *Bacteroides fragilis* in particular is directly involved in the maturation of
398 the immune system by directing the production of regulatory T cells and ensuring a balance
399 between Th1 and Th2 immunologic response [114–117]. Functional analyses revealed that the gut
400 microbes were more strongly involved in host immunity during the nursing period, highlighting
401 that microbial colonization plays an important role in priming of the host immune system in
402 geladas.

403 We detected important compositional and functional signatures of microbial rearrangement
404 around 10 months of age (i.e., 5-7 months before nursing cessation). *Bacteroides* decrease
405 substantially in the gelada gut microbiome, while two other taxa, *Lactobacillus* and *Prevotella*,
406 increase in abundance. *Lactobacillus* is a lactic acid bacterium that consumes lactose [118,119].
407 Its rise in abundance around the weaning transition indicates an increase in lactose availability in
408 the colon, likely due to the loss of endogenous lactase of infants in the upper gut [120]. *Prevotella*
409 is a keystone fiber-degrading bacterium typically enriched in individuals with a plant-based diet.
410 In two other mammalian species (vervet monkeys: [10] and northern elephant seals (*Mirounga*
411 *angustirostris*): [72]), *Prevotella* also increased in abundance during the weaning transition. The
412 abundance of *Bacteroides* and *Prevotella* are generally inversely correlated in the gut, due to the
413 trade-off between saccharolytic and proteolytic fermentation [121]. Thus, the growth of *Prevotella*
414 closer to weaning might be related to the decrease in milk degrading bacteria (i.e., *Bacteroides*)

415 and could be a good indicator of the transition from milk to solid food consumption in mammals
416 [10]. These taxonomic changes were also accompanied by important functional changes in the
417 metabolism of amino acids, vitamins, and cofactors, setting up the microbial activity characteristic
418 of the adult gut.

419 Finally, our results highlight that early-life gut microbiome composition and functionality
420 can be influenced by maternal effects, both during the nursing period, but also after weaning.
421 During the first 12 months of life, we found that infants of primiparous mothers harbored more
422 bacteria that were functionally relevant for processing milk sugars, which parallel recent findings
423 in vervet monkeys [10]. The authors in that study hypothesized that infants of primiparous mothers
424 may compensate for poor maternal investment by seeding more milk-oriented microbes that help
425 infants extract more energy from milk [10]. In support of this, *B fragilis* was more abundant in the
426 milk of low-parity vervet females, which resulted in higher abundance of milk-oriented microbes
427 in the infant gut, which in turn promoted faster growth in low-parity infants [10]. In our study,
428 vertical transmission – as assessed by fecal-fecal comparison of maternal and offspring
429 communities – was not identified as the mechanism generating such a parity effect. First, we did
430 not find evidence of vertical transmission in the first 12 months of life (infants and mothers did
431 not share more ASVs than expected by chance during nursing). Second, the microbes that *were*
432 shared by mother-offspring pairs were associated with processing grass rather than early life
433 functions such as processing milk glycans or sugars. Third, infants from primiparous females
434 actually shared fewer microbes with their mother than infants from multiparous females (since the
435 detected shared microbes are later-life microbes). This result suggests that vertical transmission of
436 early colonizers/milk-oriented microbes might be more strongly mediated by the direct transfer of
437 milk microbiota in geladas [10,49,50,54] and, in contrast to reports in humans [42,44,55,56], not

438 easily detected using fecal-fecal comparisons between infants and their mothers. In vervets, for
439 instance, infants aged 2-5 days shared more bacterial strains with their mother's milk than with
440 their mother's gut [10]. This parity effect could nonetheless result from host filtering processes
441 coming from the offspring themselves [56]. Maternal microbiomes might be similar across parity
442 status, but offspring of primiparous females might preferentially seed milk-oriented microbes from
443 milk in response to poorer maternal energetic allocation. In the absence of milk samples, evidence
444 for such mechanisms remains unclear in geladas.

445 Alternatively, the effect of maternal parity could reflect a faster pace of gut microbiome
446 maturation for offspring born to multiparous mothers. The pattern of vertical transmission might
447 be similar between primiparous and multiparous females, but offspring of multiparous females
448 might share more microbes with their mother during the first 12 months of life because they are
449 more mature for age (and because we only capture vertical transmission of grass-processing
450 microbes). This interpretation is supported by the evidence that multiparous mothers wean their
451 offspring about 5 months earlier than primiparous females in geladas (in our studied cohort and in
452 absence of takeover: multiparous=17.1 months, primiparous=21.9 months). The greater similarity
453 between multiparous mothers and their infants is thus more likely to be generated by accelerated
454 gut microbiome development, suggesting that these infants are undergoing the weaning transition
455 at a faster pace than their peers. Infants from multiparous females could be eating solid grass,
456 gaining physical independence, and becoming socially integrated earlier than their peers, all
457 of which could explain greater microbial resemblance to mothers (and other adults). Behavioral
458 and development data, such as infant growth, are needed to investigate this hypothesis of
459 accelerated development and their consequences on offspring phenotype.

460 Somewhat surprisingly, we did find that immature gut microbiomes were more similar to
461 maternal gut microbiomes than expected by chance after weaning regardless of the parity status of
462 the mother. Such an effect has been previously documented in wild red squirrels (*Tamiasciurus*
463 *hudsonicus*) [122] and chimpanzees (*Pan troglodytes*) ([74] but see [123]). Host genetics, or
464 socially transmitted microbes, may facilitate maternal-offspring gut microbiome similarities
465 beyond the early postnatal period [47]. A recent study in yellow baboons (*Papio cynocephalus*)
466 found that the gut microbiome, including both abundant and rare taxa, is highly heritable [124]
467 suggesting that the convergence of the gut microbiota between mother and offspring in geladas
468 could be due shared genes. Alternatively, or additionally, the higher similarity in later life could
469 be generated by high frequency of social contacts between mothers and offspring that extend past
470 weaning. Primate mothers and offspring form preferential social bonds long after weaning,
471 relationships that are characterized by a high degree of proximity, physical contact and grooming,
472 and are likely to represent an enduring source of maternal microbial inoculation for offspring
473 [58,125]. Further work is needed to understand the relative importance of these mechanisms in
474 explaining mother-infant similarity during juvenility.

475

476 ***Conclusion***

477 Our results highlight that early-life gut microbiome composition and function can be influenced
478 by maternal effects, both during nursing as well as after weaning. Maternal parity in particular was
479 associated with the functional maturation of the microbiome in offspring, likely reflecting faster
480 developmental pace of infants born to reproductively experienced mothers. As infants age and are
481 weaned, they converge toward an adult-like gut microbiome that is more similar to the maternal
482 gut microbiome than expected by chance. The long-term consequences of such microbially-

483 mediated maternal effects remain unknown but could potentially influence phenotypic outcomes
484 such as growth and immune function. Our work also emphasizes that early life vertical
485 transmission, mediated in large part by milk transfer, may not be detected using fecal-fecal
486 comparisons of maternal and infant communities and would ideally require data on the milk
487 microbiome whenever possible.

488

489 **MATERIAL & METHODS**

490 *Study population and study site*

491 The data for this study were collected between Jan 2015 and Jan 2019 from a population of wild
492 geladas living in the Simien Mountains National Park in northern Ethiopia (13°15'N, 38°00'E).
493 Geladas live in multi-level societies, where several reproductive units (comprising a leader male,
494 several adult females, their offspring, and occasionally 1–2 follower males) aggregate together
495 during the day to forage and sleep together forming a “band”, sharing a homerange [80]. Since Jan
496 2006, the Simien Mountains Gelada Research Project (SMGRP) has collected behavioral,
497 demographic, and genetic data on a near-daily basis from over 600 individuals living in 2 separate
498 bands of the area. All gelada subjects were habituated to human observers on foot and were
499 individually recognizable. Data were derived from 89 infants and juveniles aged between 0-3 years
500 old and 83 adult females living in 23 different reproductive units. The date of birth of each infant
501 was known within a few days' accuracy. The reproductive state of each adult female was
502 monitored daily and recorded as cycling (as indicated by the presence of sex skin swellings on the
503 neck, chest, and perineum), lactating (if she had a nursing infant), or pregnant (the date of
504 conception was inferred by removing 183 days from the date of birth of subsequent offspring)
505 [81]. Records of female reproductive history were used to assign maternal parity status for each

506 infant (first-time mother: primiparous or multi-time mother: multiparous) and to establish the date
507 at which the mother resumed cycling following the infant's birth, which we used to estimate the
508 approximate age at weaning for each infant. For 8 infants, age at weaning began on the date of
509 maternal death.

510

511 *Fecal sample collection*

512 Fecal samples (N=525; 303 females, 222 male samples) from 89 immature geladas (i.e., infants
513 and juveniles sampled pre-reproductive maturity; female: N=51; male: N=38,
514 mean±SD=5.90±5.53 samples per individual, range=1-18) were collected opportunistically from
515 2015-2016, and then regularly from 2017 to 2018) during the development using targeted protocols
516 (**Figure S1**). These samples come from individuals residing in 17 different reproductive units
517 (mean±SD= 5.65±4.44 number of individuals sampled per unit, range=1-17). For a subset of
518 immature samples (N=398 samples from 61 infants), we also collected a matched fecal sample
519 from the mother (N=398 samples from 44 mothers) on the same day or on the following day of the
520 infant sample collection. Fecal samples of known adult females in all reproductive states were also
521 routinely collected (N=222 samples from 79 females) and were used to generate a random
522 distribution of gut microbiome composition similarity between females of the population and
523 immatures. Immediately upon defecation, approximately 1.5 g of feces was collected in 3 ml of
524 RNA later [126] stored at room temperature for up to 2 months, and subsequently shipped to the
525 University of Washington (UW). At UW, samples were stored at -80°C until the sequencing
526 libraries were prepared.

527

528 *Maternal dominance ranks*

529 Female dominance ranks were established using *ad libitum* and focal observations of agonistic
530 interactions between all adult females belonging to the same unit with an Elo-rating procedure
531 [127] implemented in the R package EloRating [128]. Agonistic interactions included physical
532 aggression (hit, bite), chase, threats (vocal threats, non-vocal gestures), approach-avoid
533 interactions (displacements) and submissive behaviors (fear bark, crouch, grimace). In geladas,
534 agonistic interactions usually consist of a sequence of several behaviors in a row emitted and
535 received by both parties. Since it can be difficult to establish the winner of each agonistic sequence,
536 we consider each behavior of a sequence as a separate event and assign the winner and loser based
537 on the directionality of the behavior. We obtained a daily Elo-score that we then averaged per
538 month. Since Elo-scores can be sensitive to differences in sampling effort, we then converted this
539 monthly Elo-rank into a monthly proportional rank and controlling for female group size
540 (0=lowest-ranking females and 1= highest ranking female). In the analyses, we used maternal
541 dominance rank during the month of the infant's birth since we expect microbially-mediated
542 maternal effects to be the strongest in the postnatal period (during nursing). However, we also
543 investigated maternal rank during pregnancy and at the date of immature sample collection, which
544 led to similar results (not reported here).

545

546 **Environmental data**

547 The study area is located at 3200 m above sea level and is characterized as an Afroalpine grassland
548 ecosystem, consisting of grassland plateaus, scrublands, and Ericaceous forests [129]. The climate
549 in the Simien Mountains National Park exhibits marked inter- and intra-annual fluctuation in
550 rainfall and temperature and can be broadly divided into 3 distinct seasons : a cold-dry season (Oct
551 to Jan), a hot-dry season (Feb to May) and a cold-wet season (Jun to Sep) [130]. Fecal samples of

552 immatures and adult females were collected across the year, with roughly equal coverage across
553 seasons (406 in cold-dry, 426 in cold-wet and 313 in hot-dry season). Daily cumulative rainfall
554 and minimum and maximum temperature are recorded on a near-daily basis by the SMGRP.
555 Geladas are graminivorous, with up to 90% of their diet composed of graminoids [76]. They eat
556 primarily graminoid leaves (i.e., grasses and sedges) all year long, but increase substantially their
557 consumption of underground storage organs (rhizome, corms, roots) in the dry season, as above-
558 ground graminoid leaves become less abundant [76]. A previous study established that the gut
559 microbiome composition of adults shifts in response to environmental variation, in particular with
560 cumulative rainfall which is a good proxy of diet. [79]. Thus, in all models we controlled the total
561 cumulative rainfall over the 30 days prior to the date of fecal sample collection (as a proxy for
562 grass availability) and the average minimum daily temperatures in the 30 days preceding the date
563 of sample collection (as a proxy of thermoregulatory constraints).

564

565 ***16S rRNA gene sequencing***

566 We performed 16S rRNA gene amplicon sequencing on the immature and female fecal samples to
567 establish gut microbial composition. We first extracted microbial DNA using Qiagen's
568 PowerLyzer PowerSoil DNA Isolation kit (Qiagen #12855) following standard protocols. We then
569 amplified the hypervariable V4 region of the 16S rRNA gene using PCR primer set 515F and 806R
570 from The Human Microbiome Project and a dual-indexing approach [131]. Details of the
571 amplification protocol can be found in [79] (see also: <https://smack-lab.com/protocols/>). The
572 libraries were then pooled in roughly equimolar amounts (each with their own unique indexing
573 primer combination), spiked with 10% PhiX to increase library complexity, and sequenced

574 together on a single Illumina NovaSeq 6000 SP 250 bp paired-end sequence flowcell at the
575 Northwest Genomics Sequencing Core at the University of Washington, Seattle.

576 Data were processed using the Quantitative Insights Into Microbial Ecology 2 (QIIME2)
577 platform [132] using the demux command to demultiplex raw reads and the DADA2 pipeline [133]
578 to generate amplicon sequence variants (ASVs) feature tables. Forward and reverse reads were
579 trimmed to 220 and 180 bases, respectively, to remove the low-quality portion of the sequences.
580 Only samples with more than 20,000 reads were retained for analyses following observation of
581 rarefaction curves. After filtering, trimming, merging, and chimera removal, we retained a total of
582 219,125,888 reads across the 525 immature fecal samples ($417,382 \pm 645,328$ reads per sample,
583 range= 21,256- 7,976,983) and 293,003,271 reads across the 620 adult female fecal samples
584 ($472,586 \pm 869,181$ reads per sample, range= 20,068- 10,723,460). ASVs were taxonomically
585 assigned using the q2-feature classifier in QIIME2 against version 132 of the SILVA database
586 (updated December 2017) [134] based on 100% similarity.

587

588 *Statistical analyses*

589 The count and taxonomy files generated by QIIME2 were imported into R version 3.5.2 [135]
590 using the qiime2R package [136]. We filtered the count table to retain only ASVs that had at least
591 500 reads total in the dataset to eliminate potentially artifactual sequences. With this filtering
592 criteria, only 3,884 ASVs remained (out of the 29,686 initially observed). In total, 3,784 different
593 ASVs were found across the 525 immature fecal samples (mean \pm SD number of ASVs per sample:
594 728 ± 261 , range: 65-1498), while the 620 female samples contained 3,679 ASVs (mean \pm SD
595 number of ASVs per sample: 829 ± 248 , range: 98-1761). Most ASVs could be taxonomically

596 assigned to the phylum (100%), class (99%), and order levels (99%), with assignments decreasing
597 substantially at the family (88%) and genus (63%) levels.

598

599 Alpha-diversity analyses

600 We calculated three complementary metrics of alpha diversity for each sample: the observed
601 richness (the total number of unique ASVs per sample), Shannon Index (taking into account both
602 richness and evenness in abundance of ASVs), and Faith's phylogenetic diversity (a measure of
603 the diversity of phylogenetic lineages within a sample) using the "phyloseq" [137] and "picante"
604 package [138]. To assess which predictors affected immatures' gut microbial alpha diversity, we
605 used generalized additive mixed models (GAMMs) with the 'mgcv' package in R [139]. Such
606 models allow fitting of a nonlinear relationship between the response variable and the fixed effect
607 (by adding a smooth term), such as between alpha diversity and immature age (**Figure 1C**). Fitted
608 predictors included: immature age at the date of fecal sample collection (modeled as a smooth
609 term), immature sex, the parity status of mother, maternal dominance rank in the month of infant's
610 birth, cumulative monthly rainfall, average monthly minimum temperature and the log-
611 transformed sequencing depth (i.e., the number of reads per sample). The use of rarefaction (i.e.,
612 subsampling of the read counts in each sample to a common sequencing depth) has been strongly
613 discouraged on microbiome dataset because it discards too much sequencing information and leads
614 to high rate of false positives [140], so we calculated alpha diversity on raw counts but controlled
615 for sequencing depth in our model. Graphical representation of alpha diversity metrics are
616 nonetheless displayed using a rarefied dataset at 20,000 reads. Individual identity and unit
617 membership were included as random effects. Model residual checks were performed using the
618 qq.gamViz and check.gamViz functions. Given that GAMMs models can not accommodate the

619 test of the interaction between a smooth and fixed term, we ran those models including all
620 immature samples or on only young infants (0-12 months) to test for the significance of maternal
621 effects in early life (i.e., when the infant is still nursing).

622 To quantitatively assess the age at which alpha diversity reaches a plateau (i.e., converges
623 to adult-like pattern), we used quadratic plateau models (formula: $y \sim (a + b * x + c * I(x^2)) * (x$
624 $\leq -0.5 * b/c) + (a + I(-b^2/(4 * c))) * (x > -0.5 * b/c)$) fitted using the `nlsfit()` function of the
625 `easynls` package [141] and extracted the critical point of inflexion and r-squared of the optimized
626 model (i.e., with the values of a, b and c fit best the data). Since it is not possible to control for
627 covariates in those analyses (e.g., sequencing depth), we ran those models on a rarefied dataset at
628 20,000 reads.

629

630 Beta-diversity analyses

631 Beta-diversity (between-sample dissimilarity in composition) was computed as the Aitchison
632 distance [142], which is simply the Euclidean distance between samples after centered log-ratio
633 (clr) transformation of the raw counts (a pseudo-count was added to the zeros using the imputation
634 based on a Bayesian-multiplicative replacement from the `cmultRepl()` function in the package
635 `zCompositions` [143]). The clr transformation allows us to account for differences in sequencing
636 depth between samples and is a better practice than rarefaction of the counts [144]. Principal
637 components analysis (PCA) on the Aitchison dissimilarity matrix (function “`prcomp`”) was used
638 to examine how immatures samples clustered by age. We extracted the loading scores for each
639 ASV onto the first Principal component (PC1) of the PCA to determine which specific ASVs have
640 the highest influence on the clustering by age of samples. A quadratic plateau model was
641 implemented to find the age at which Aitchison beta diversity reaches a plateau.

642 Permutational Multivariate Analysis of Variance (PERMANOVA) was then carried out on
643 the Aitchison dissimilarity matrix using the `adonis2` function in the `vegan` package [145] (with
644 10,000 permutations) to test for associations among gut microbial beta-diversity and the variables
645 of interest (immature age, sex, maternal parity, maternal rank, environmental variables, the log-
646 transformed sequencing depth, and unit membership). Individual identity was included as a
647 blocking factor (“strata”) to control for repeated sampling among individuals. PERMANOVA
648 models were run when including all immatures samples or on only young infants (0-12 months) to
649 test for the significance of maternal effects in early life. We also replicated those PERMANOVA
650 analyses using more classical measures of beta diversity (unweighted and weighted UniFrac
651 dissimilarity) on a rarefied dataset at 20,000 reads and found essentially similar results (**Table**
652 **S13**).

653

654 Mother-infant comparison of gut microbiome composition

655 To assess similarity in gut microbiome composition between mother and offspring, we calculated
656 (1) the number of shared ASVs across maternal and immature communities, and (2) the beta
657 diversity dissimilarity (unweighted and weighted UniFrac distances) between the matched infant-
658 mother fecal samples collected the same day (N=398). The dataset of immature and mother fecal
659 samples was rarefied at 20,000 reads to calculate these metrics since sequencing depth is likely to
660 affect the similarity between paired samples. Quadratic plateau models were implemented on the
661 three metrics to identify the age at which infants converged toward the maternal (i.e., adult-like)
662 gut microbial composition. To assess which predictors affected the compositional similarity
663 between mother-offspring pairs, we used GAMMs to model those three metrics as a function of
664 immature age (as a smooth term), immature sex, maternal parity and maternal dominance rank,

665 climatic variables (cumulative monthly rainfall and average monthly minimum temperature),
666 while individual identity and unit membership were included as random effects. These GAMMs
667 were also run separately on young infant samples (<12 months) or only on old immatures (>18
668 months) to assess how the strength of vertical transmission varies with maternal traits.

669

670 Individuality of the microbiomes in immatures

671 To capture the compositional divergence between immature samples, we calculated a measure of
672 “individuality” of the microbiomes among the 525 immature samples, as defined in [146], which
673 corresponds to the beta diversity dissimilarity value between a sample and the most similar sample
674 (i.e., the minimum pairwise values from a beta diversity dissimilarity matrix, based on unweighted
675 and weighted UniFrac metrics). The higher the value, the more distinct the gut microbiome
676 composition is from all other immature samples in the cohort. This was calculated using the
677 rarefied dataset at 20,000 reads.

678

679 Age-associated changes in microbial taxonomic composition

680 To identify the microbial taxa that vary significantly in abundance as immatures age, we used a
681 statistical framework that is commonly used to analyze time series (and, in our case, longitudinal
682 dataset). Autoregressive Integrated Moving Average (ARIMA) models allowed us to model and
683 test for chronological trends in temporal data [147]. First, raw microbial counts were aggregated
684 at the family or genus level, normalized using a clr-transformation, and z-transformed per taxon
685 (i.e., across samples) to correct for variation in library size and unaccounted variance due to other
686 covariates. Only microbial families or genera $\geq 0.01\%$ relative abundance across the samples were
687 selected for further analyses. Second, the counts were averaged across samples belonging to the

688 same chronological age and converted into z-ordered objects (using R package zoo [148]) and into
689 time series objects. Formatted time series were then analyzed using auto.arima (from the forecast
690 R package [149]), using stepwise search and Akaike Information Criterion (AIC) to select the best
691 model. This algorithm scans a wide range of possible ARIMA models and selects the one with the
692 smallest AIC. ARIMA models that exhibited significant non-stationary trends (as opposed to
693 unstructured “noise” fluctuations indistinguishable from stationary data) were selected following
694 the criteria in [147]: (1) the difference order from stationary was higher than zero, and (2) at least
695 one autoregressive (AR) and moving average (MA) coefficient was included in the model. LOESS
696 regressions were then fitted to re-predict the count of each taxon as a function of age.

697 We then grouped bacterial taxa into clusters based on similarities in age-associated
698 abundance trajectories. Pairwise distances between microbial taxa trajectories (i.e., the predicted
699 values of the LOESS regression) were computed using correlation coefficients as a distance
700 measure [150], and hierarchical clustering was performed using the complete method (using the
701 function hclust from the stats R package). The optimal number of clusters was determined using
702 the Elbow method (i.e., choosing a number of clusters so that adding another cluster does not
703 highly improve the total within-cluster sum of squares) [151]. Results of hierarchical clustering
704 were visualized using the R package heatmap3 [152] to provide an overview of gut microbiome
705 composition changes with age.

706

707 Age-associated changes in microbial functional composition

708 To predict the microbial functional metagenomes of each sample from 16S rRNA data, we used
709 Phylogenetic Investigation of Communities by Reconstruction of Unobserved States 2
710 (PICRUSt2) v.2.1.3-b software [84] with default options (picrust2_pipeline.py). We then

711 computed the relative abundance of Kyoto Encyclopedia of Genes and Genomes (KEGG)
712 Orthologs (KOs) (agglomerated at level 2 or 3 of the BRITE map) and of Enzyme Commission
713 (EC) numbers for each sample. The accuracy of the PICRUSt2 predictions for each sample were
714 assessed by calculating the weighted Nearest Sequence Taxon Index (NSTI) score, a measure of
715 how similar the bacteria from the sample are to reference genome sequences. The mean \pm SD NSTI
716 score across the 525 immature samples was 0.49 ± 0.19 (range: 0.01-0.89).

717 The age-related temporal trajectory of each KO pathway and EC was assessed using
718 ARIMA models in a similar fashion than described above. The only difference is that the raw
719 metagenome counts were transformed into relative abundance (instead of clr transformed). Only
720 microbial pathways $\geq 0.01\%$ relative abundance across the samples were included. Hierarchical
721 clustering was used to group the pathways with similar aging trajectories.

722

723 Maternal effects on offspring's gut microbiome development

724 We examined how maternal traits (dominance rank, parity) were associated with differences in
725 offspring gut microbiome (1) composition (at the family and genus levels) and (2) function (KO
726 pathways at level 2 or 3 and EC numbers) using GAMMs models. We modelled the relative
727 abundance of each taxon and each functional pathway as a function of maternal parity and maternal
728 dominance rank in the month of infant's birth, while controlling for immature age (as a smooth
729 term), immature sex, climatic variables (cumulative monthly rainfall and average monthly
730 minimum temperature. For (1), the logarithm of the relative abundance of each taxon was fit
731 (adding a pseudo-count of 0.001% to include zero counts). In all models, individual identity and
732 unit membership were included as random effects. P-values were adjusted for multiple hypothesis
733 testing by calculating the Benjamini-Hochberg FDR multiple-test correction. Only taxa that had

734 an average relative abundance across samples $\geq 0.01\%$ were tested. Given the number of metabolic
735 pathways and the correction of p-values for multiple testings, only pathways that had an average
736 relative abundance across samples $\geq 0.10\%$ were tested. Taxa or functional pathways with a p-
737 value < 0.05 were considered statistically significant. These analyses were run including all
738 immatures samples (0-3 years), only young infant samples (<12 months) or only old samples (>18
739 months).

740

741 Mother-to-infant vertical transmission

742 To assess if maternal and infant gut microbiome communities were more similar than expected by
743 chance, we took a resampling approach (with 1000 repetitions) to compare the number of shared
744 ASVs and beta diversity dissimilarity metrics (unweighted and weighted UniFrac) between (1)
745 actual mother-infant matched samples (the observed value) and (2) random pairs of fecal samples
746 of an infant and an adult female of the population (the random distribution). Since mother-infant
747 pairs always shared the same social unit and were always collected 0-1 day apart (i.e., in the same
748 season), we needed to match the random female samples accordingly to avoid introducing
749 consistent bias in the random distribution. The random matching was thus done by either matching
750 the infant sample to (i) a female of the same unit (to control for higher similarity only due to
751 sharing the same social group) or (ii) a female sample collected in the same season (to control for
752 higher similarity only due to seasonality). We did not have enough female samples to match by
753 both criteria simultaneously. After we created the set of random pairs, we use GAMMs to compare
754 the observed and random distribution of the metrics (number of shared ASVs or beta diversity
755 dissimilarity) (response variable) by fitting a variable (“type of pairs?”) coding whether the value
756 comes from an actual mother-offspring pair (1=observed) or a random infant-female pair

757 (0=random), and controlling for immature age (as a smooth term) and immature sex. Infant and
758 female identity were included as random effects to account for repeated observations of the same
759 individuals. We extracted the estimate of the “type of pairs?” variable for the model and re-ran the
760 model on a different set of random pairs (1000 times in total). We thus obtained a distribution of
761 1000 estimates for the “type of pairs?” variable. We report the exact p-value (calculated as the
762 proportion of models with positive estimates for the number of shared ASVs and the proportion of
763 models with negative estimates for beta dissimilarity) and the 95% confidence interval of the
764 estimates of the “type of pairs?” variable. Fecal samples were rarefied at 20,000 reads to control
765 for differences in sequence depth between infant and female samples. These analyses were run
766 including all immatures samples (0-3 years), only young infant samples (0-12 months), or only old
767 immatures (>18 months) to compare the strength of the effect among the different age categories.

768 To examine the nature of the shared microbes between mother and offspring in early life
769 (when infants are <12 months), we extracted all ASVs in common between the 136 mother-
770 offspring pairs (on the rarefied dataset). For each ASV found in the young infant samples (<12
771 months, N=3,402 ASVs total), we simply computed its relative abundance and prevalence across
772 samples and how many pairs shared this given ASV. We then plotted the loading score of the ASV
773 on PC1 of the beta diversity ordination (PC1 correlates strongly with age, so ASVs with the most
774 negative versus positive loading scores are found in early versus later life) according to the
775 percentage of mother-offspring pairs sharing this ASV.

776

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787 **AUTHORS' CONTRIBUTIONS**

788 Conceptualization, Data curation, Formal analysis, Investigation, Visualization: A.B, A.L., N.S.M;
789 Methodology/Laboratory work: A.B., S.S., A.M., Writing – Original Draft: A.B, A.L., N.S.M;
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799

800 **AVAILABILITY OF DATA AND MATERIALS**

801 All 16S sequence data used in this study are available at the NCBI Sequence Read Archive under
802 BioProject ID: PRJNA772269 (<http://www.ncbi.nlm.nih.gov/bioproject/772269>) for the immature

803 samples [temporary note: the sequences coming from these samples have been deposited on
804 NCBI, but will only be released upon acceptance of the manuscript] and PRJNA639843
805 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA639843>) for the adult female samples. Data
806 (including the ASV table and metadata) and R code to reproduce all the analyses are available at:
807 https://github.com/GeladaResearchProject/Baniel-et-al_2022_Infant-gut-microbiome.

808

809 **COMPETING INTERESTS**

810 The authors declare no competing interests.

811

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Figure Legends

Figure 1. Gut microbiome taxonomic assembly in the first three years of life in immature geladas.

(A, B) Taxonomic composition of the immature gelada gut microbiome at the phylum and family level as a function of age. The relative abundance of each taxon was calculated per sample by dividing the counts of the taxa by sequencing depth, and then averaged across samples belonging to the age category of interest. Age was split into categories for visualization purposes, but analyses treated age as a continuous variable. (C) Age-associated pattern of alpha diversity within samples, as calculated by the Shannon index (richness and evenness of Amplicon Sequencing Variants, ASVs). The vertical line represents the critical point of inflexion (calculated using quadratic plateau models) representing the age at which alpha diversity converges to adult-like patterns. The dataset was rarefied at 20,000 reads for the figure. (D,E) Age-associated pattern of beta diversity. A Principal Component Analysis (PCA) was used to ordinate the samples based on the Aitchison dissimilarity index (which is simply the Euclidean distance after centered-log-ratio transformation of the raw counts). Panel D represents the projection of the first principal component (PC1) that is best explained by the age of immatures. The vertical line represents the critical point of inflexion (calculated using quadratic plateau models) representing the age at which beta diversity converges to adult-like patterns. Panel E shows how age structures the gut microbiome composition of immatures on the two first principal components. (F) Comparison of gut microbiome composition between mother and offspring, as assessed using 398 matched mother-infant pairs of fecal samples collected on the same day. Here, the number of shared ASVs between pairs of samples is represented. The vertical line represents the critical point of inflexion (calculated using quadratic

1226 plateau models) representing the age at which the number of shared ASVs stabilizes to its maximal
1227 value. The dataset was rarefied at 20,000 reads for the calculation. (G) Age distribution inter-
1228 individual variability in gut microbiomes using the ASV-level unweighted and weighted UniFrac
1229 distances. This score was calculated as the minimum pairwise dissimilarity value from a beta
1230 diversity matrix for a given immature sample, and captures how dissimilar a sample is from its
1231 nearest neighbor, given all other gut microbiome samples in the immature cohort. Higher values
1232 indicate a more distinct gut microbiome from the population. The dataset was rarefied at 20,000
1233 reads for the calculation.

1234

1235 **Figure 2. Age-associated changes in microbial composition at the family level.**

1236 (A) Heatmap of the microbial families exhibiting a significant chronological trend as a function of
1237 age (fitted values from ARIMA models and predicted using LOESS regression per taxa as a
1238 function of age). Values represent z-score normalized counts after centered-log-ratio (clr)
1239 transformation. Hierarchical clustering was used to group age-dependent trajectories into four
1240 clusters exhibiting similar chronological trends. Color bars on the left side identify the clusters.
1241 Taxa (i.e. rows) are ordinated on the heatmap using correlation as distance function. All microbial
1242 families above 0.01% abundance were analyzed (N=55) and the 53 that displayed a significant
1243 trend are represented. (B) Relative abundance of 8 functionally important microbial families that
1244 are enriched in early life (belonging to cluster 1), as a function of age. The age-dependent
1245 trajectories were calculated on clr-transformed counts, but here for interpretation purposes we
1246 represent the LOESS regression on the raw relative abundance across samples (same for panels C
1247 and D). (C) Relative abundance of 5 functionally important microbial families that peak in
1248 abundance around 10 months of age (belonging to cluster 2). Relative abundance is represented

1249 on a log-scale to accommodate high and low abundance families together. (D) Relative abundance
1250 of 8 functionally important microbial families that are enriched in later life (belonging to cluster
1251 4). Relative abundance is represented on a log-scale to accommodate high and low abundance
1252 families together.

1253

1254 **Figure 3. Composition of the early-life microbiota at the genus level.**

1255 Relative abundance of functionally important genera in early life, as a function of age. The age-
1256 dependent trajectories were calculated on clr-transformed counts. For visualization purposes
1257 however, we represent the LOESS regression on the raw relative abundance across samples (on a
1258 log-transformed scale).

1259

1260 **Figure 4. Age-associated changes in the functional profile of the gut microbiome of**
1261 **immatures based on predicted KEGG orthologs (KO) metagenomes.**

1262 (A) Relative abundance of metabolic pathways (left: KO level 2 and right: KO level 3 of the
1263 carbohydrate metabolism) enriched in early life, as a function of age. (B) Relative abundance of
1264 metabolic pathways (KO level 3) that peak in abundance or decrease in abundance at 10 months
1265 of age. (C) Relative abundance of metabolic pathways (at KO level 2) enriched in later life, as a
1266 function of age. In all plots, the average curve is the LOESS regression on the raw relative
1267 abundance across samples.

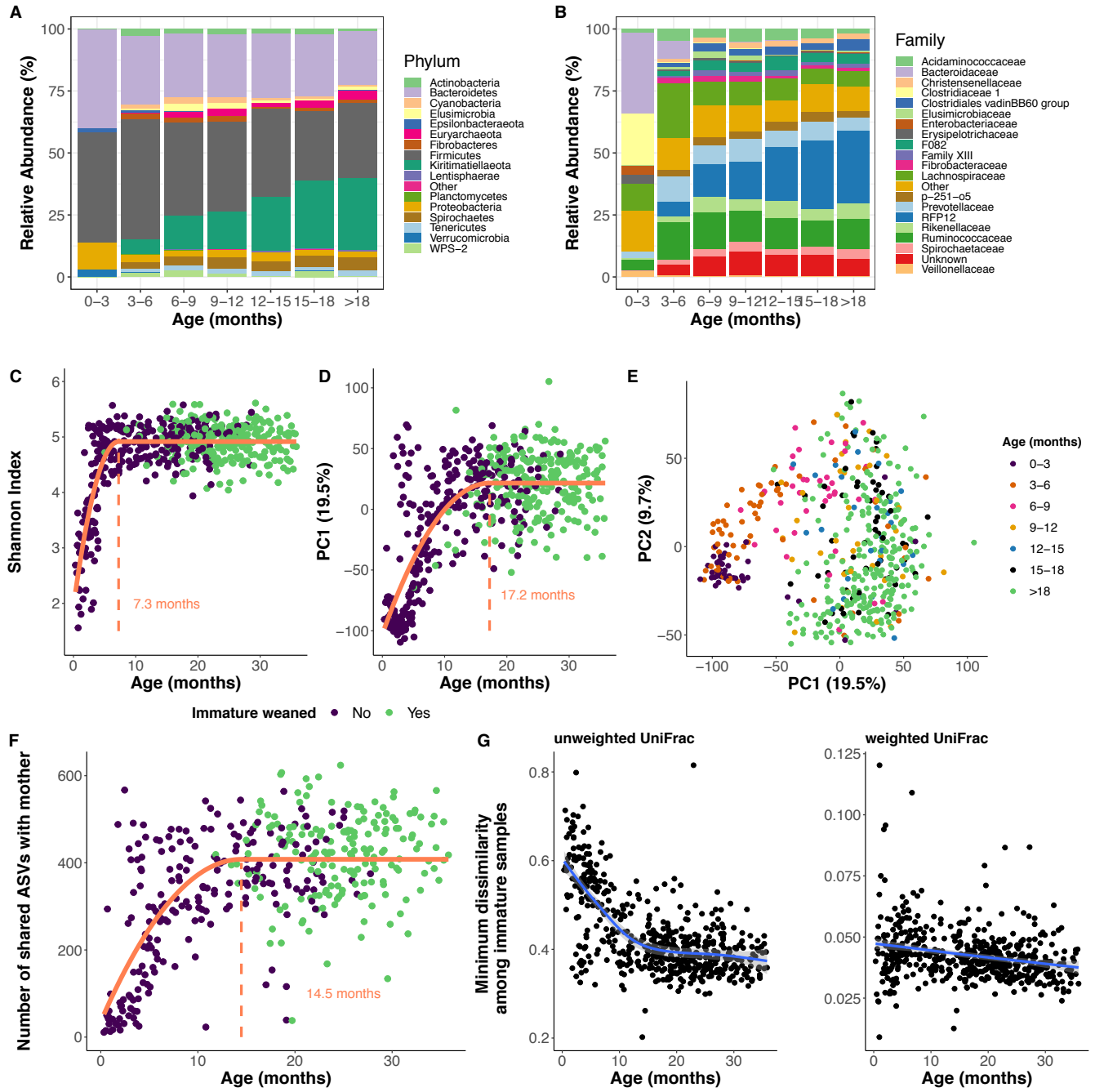
1268

1269 **Figure 5. The effect of maternal parity on offspring's gut microbiota functional capacity.**

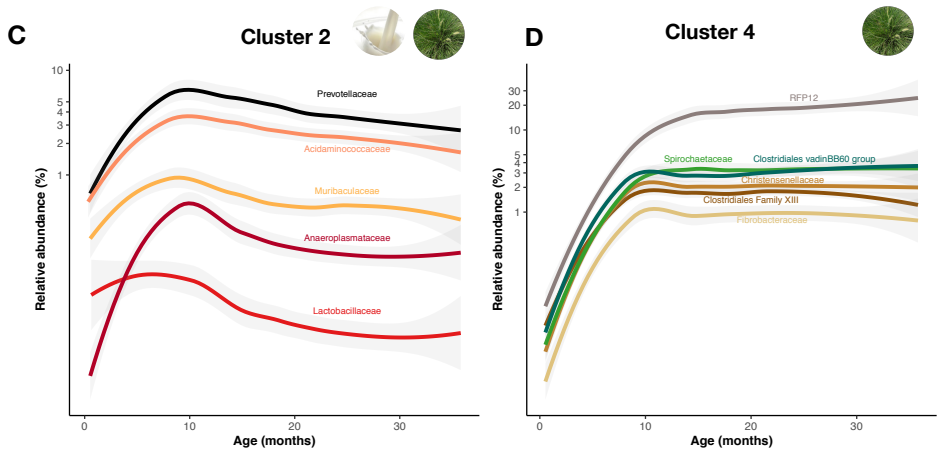
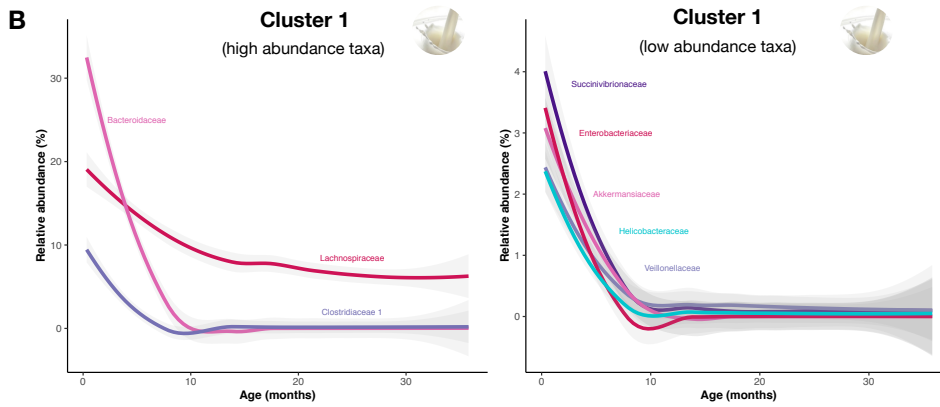
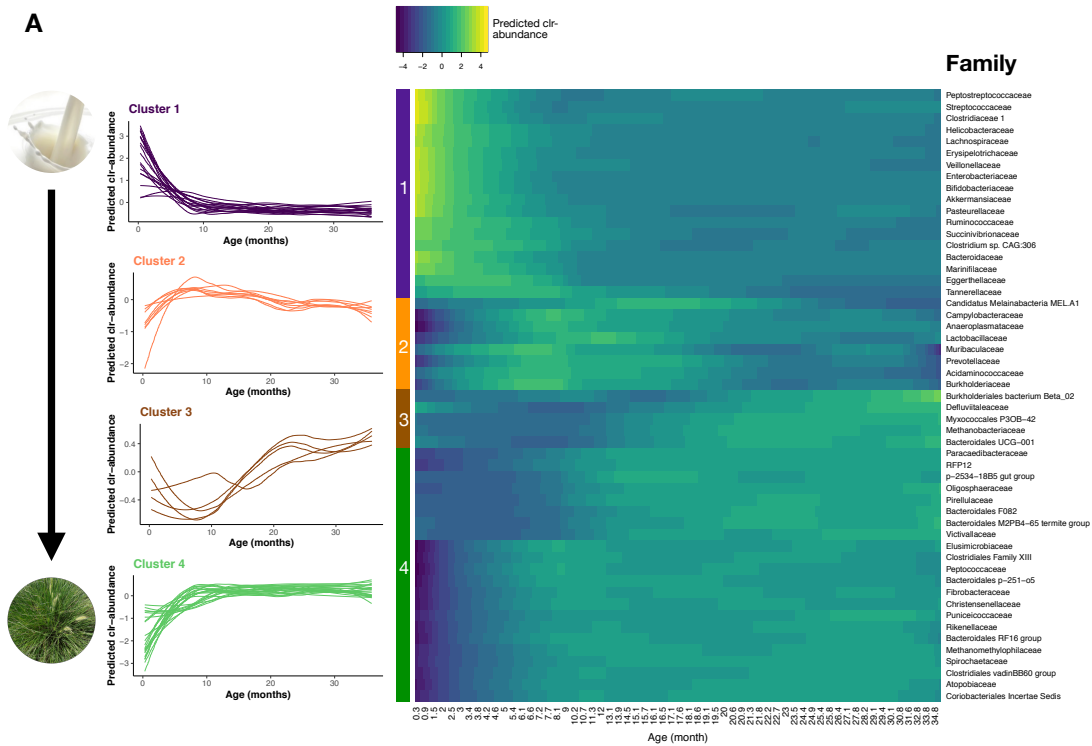
1270 (A) Metabolic pathways (KO level 2 for upper row and KO level 3 for lower row) that are more
1271 abundant in infants <12 months) born to primiparous females than infants of multiparous females.

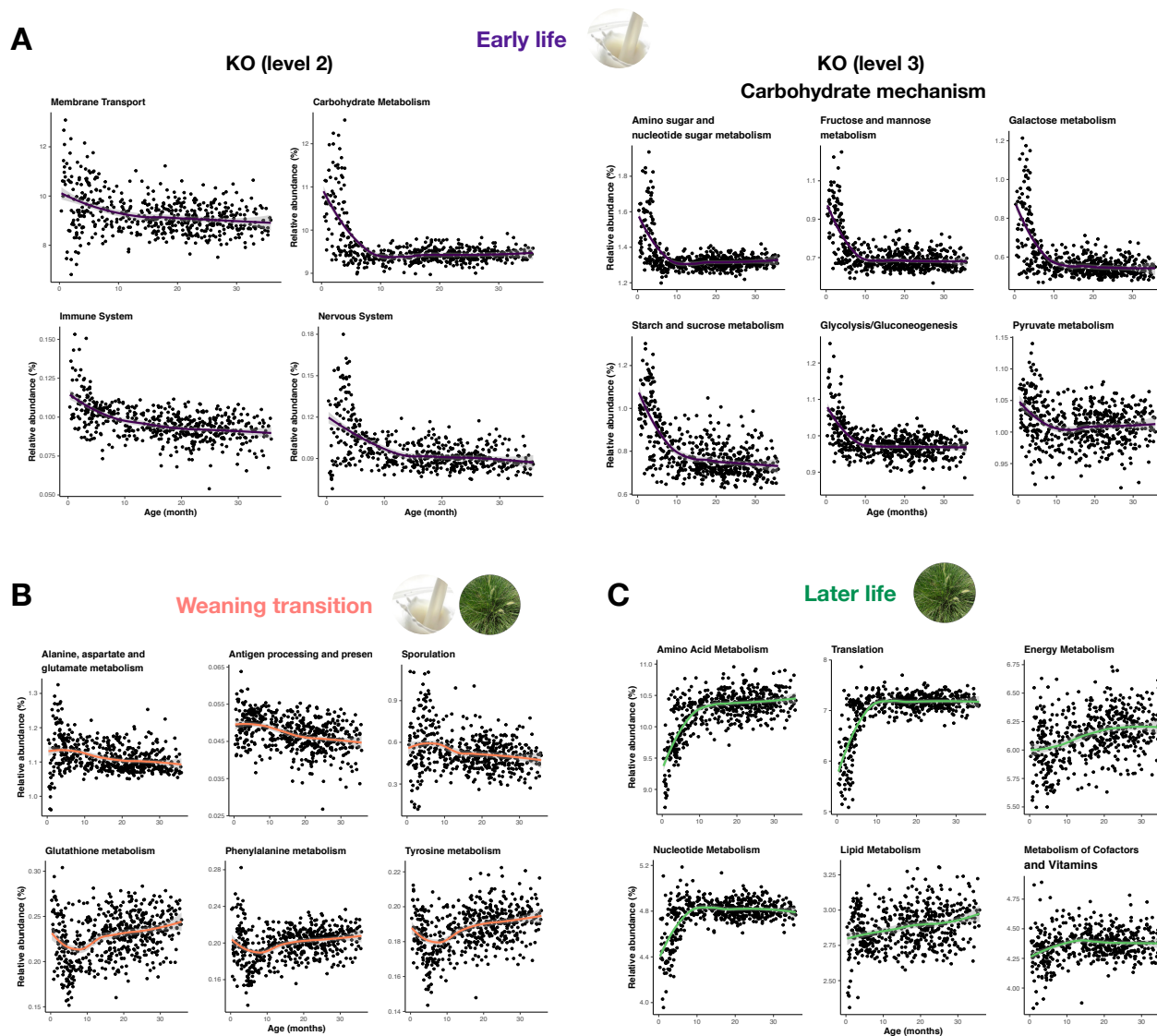
1272 (B) Metabolic pathways (KO level 2) that are less abundant in infants (<12 months) born to
1273 primiparous females than infants of multiparous females. (C) Results of the nonparametric
1274 resampling approach testing if offspring share more Amplicon Sequencing Variants (ASVs) and
1275 have a more similar gut microbiome composition (unweighted UniFrac dissimilarity) to their
1276 mother than to random adult females of the population. The histograms show the random
1277 distribution of the metric of interest (i.e. when matching each infant sample to a random female
1278 sample collected during the same season, with 1000 repetitions). The vertical line shows the
1279 observed value of the metric (i.e. between the actual mother-offspring pairs of fecal samples
1280 collected the same day). This analysis was performed separately on young (nursing) infants (aged
1281 0-12 months, N=136 samples) and old immatures (>18 months, N=201) that were likely weaned.
1282 (D) Composition of the shared ASVs between young infants (<12 months) and their mothers. The
1283 ASVs that are commonly shared between mother-offspring pairs (e.g. among > 70% of the pairs)
1284 in early life tend to have high loading scores of the first principal component (PC1) of a Principal
1285 Component Analysis ordination (based on Aitchison distance). Since PC1 strongly correlates
1286 positively with age, these shared ASVs are characteristic of later life. (E) Vertical transmission
1287 differs for primiparous and multiparous females in early life. Young infants (<12 months) born to
1288 primiparous females share fewer ASVs with their mother and have a more dissimilar gut
1289 microbiome composition (unweighted UniFrac dissimilarity) compared to their mother than
1290 offspring born to multiparous females. Later in life (>18 months), immatures born to primiparous
1291 and multiparous females are equally similar to their mother in terms of gut microbiome
1292 composition.

1293 **Figure 1.**



1295 **Figure 2.**





1301 **Figure 5.**

