Parity modulates impact of BMI and Gestational Weight Gain on gut microbiota in human pregnancy

Katherine M. Kennedy[1-5], Andreas Plagemann[6], Julia Sommer[6], Marie Hofmann[6], Wolfgang Henrich[6], Jon F.R. Barrett[2], Michael G. Surette[1,3,7], Stephanie Atkinson[1,8], Thorsten Braun[6]* and Deborah M. Sloboda*[1,2,3,7]

[1]Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Canada

[2]Department of Obstetrics and Gynecology, McMaster University, Hamilton, Canada

[3]Farncombe Family Digestive Health Research Institute, McMaster University, Hamilton, Canada

[4]McMaster Immunology Research Centre, McMaster University, Hamilton, Canada

[5]M.G. DeGroote Institute for Infectious Disease Research, McMaster University, Hamilton, Canada

[6]Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Department of Obstetrics and Department of 'Experimental Obstetrics', Augustenburger Platz 1, 13353 Berlin, Germany

[7]Department of Medicine, McMaster University, Hamilton, Canada.

[8]Department of Pediatrics, McMaster University, Hamilton, Canada.

*shared senior authorship

Corresponding Authors

D. M. Sloboda
McMaster University
Department of Biochemistry and Biomedical Sciences
1280 Main St West, Hamilton, Canada
Tel: +1-905-525-9140
Fax: +1-905-522-9033
Email: sloboda@mcmaster.ca

T. Braun
Charité – Universitätsmedizin Berlin Departments of Obstetrics and Experimental Obstetrics
Augustenburger Platz 1, 13353 Berlin
Germany
Tel: +49-30-664309
Fax: +49-30-564901
Email: thorsten.braun@charite.de

Abstract

Pregnancy requires maternal adaptations to support fetal growth: whether these adaptations include temporal shifts in the gut microbiome is still unclear. We investigated the maternal gut microbiome longitudinally over the course of pregnancy and the impact of pre-pregnancy BMI (pBMI) and GWG. We also determined whether parity modulated observed associations. We show that the gut microbiota of participants with higher pBMI changed less over the course of pregnancy in primiparous, but not multiparous participants. This suggests that previous pregnancies may have persistent impacts on maternal adaptations to pregnancy. This ecological memory appears to be passed to the next generation, as parity modulated the impact of maternal GWG on the infant gut microbiome. This work supports a role for the gut microbiome in maternal adaptations to pregnancy and highlights the need for longitudinal sampling and accounting for parity as key considerations for studies of the microbiome in pregnancy and infants.

Background

Pregnancy requires physiological, metabolic, and immune adaptations to support fetal growth and development[1]. Pregnancy-induced metabolic adaptations mirror those seen in non-pregnant metabolic syndrome; including hyperglycemia, hyperinsulinemia, and increased adiposity. When combined with high pre-pregnancy body mass index (pBMI) or excess gestational weight gain (GWG) these adaptations can often reach unhealthy levels. Indeed, high pBMI is a known risk factor for maternal and infant health complications[2]. Although some data suggest that pregnancy-associated metabolic adaptations may influence[3] and/or be influenced by[4] shifts in the gut microbiota over the course of pregnancy, most studies have investigated only one (sometimes two) timepoints during gestation[4–7]. An evaluation of whether gut microbiota change temporally across pregnancy, birth and the postnatal period is required to robustly assess whether host-microbe interactions could govern pregnancy adaptations. Of note, we currently lack even a foundational understanding of whether the maternal microbiome does indeed change over the course of pregnancy.

One of the first studies of the gut microbiome during pregnancy reported an increase in diversity between samples (beta-diversity) and a decrease in diversity within samples (alpha-diversity) in healthy pregnant individuals during the third trimester of pregnancy compared to the first[4]. In contrast, others have found the maternal gut microbiota to be stable over the course of pregnancy[8–10]. Few studies have investigated

63 microbial shifts in the context of pregnancy complications like high pBMI or excess GWG[11–13]. Although
64 excess GWG is more likely to occur in cases where maternal pBMI is high, 50% of all pregnant
65 individuals experience excess GWG. Thus, these two factors both independently and together influence
66 maternal and infant health. To date, no human studies have investigated whether pregnancy-associated
67 microbiotal shifts are influenced by previous pregnancies (parity).

68 Both high maternal pBMI and excess GWG are associated with pregnancy complications[2] and impaired
69 offspring metabolism[14,15]. Reports suggest that some of these maternal and infant associations may be
70 mediated by obesity-associated "dysbiosis" of the maternal gut microbiome during pregnancy[7,11]. As
71 humans are not colonized by microbes before birth[16], the maternal microbiome may influence offspring
72 development via changes to the *in utero* environment (e.g. altered maternal metabolic adaptations to
73 pregnancy) and/or altered seeding of the infant microbiome during and after birth. In this prospective,
74 observational cohort study we set out to determine whether temporal changes in maternal gut microbial
75 composition occur with advancing gestation and the postpartum period. We also investigated whether
76 maternal BMI and/or excess GWG and parity impact maternal microbiotal adaptations and how these
77 contexts influence the maternal and infant microbiomes at 6 months of age.

78 Results

79 Study cohort

80 A total of 89 participants were recruited; after we excluded participants who reported any nicotine or
81 antibiotic use during pregnancy, 65 participants were included in the study (primiparous n=40;
82 multiparous n=25). The majority of participants with a pre-pregnancy BMI (pBMI) of 25-30 or >30 had
83 gestational weight gain (GWG) above the Institute of Medicine (IOM) recommended range[17] (83% and
84 86% respectively), compared to less than half of participants with a pBMI 18.5-25 (33%; Extended Data
85 Table 1). Most participants delivered vaginally (79%), and mode of delivery was similar across pBMI
86 categories (q=0.8). Multiparous participants had either one (88%) or two previous pregnancies (12%).
87 Multiparous participants were older than primiparous participants (32.8±3.1 vs 29.6±4.4, q=0.009), but
88 there was no significant effect of parity or maternal age on pBMI, GWG, post-partum weight retention,
89 length of gestation, or delivery mode.

Parity modulates the impact of pBMI and GWG on maternal gut microbiota

Maternal stool samples were collected longitudinally: in the first (10-17 weeks gestation), second (26-28 weeks gestation), and third trimesters (>36 weeks gestation) of pregnancy, at delivery, and at 6 months postpartum. We assessed the maternal gut microbiota using 16S ribosomal RNA gene sequencing of the combined V3-V4 region. We identified minimal sporadic contamination in negative controls, likely originating from samples. Of 13 sequenced gDNA extraction negative controls, 4 had no reads and an additional 4 had fewer than 20 reads. The 5 remaining negative controls had 45, 55, 80, 130, and 384 reads: 65 of 67 ASVs were detected in a single negative control (*Escherichia/Shigella* and *Bacteroides* were each detected in 2 negative controls). These negative control data show no consistent contamination signal. We identified a total of 7577 ASVs in maternal samples after removal of host-associated reads (Kingdom Eukaryota, Order Chloroplast, Family Mitochondria, or no assigned Phylum) and the median sample read count was 62,853 (min = 14,872; max = 136,000).

Alpha diversity was positively correlated with pBMI (R^2 = 0.11, p=0.010), particularly in participants with GWG above the recommended range overall (R^2 = 0.12, p=0.0069) and at each pregnancy time point (R^2 = 0.14-0.21, p=0.012-0.046; Extended Data Fig. 1), but not at 6 months post-partum (R^2=0.014, p=0.57). To assess differences in overall community composition between samples (beta-diversity) we performed principal coordinate analysis (PCoA) of Bray-Curtis dissimilarity (Fig. 1a, b). Beta-diversity was modestly impacted by GWG category (R^2 = 2.6%, p=0.0001; Fig. 1a), pBMI category (R^2 = 2.8%, p=0.0001; Fig. 1b), parity (R^2 = 1.4%, p=0.0001; Extended Data Fig. 2a), and sample time point (R^2 = 1.0%, p=0.0001; Extended Data Fig. 2b). These modest effects were overshadowed by the impact of interindividual variability, which explained the majority of variation between samples (PERMANOVA; R^2=62.3%, p=0.0001). Longitudinal sampling within individuals is necessary to disentangle these large effects of inter-individual variability[18] from factors impacting pregnancy-associated shifts in the microbiota (intra-individual variability). We assessed beta-diversity longitudinally across time points (Bray-Curtis dissimilarity) *within* individuals and found that the impact of pBMI differed by parity. Beta-diversity was negatively correlated with pBMI at every time point in primiparous (R^2 = 0.041-0.19, p < 0.0001-0.029) but not multiparous participants (R^2 < 0.0001-0.016, p = 0.34-0.99; Fig. 1c). These data suggest that in primiparous (first) pregnancy, the gut microbiota is more stable in individuals with a high pBMI over the course of pregnancy and postpartum. Together these observations illustrate that when considering temporal changes in microbial composition related to pregnancy, longitudinal sampling within individuals is essential in uncovering patterns of change that exist with advancing gestation and persist postpartum. These data also highlight a novel observation; that gut microbial diversity during

122 pregnancy and postpartum is influenced by previous pregnancies. Parity therefore is a key confounder
123 that must be considered in all future studies investigating the maternal microbiome.

124 Parity modulates impact of pBMI and GWG on fecal SCFA levels

125 Because of their functional role in metabolic signalling pathways[19], we assessed fecal SCFA level
126 associations with pBMI and GWG across all timepoints in a subset of maternal fecal samples
127 (primiparous, n=12; multiparous, n=11). This subset included samples that had enough starting material
128 for SCFA quantification and was not biased in terms of pBMI, GWG, parity, maternal age, gestational
129 length, or mode of delivery compared to participants not included in SCFA analyses. To account for
130 variability in recovery between samples, the relative concentration of each SCFA was assessed as a
131 percentage of total SCFAs (Fig. 2). Due to limited sample size, we assessed fecal SCFA levels by both
132 GWG and pBMI—and the interaction of each of these factors with parity—across all samples.

133 Fecal SCFA levels differed by GWG, and this effect was dependent upon parity as differences occurred
134 only in primiparous participants (Supplementary Tables 1 and 2). Fecal acetate was decreased by GWG
135 above the recommended range (main effect of GWG-by-parity interaction p=0.036) in primiparous
136 participants (p=0.0041), and this decrease was accompanied by an increase in propionate (p=0.027, main
137 effect of interaction p=0.028). Overall, lactate was decreased in participants with GWG above the
138 recommended range (p=0.0042), particularly in primiparous participants (p=0.0047). Together, these data
139 suggest that differences in non-digestible carbohydrate fermentation processes that produce SCFAs, and
140 by inference the biochemical pathways between them, appear to be driven by gestational weight gain in
141 primiparous women.

142 In contrast, differences in branched SCFA (BCFA) levels were driven by pBMI. Overall, isobutyrate was
143 decreased by pBMI >25 (main effect of pBMI p=0.0040 vs pBMI <25), particularly in primiparous
144 participants (p=0.013). Previous studies have shown BCFAs contribute to intestinal barrier function[20] and
145 facilitate the storage of diet-derived lipids in adipocytes by inhibiting lipolysis and increasing glucose
146 uptake[21]. Thus, decreased BCFAs may contribute to a decrease in appropriate maternal metabolic
147 adaptive flexibility leading to increased risk of pregnancy complications in participants with a pBMI >25
148 and excess GWG, though this hypothesis remains to be tested.

149 ## Maternal taxonomic associations with pBMI, GWG, and parity

150 Taxa that were differentially abundant during pregnancy between pBMI and GWG categories were
151 identified using DESeq2 (RRID: SCR_015687). Overall, 14 genera with a mean abundance > 150
152 differed by pBMI category (Fig. 3a-c, Supplementary Table 3) compared to participants with a pBMI <
153 25. Participants with a pBMI 25-30 had 6 differentially abundant genera, whereas participants with a
154 pBMI > 30 had 11 genera that were differentially abundant (3 genera were differentially abundant for
155 pBMI of both 25-30 and > 30). The relative abundance of *Blautia* was decreased in participants with a
156 pBMI 25-30 or > 30 (Fig. 3a-c). This was accompanied by decreased *Bacteroides* and *Anaerostipes* in
157 participants with pBMI >30. In contrast, *Prevotella 9* was markedly increased in participants with pBMI
158 > 25 or > 30. In primiparous participants a pBMI of 25-30 or >30 was associated with increased
159 *Rikenellaceae RC9 gut group*, which has been linked to higher propionate levels in high-fat fed mice[22],
160 consistent with the increase we observed in fecal propionate in participants with pBMI >25 and/or excess
161 GWG.

162 The relative abundances of 13 genera with a mean abundance > 150 differed by GWG category (Fig. 4 a-
163 c, Supplementary Table 3): compared to participants with GWG within the recommended range,
164 participants with excess GWG (above) had 11 genera and participants with insufficient GWG (below) had
165 2 genera that were differentially abundant overall. *Prevotella 9* was markedly decreased by excess GWG,
166 while insufficient GWG (below) was associated with decreased *Lachnospiraceae NK4A136 group*, the
167 abundance of which has previously been associated with gut barrier function in mice[23]. Excess GWG was
168 also associated with decreased relative abundances of 6 genera of the SCFA-producing family
169 Ruminococcaceae (Fig. 4c), including uncultured genus (UCG) 005 which has previously been reported
170 to decrease with gestational diabetes mellitus[24]. In primiparous participants excess GWG (above) was
171 associated with a decreased in *Coprococcus 1*, which prior studies have found to be inversely associated
172 with circulating triglyceride levels in non-pregnant individuals[25]. In multiparous participants, excess
173 GWG was associated with increased *Bifidobacterium*. Overall, *Bifidobacterium* was also increased in
174 multiparous participants compared to primiparous participants (Supplementary Table 3).

175 ## Maternal GWG and the infant gut microbiota at 6 months of age

176 Of the 63 total mother-infant dyads who provided an infant fecal sample at 6 months of age, we excluded
177 3 for nicotine exposure during pregnancy, 1 for pre-term birth, and 1 for low sequencing read count,
178 which left 58 infants for full analysis. The majority of infants delivered by C-section were exposed to

179 perinatal antibiotics (12/14; 86%;) while relatively few vaginally delivered infants were exposed (9/44;
180 20%). As perinatal antibiotic exposure has previously been shown to disturb vertical (maternal-to-infant)
181 transmission of the microbiota during birth[26], we assessed the impact of perinatal antibiotics (between 33
182 weeks gestation and birth, including antibiotics given during delivery) on the infant gut microbiota in this
183 cohort. There was no significant impact of antibiotics on alpha diversity (Observed p=0.70, Shannon
184 p=0.39; Extended Data Fig. 3a). Infant gut microbiota beta-diversity differed by antibiotic exposure in
185 those born to primiparous participants (PERMANOVA, R^2=0.068, p=0.019), but not in those born to
186 multiparous participants (R^2=0.043, p=0.56; Extended Data Fig. 3b). This may reflect the a rescue effect
187 due to horizontal transmission from older siblings[27]. Overall, perinatal antibiotic exposure was associated
188 with a decreased abundance of 8 genera, including *Bifidobacterium*, *Streptococcus*, *Blautia*, and
189 *Parabacteroides* (Extended Data Fig. 4 a-c). Three additional genera (*Lachnoclostridium*,
190 *Flavonifractor*, *Intestinibacter*) were significantly decreased by antibiotic exposure only in primiparous
191 participants. *Akkermansia* and an unidentified genus of the family Lachnospiraceae were the only genera
192 that increased with antibiotic exposure, although only in primiparous participants. In contrast, both these
193 genera were decreased by antibiotic exposure in multiparous participants. These data are consistent with
194 others that show that perinatal antibiotic exposure significantly perturbs the infant gut microbiota[26] but
195 extend these observations to show that parity influences these effects.

196 In infants that were not exposed to antibiotics (female, n=19; male, n=18) the majority were delivered
197 vaginally (35/37; 95%). Infant characteristics at birth did not differ by maternal pBMI category overall or
198 within primiparous and multiparous participants (Extended Data Table 2). Although infant birth weight
199 did not differ by GWG overall, excess GWG was associated with higher birth weight in multiparous
200 participants (p=0.028; Extended Data Table 2); although this difference was not significant after adjusting
201 for multiple testing. Overall, at 6 months of age, infant weight did not differ significantly by maternal
202 pBMI, GWG, or parity. Among infants with available feeding data, at 6 months of age the majority were
203 both breastfed (27/30; 90%) and eating solid foods (23/28; 82%).

204 We evaluated the impacts of maternal pBMI and GWG on the gut microbiota of infants at 6 months of
205 age. We identified a total of 1063 ASVs after removal of host-associated reads (Kingdom Eukaryota,
206 Order Chloroplast, Family Mitochondria, or no assigned Phylum). The median sample read count was
207 59,561 (minimum 6,045; maximum 124,303). Overall, infant microbiota alpha diversity (number of
208 observed ASVs) differed by maternal pBMI (p = 0.036) and GWG category (p = 0.047; Fig. 5a) but did
209 not differ by infant sex (p=0.78). There was a significant effect of maternal GWG on beta-diversity in
210 infants of multiparous (R^2=0.378, p=0.0009), but not primiparous, mothers (R^2=0.0950, p=0.377; Fig.
211 5b). Infant beta-diversity did not differ by maternal pBMI category (R^2=0.0511, p=0.565) or infant sex

212 (R^2 = 0.0126, p=0.914). Therefore, in addition to previous reports linking maternal GWG and BMI to
213 shifts in the infant gut microbiota[13,28–33], we now show that that parity modulates the impact of maternal
214 GWG on the infant gut microbiota.

215 Taxa that were differentially abundant in infants between maternal pBMI and GWG categories were
216 identified using DESeq2. Overall, maternal excess GWG (above) was associated with an increased
217 abundance of *Bifidobacterium* (Fig. 6 a-c)—echoing similar shifts in the maternal gut microbiota (Fig. 4
218 a-c). In infants of multiparous, but not primiparous, participants this was accompanied by a decrease in
219 *Bacteroides*. Compared to a maternal pBMI 18.5-25, a maternal pBMI >25 was associated with a
220 decreased relative abundance of Clostridiales genera *Blautia* and *Intestinibacter* in the infant gut
221 microbiota, which were also decreased by pBMI >25 in the maternal gut microbiota. Depleted *Blautia* in
222 obese children has been linked to intestinal inflammation[34] which is consistent with an observed
223 association between excess maternal adiposity with childhood obesity and inflammation[35]. This overlap
224 in differentially abundant taxa between the maternal and infant gut microbiota is consistent with vertical
225 transmission of the maternal gut microbiota[36].

226 Discussion

227 Pregnancy-associated shifts in maternal gut microbiota have been implicated in mediating metabolic
228 adaptations to pregnancy[4], but few studies have investigated the gut microbiota longitudinally over the
229 course of healthy pregnancy. Because of this, there exists contrasting data regarding whether these
230 temporal shifts do[4] or do not[9,37] occur. In this study, we investigated the independent and combined
231 impacts of maternal pre-pregnancy BMI (pBMI) and gestational weight gain (GWG) on the maternal gut
232 microbiota over the course of pregnancy and lactation, and infant gut microbiota at 6 months of age. We
233 show pregnancy-associated remodeling of the gut microbiota is individual-specific and therefore
234 longitudinal sampling within individuals is essential. We found that pBMI and GWG impact the maternal
235 gut microbiome during pregnancy—both the microbial composition and possible function via SCFA
236 metabolites—and also impact the infant gut microbiota at 6 months of age. We also show for the first
237 time in humans that parity modulates the impact of pBMI on pregnancy-associated remodeling of the
238 maternal gut microbiota and also modulates the impact of perinatal antibiotic exposure and GWG on the
239 infant gut microbiota. We now suggest that the maternal gut microbiota may retain a "ecological
240 memory"[38] of previous pregnancies.

241 We found that in primiparous (first) pregnancy, the gut microbiota of participants with higher pBMI
242 changed less over the course of pregnancy and postpartum than that of participants with a lower pBMI.
243 Previous studies of the gut microbiota have found little-to-no overall effect of pBMI on overall beta-
244 diversity in the first trimester[39] and across pregnancy[8,37] but have not investigated beta-diversity within
245 individuals over the course of pregnancy. Shifts in the gut microbiome due to diet or antibiotic
246 perturbation are highly individual-specific[40] and our data now suggest pregnancy-associated remodeling
247 of the gut microbiota is also individual-specific. Our observed decrease in intra-individual beta-diversity
248 with increasing pBMI in primiparous participants is consistent with a decreased magnitude of metabolic
249 adaptation to pregnancy with increasing pBMI[41–43], and highlights the necessity of longitudinal sampling
250 for investigations of pregnancy-associated microbiota remodeling.

251 Our novel observation that this remodeling was not associated with pBMI in multiparous participants
252 suggests that microbiotal adaptations to first pregnancy may have persistent impacts that modulate
253 microbiotal adaptations to subsequent pregnancies. The impact of parity on the gut microbiome and its
254 contribution to metabolic adaptations to pregnancy has not previously been investigated in human
255 pregnancy. Recently, Berry et al.[44] found that maternal microbiotal adaptations to pregnancy occurred
256 more rapidly with increasing parity in pigs. This may reflect increased adaptive plasticity resulting from
257 adaptations to first pregnancy[45]. Indeed, it is known that first-pregnancies are associated with greater
258 maternal constraint[46,47] compared to subsequent pregnancies, which may include reduced or delayed
259 metabolic adaptations to pregnancy.[48] Whether a functional ecological memory exists from one pregnancy
260 to the next in humans is still unclear, but our data support the notion that parity influences microbial
261 community composition and must be considered when investigating human pregnancy-related microbiotal
262 (and intestinal) adaptations.

263 Maternal adaptations to pregnancy include increased intestinal surface area and increased transit time,
264 allowing increased nutrient uptake to provide the energy required by fetal development and lactation[49].
265 SCFAs may support these intestinal adaptations by promoting proliferation of the intestinal epithelium[50]
266 and improved intestinal barrier funciton[20,51]. Although human data on intestinal adaptations to pregnancy
267 are limited, in animal models we have found that excess adiposity during pregnancy is associated with
268 decreased intestinal length and increased intestinal permeability[52]. In this study, we found that parity
269 modulated the impact of pBMI and GWG on the relative abundance of maternal fecal SCFAs. In
270 primiparous participants, GWG above the recommended range was associated with decreased acetate and
271 increased propionate. As fecal SCFAs levels may be more strongly correlated to intestinal absorption than
272 production[53], these changes could be due to altered epithelial cell uptake resulting from reduced intestinal
273 adaptations to pregnancy, but this hypothesis remains to be tested.

274 Alternatively, differences in fecal SCFA levels may result from shifts in microbial production due to
275 altered microbiota community composition. We found increased propionate in primiparous participants
276 with excess GWG and/or pBMI>25, consistent with a prior study that found increased fecal propionate in
277 pregnant individuals with a pBMI>25 compared to pBMI<25, although parity and GWG were not
278 reported[54]. *Prevotella*-dominant microbiota produce more propionate—and more SCFAs overall—than
279 *Bacteroides*-dominant microbiota[55]. This is consistent with our finding that *Prevotella* was increased in
280 participants with a pBMI>25, while *Bacteroides* was decreased, particularly in primiparous participants.
281 A previous study also found decreased *Bacteroides* in the first and third trimester with pre-pregnancy
282 overweight[11], although others have reported increased levels in the third trimester with pre-pregnancy
283 overweight but not obesity[12,56]. In independent studies, *Prevotella* has been associated with improved
284 glucose tolerance[57] and impaired glucose tolerance[58]. This functional diversity mirrors that of *Bacteroides*
285 and suggests the impacts of these genera depend on host physiology including pregnancy history. Future
286 studies are needed to longitudinally investigate pregnancies within participants as well as in the
287 intrapartum period, to test this hypothesis.

288 Shifts in the maternal gut microbiota can impact the infant gut microbiome by (1) altering fetal intestinal
289 development through the *in-utero* environment and (2) direct transmission of maternal microbes to the
290 infant during and after birth. Disruption of this vertical transmission may have persistent effects on the
291 infant gut microbiota, as we found perinatal antibiotic exposure was associated with a decrease in
292 *Bifidobacterium* in the infant gut microbiota at 6 months of age. We show novel data, that supports the
293 position that parity modulates the impact of maternal GWG on infant characteristics at birth and the infant
294 gut microbiota at 6 months of age. Gestational weight gain above the recommended range is often
295 associated with a reduced risk of small for gestational age (SGA) birth weight and an increased risk of
296 large for gestational age (LGA) birth weight[59], although some data suggest that maternal obesity can also
297 be associated with intrauterine growth restriction (IUGR)[60]. Little to no data exist on the modulating
298 effect of parity. We found excess GWG was associated with higher birth weight in infants of multiparous
299 participants, and a tendency towards lower birthweight in infants of primiparous participants. These
300 impacts of excess GWG were also associated with a shift in microbiota beta-diversity at 6 months of age
301 in infants of multiparous—but not primiparous—participants. These same infants also had a decrease in
302 *Bacteroides* and increase in *Bifidobacterium* reflecting similar shifts in the maternal gut microbiota
303 consistent with the high rates of vertical transmission previously found for these genera[61]. Therefore,
304 health behaviours during pregnancy and the postpartum/intrapartum interval should be supported not only
305 for maternal health but also infant microbiotal health.

306 Our study has both strengths and limitations. Our longitudinal sampling of a prospective pregnancy
307 cohort allowed us to explore beta-diversity within participants to overcome the overwhelming effect of
308 inter-individual variation. However, as we recruited participants in the first trimester of pregnancy, we are
309 not able to address pregnancy-associated shifts in the gut microbiota that may occur very early in
310 gestation. We also show potential functional outcomes of observed microbial shifts in our analysis of
311 fecal SCFA levels, but did not quantify circulating SCFA levels, which may more directly modulate host
312 metabolism[62]. We also cannot measure whether production versus absorption on these SCFAs is altered
313 with pregnancy, GWG, BMI or parity. Finally, although our study is unique among studies of the
314 pregnancy microbiome in its design and sample size and relationship to parity, we were unable to
315 examine any interacting effects of pBMI and GWG as the vast majority of participants with high pBMI
316 also had GWG above the recommended range.

317 In conclusion we show that parity modulates impact of pBMI on pregnancy-associated remodeling of the
318 maternal gut microbiota, colonization of the neonatal gut, and the impact of GWG on the infant gut
319 microbiota. We also show that in women in their first pregnancy, high pBMI is associated with
320 diminished remodeling of the gut microbiome during pregnancy, which may reflect a reduced
321 requirement for metabolic adaptations to pregnancy. This is accompanied by functional impacts via
322 altered SCFA levels, which may reflect reduced intestinal adaptations to pregnancy. These outcomes are
323 important not only to maternal health, but also to infant health as infants born to multiparous women with
324 excess gestational weight gain show distinct shifts in their microbiota. Finally, our data suggest the
325 human gut microbiome may retain an "ecological memory"[38] of prior pregnancies[44] which may serve as
326 an adaptation that minimizes maternal constraint in subsequent pregnancies.

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Author Contributions

Conceptualization, K.M.K., S.A., T.B., A.P., and D.M.S.; Investigation, K.M.K, J.S., M.H., and T.B.; Data Curation, K.M.K; Formal Analysis, K.M.K.; Visualization, K.M.K; Writing – Original Draft, K.M.K; Writing – Review & Editing, K.M.K, M.G.S., T.B., and D.M.S; Supervision, J.F.R.B., T.B., A.P., W.H., and D.M.S.; Project Administration, T.B., A.P., W.H., and D.M.S.; Resources, T.B., A.P., W.H., M.G.S., and D.M.S.; Funding Acquisition, T.B. and D.M.S.

Competing Interests statement

The authors declare that they have no competing interests.

Methods

Data and code availability

Sequencing data have been deposited at the NCBI Sequence Read Archive (SRA; PRJNA878704) and are publicly available as of the data of publication. All original code has been deposited on GitHub and is publicly available as of the date of publication. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

Study design

The study protocol was reviewed and approved by the Charité ethics committee (EA 4/059/16). All participants provided written informed consent. Study participants were part of an ongoing research program at the Charité University Berlin. Healthy females >18 years of age with singleton pregnancies were prospectively recruited between 9-17 weeks of gestation. We excluded participants who (1) had severe chronic gastrointestinal diseases or conditions, (2) had any significant heart, kidney, liver, or pancreatic diseases, (3) had pre-existing diabetes, (4) had depression, or (5) delivered infants with severe malformations noted at birth. A total of 89 participants were included in the study. We additionally excluded maternal and infant samples from participants who smoked during pregnancy and excluded maternal samples from participants who took antibiotics during pregnancy. After these exclusions, this study included infant samples from 58 participants, and maternal samples from 65 participants.

Sample collection

Participants were evaluated at the end of the first trimester (9-17 weeks gestation), second trimester (26-28 weeks), and third trimester (>33 weeks) of pregnancy, at delivery, and at 6 months postpartum. Pre-pregnancy body mass index (pBMI) was obtained from measured height recruitment and recalled pre-pregnancy weight. BMI was categorized into normal weight (18.5-24.9kg/m2), overweight (25-29.9 kg/m2), and obese (>30<40 kg/m2). At each study visit, participant weight was measured. Participant collected fecal samples at home prior to each study visit following a standardized sterile stool collection protocol. Stool was frozen at -20°C immediately after collection in provided sample collection bags and stored at -80°C upon arrival until further use. None had taken prebiotics, laxatives, or diarrhea inhibitors in the days before the sampling.

370 ## DNA extraction and amplification

371 Genomic DNA (gDNA) was extracted from fecal samples as described previously[63] on a MagMAX
372 Express semi-automatic robot (MagMAX-96 DNA Multi-Sample Kit; Invitrogen, cat# 4413022) with the
373 addition of a mechanical lysis step using 0.2 g of 2.8 mm ceramic beads to improve extraction efficiency
374 and without mutanolysin. Four negative controls were included on each extraction plate. PCR
375 amplification of the variable 3 and 4 (V3-V4) regions of the 16S rRNA gene was performed on the
376 extracted DNA using methods previously described[64]. Each reaction contained 5 pmol of primer (341F –
377 CCTACGGGNGGCWGCAG, 806R – GGACTACNVGGGTWTC-TAAT), 200 mM of dNTPs, 1.5µl 50
378 mM MgCl2, 2 µl of 10 mg/ml bovine serum albumin (irradiated with a transilluminator to eliminate
379 contaminating DNA) and 0.25µl Taq polymerase (Life Technologies, Canada) for a total reaction volume
380 of 50 µl. 341F and 806R rRNA gene primers were modified to include adapter sequences specific to the
381 Illumina technology and 6-base pair barcodes were used to allow multiplexing of samples. Lack of
382 amplification in all negative controls was confirmed by gel electrophoresis and one negative control from
383 each extraction plate was sequenced.

384 ## 16S rRNA gene sequencing

385 16S DNA products of PCR amplification were sequenced using the Illumina MiSeq platform (2x300bp)
386 at the Farncombe Genomics Facility (McMaster University, Hamilton ON, Canada). Primers were
387 trimmed from FASTQ files using Cutadapt[65] (RRID: SCR_011841) and DADA2[66] was used to derive
388 amplicon sequence variants (ASVs). Taxonomy was assigned using the Silva 132 reference database[67].
389 Non-bacterial ASVs were culled (kingdom Eukaryota, family Mitochondria, order Chloroplast, or no
390 assigned phylum), as was any ASV to which only 1 sequence was assigned.

391 ## Quantification of short-chain fatty acids

392 SCFA levels were measured in a subset of fecal samples by gas-chromatography mass spectroscopy (GC-
393 MS) at the McMaster Regional Centre of Mass Spectrometry. A weight equivalent amount of 3.7% HCl,
394 10 µL of internal standard, and 500 µL of diethyl ether was added to each fecal sample and vortexed for
395 15 min. After vortexing, 400 µL of diethyl ether fecal extract was transferred to a clean 1.5 mL Eppendorf
396 tube. In a chromatographic vial containing an insert, 20 µL of N-tert-butyldimethylsilyl-N-
397 methyltrifluoroacetamide was added, after which 60 µL of diethyl ether fecal extract was added. The
398 mixture was incubated at room temperature for 1 hour and analyzed using gas chromatography-mass

399 spectrometry (6890N GC, coupled to 5873N Mass Selective Detector; Agilent Technologies, Santa Clara,
400 CA, USA). Statistical significance was assessed by mixed linear model with pBMI, GWG category, and
401 parity as fixed effects and participant ID as a random effect, and multiple comparisons by pBMI and
402 GWG category were performed within primiparous and multiparous participants (Kenward-Rogers
403 degrees of freedom with Tukey adjustment for multiple comparisons).

404 Statistical analysis

405 Participant characteristics

406 Participant characteristic variables (Extended Data Tables 1 and 2) were analyzed in R using the
407 gtsummary package (RRID: SCR_021319). Normality was determined by Shapiro-Wilk test (rstatix;
408 RRID: SCR_021240) and significance was assessed by Kruskal-Wallis test and false discovery rate
409 correction for multiple testing.

410 16S analysis

411 We performed alpha and beta-diversity analyses in R using phyloseq[68] (RRID: SCR_013080). Alpha
412 diversity analyses were performed on rarefied data (14872 reads per sample). Significance of alpha
413 diversity was analyzed by linear mixed model (Kenward–Roger degrees of freedom) with time point,
414 pBMI, or GWG category as a fixed effect and participant ID as a random effect. Beta-diversity analyses
415 were performed on proportionally normalized data. We tested for whole community differences across
416 groups using vegan's[69] (RRID: SCR_011950) implementation of permutational multivariate analysis of
417 variance (PERMANOVA) in the adonis2 command blocked by sample time point as strata to account for
418 repeated measures. These results were visualized via Principal Coordinate Analysis (PCoA) ordination
419 using R's ggplot2 package (RRID: SCR_014601)[70]. Significance of Bray-Curtis dissimilarity was
420 analyzed within primiparous and multiparous participants by mixed linear model with pBMI, GWG
421 category, and sample time point as interacting fixed effects and participant ID as a random effect.
422 Differential abundance analysis was performed using DESeq2 (RRID: SCR_015687) across all pregnancy
423 time points. We did not assess interacting effects of pBMI and GWG as (1) pBMI category and GWG
424 category were collinear (χ-squared $p < 0.0001$) and (2) all but 4 participants with a pBMI > 25
425 experienced excess GWG. These 4 participants were excluded and the impact of pBMI was assessed
426 within participants with excess GWG and the impact of GWG was assessed in participants with a pBMI
427 18.5-25.

428 ## SCFA analysis

429 Statistical significance was assessed by mixed linear models with pBMI category and parity or GWG
430 category and parity as fixed effects and participant ID as a random effect, and multiple comparisons by
431 pBMI and GWG category were performed within primiparous and multiparous participants (Kenward-
432 Rogers degrees of freedom with Tukey adjustment for multiple comparisons). All statistical details,
433 including statistical tests used, sample sizes, and definition of center and dispersion can be found in the
434 figure legends.

435

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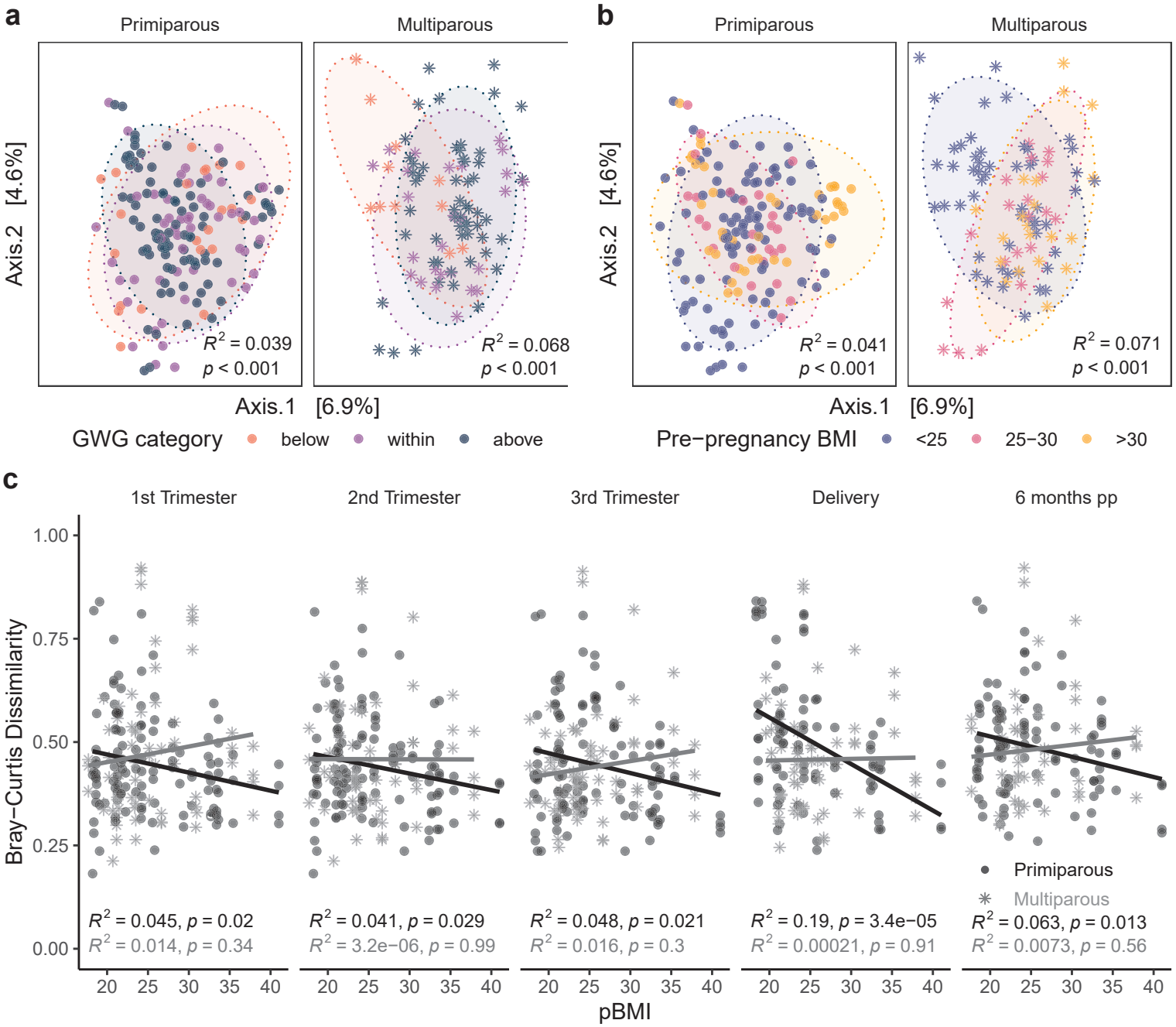


Figure 1. Parity modulates impact of pBMI and GWG on pregnant gut microbiota. PCoA of Bray-Curtis distances in primiparous (n=39, one excluded - no GWG data) and multiparous (n=23, two excluded – no GWG data) participants shows modest clustering by **a**, GWG category (primiparous, R2=0.039, p<0.0001; multiparous R2=0.068, p<0.0001) and **b**, pBMI category (primiparous, R2=0.041, p<0.0001; multiparous R2=0.071, p<0.0001). Significance was assessed by PERMANOVA blocked by sample time point as strata to acco unt for repeated measures. **c**, Bray-Curtis dissimilarity at each timepoint to all other timepoints within individual participants are negatively associated with pBMI in primiparous participants (n=40), but not in multiparous participants (n=25). Significance was analyzed within primiparous and multiparous participants by mixed linear model with pBMI, GWG category, and sample time point as interacting fixed effects and participant ID as a random effect.

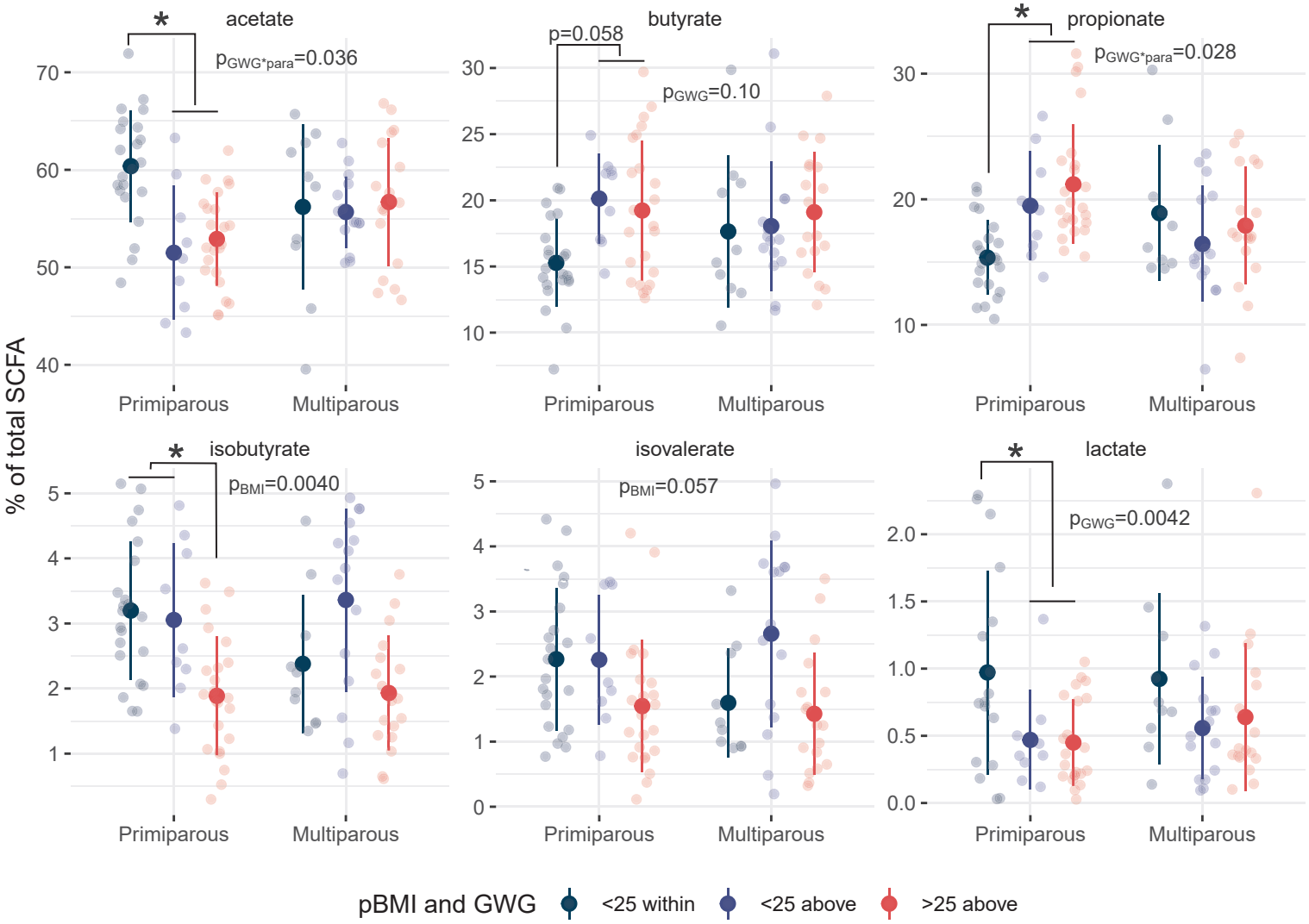


Figure 2. Relative SCFA concentrations differ with pBMI >25 and excess GWG. Dot plots of relative SCFA concentrations as a percentage of total SCFAs show decreased acetate (p=0.014) and lactate (p=0.034) with GWG above the recommended range and decreased isobutyrate with pBMI >25 (p=0.044). Primiparous (<25 within, n=7; <25 above, n=2; >25 above, n=7); Multiparous (<25 within, n=6; <25 above, n=6; >25 above, n=5). Individual points are shown (transparent dots) as well as the mean (solid black dot) and standard deviation (whiskers). Significance was assessed by mixed linear model with pBMI category or GWG category as fixed effects and participant ID as a random effect.

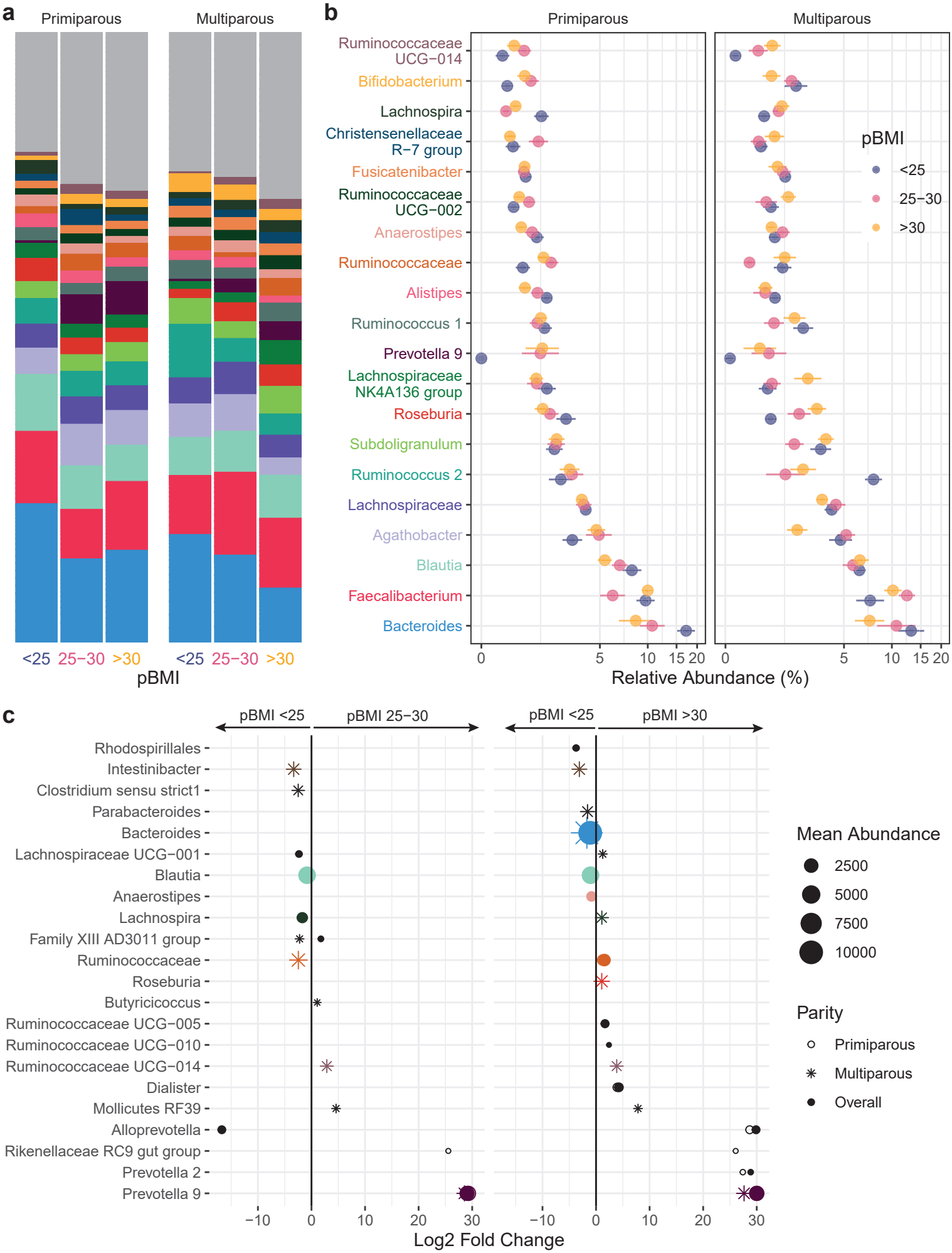

a
Primiparous
Multiparous
<25 25−30 >30
<25 25−30 >30
pBMI
b
Primiparous
Multiparous
Ruminococcaceae UCG−014
Bifidobacterium
Lachnospira
Christensenellaceae R−7 group
Fusicatenibacter
Ruminococcaceae UCG−002
Anaerostipes
Ruminococcaceae
Alistipes
Ruminococcus 1
Prevotella 9
Lachnospiraceae NK4A136 group
Roseburia
Subdoligranulum
Ruminococcus 2
Lachnospiraceae
Agathobacter
Blautia
Faecalibacterium
Bacteroides
pBMI
<25
25−30
>30
0 5 10 15 20
Relative Abundance (%)
c
pBMI <25
pBMI 25−30
pBMI <25
pBMI >30
Rhodospirillales
Intestinibacter
Clostridium sensu strict1
Parabacteroides
Bacteroides
Lachnospiraceae UCG−001
Blautia
Anaerostipes
Lachnospira
Family XIII AD3011 group
Ruminococcaceae
Roseburia
Butyricicoccus
Ruminococcaceae UCG−005
Ruminococcaceae UCG−010
Ruminococcaceae UCG−014
Dialister
Mollicutes RF39
Alloprevotella
Rikenellaceae RC9 gut group
Prevotella 2
Prevotella 9
−10 0 10 20 30
Log2 Fold Change
Mean Abundance
2500
5000
7500
10000
Parity
Primiparous
Multiparous
Overall

Figure 3. Taxonomic shifts with pBMI in pregnant gut microbiota. a-b, The relative abundances of the 20 most abundant genera in the maternal gut microbiota by pBMI <25 (blue, n=12), 25-30 (red, n=10), and >30 (yellow, n=12) are shown as a, mean relative abundance in taxa bar plots and b, mean ± standard error of the mean for primiparous (pBMI <25, n=7; 25-30, n=6; >30, n=7) and multiparous (pBMI <25, n=5; 25-30, n=4; >30, n=5) participant with excess GWG. **c**, Dot plots showing DESeq2 results of differentially abundant genera (mean abundance >150) by pBMI category overall (filled dots) and in primiparous (no fill dots) and multiparous (stars) participants (dot size based on mean abundance, colours correspond to genus colour in a where a differentially abundant genus is among the 20 most abundant genera).

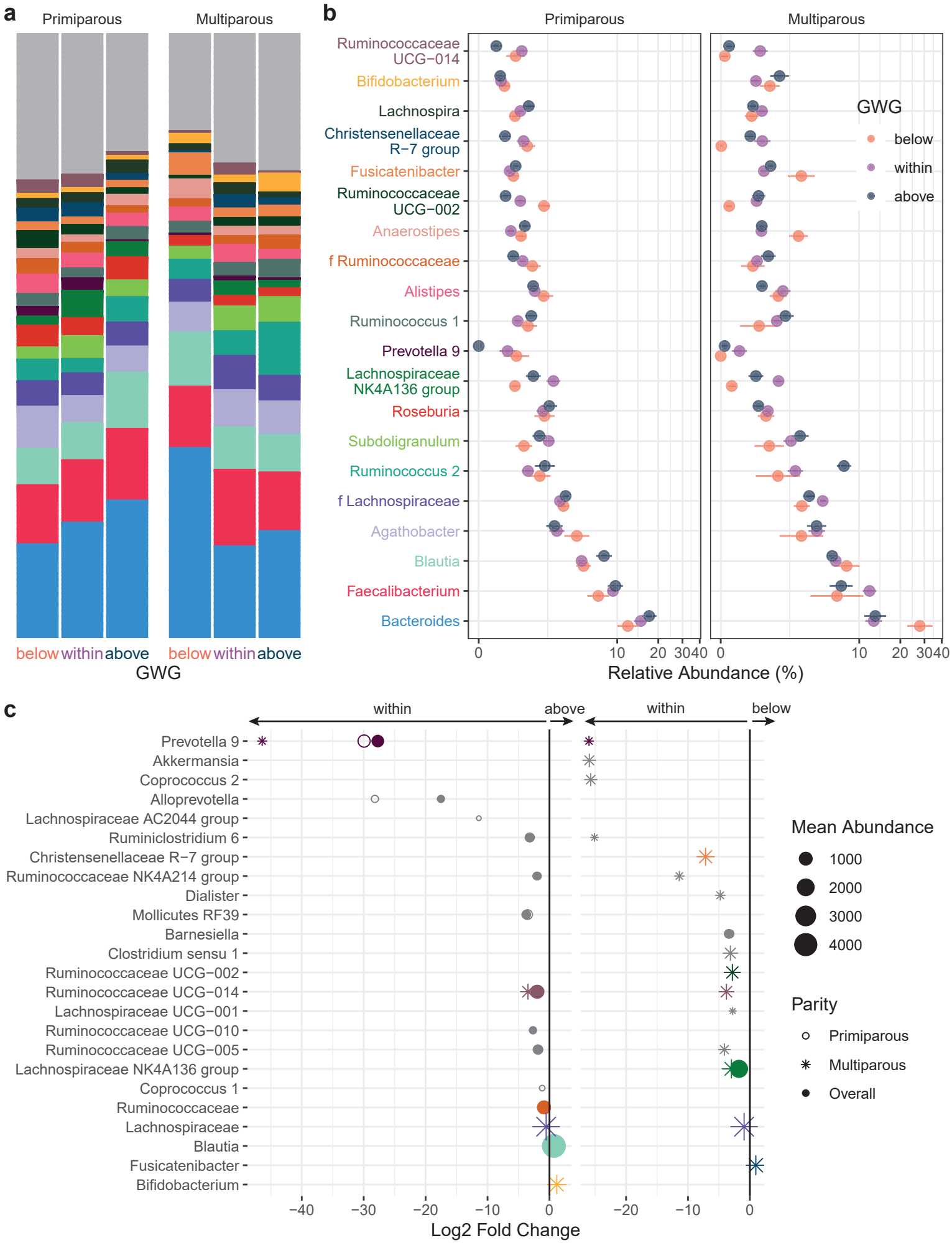

a
Primiparous
Multiparous
below within above
below within above
GWG
b
Primiparous
Multiparous
Ruminococcaceae UCG−014
Bifidobacterium
Lachnospira
Christensenellaceae R−7 group
Fusicatenibacter
Ruminococcaceae UCG−002
Anaerostipes
f Ruminococcaceae
Alistipes
Ruminococcus 1
Prevotella 9
Lachnospiraceae NK4A136 group
Roseburia
Subdoligranulum
Ruminococcus 2
f Lachnospiraceae
Agathobacter
Blautia
Faecalibacterium
Bacteroides
GWG
below
within
above
0
10
20
30
40
Relative Abundance (%)
c
within
above
within
below
Prevotella 9
Akkermansia
Coprococcus 2
Alloprevotella
Lachnospiraceae AC2044 group
Ruminiclostridium 6
Christensenellaceae R−7 group
Ruminococcaceae NK4A214 group
Dialister
Mollicutes RF39
Barnesiella
Clostridium sensu 1
Ruminococcaceae UCG−002
Ruminococcaceae UCG−014
Lachnospiraceae UCG−001
Ruminococcaceae UCG−010
Ruminococcaceae UCG−005
Lachnospiraceae NK4A136 group
Coprococcus 1
Ruminococcaceae
Lachnospiraceae
Blautia
Fusicatenibacter
Bifidobacterium
−40
−30
−20
−10
0
−20
−10
0
Log2 Fold Change
Mean Abundance
1000
2000
3000
4000
Parity
Primiparous
Multiparous
Overall

Figure 4. Taxonomic shifts with GWG in pregnant gut microbiota. a-b, The relative abundances of the 20 most abundant genera in the maternal gut microbiota by GWG below (orange, n=7), within (purple, n=17), and above (dark blue, n=12) the recommended range are shown as a, mean relative abundance in taxa bar plots and b, mean ± standard error of the mean for primiparous (below, n=5; within, n=11; above, n=7) and multiparous (below, n=2; within, n=6; above, n=5) participant with pBMI <25. **c**, Dot plots showing DESeq2 results of differentially abundant genera (mean abundance >150) by GWG category overall (filled dots) and in primiparous (no fill dots) and multiparous (stars) participants (dot size based on mean abundance, colours correspond to genus colour in a where a differentially abundant genus is among the 20 most abundant genera).

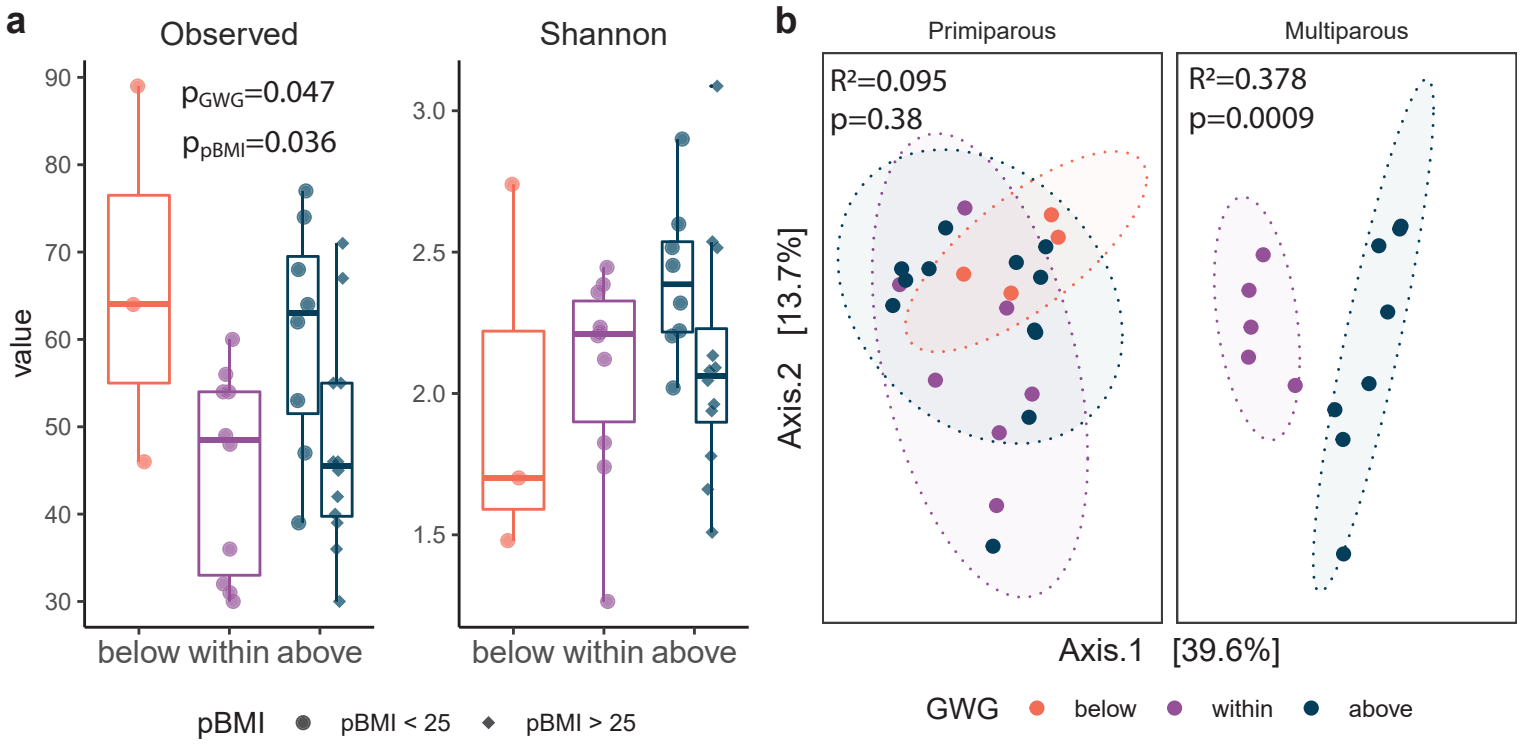
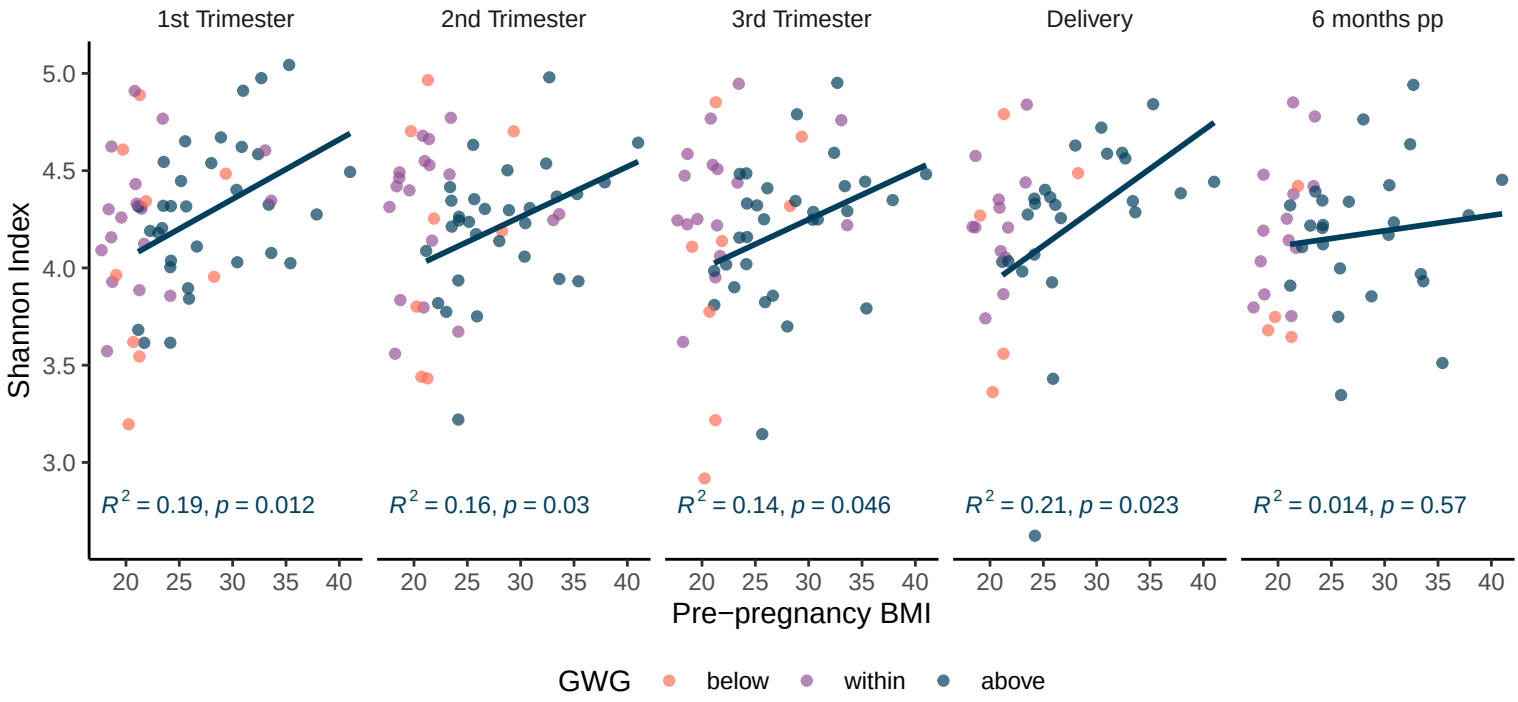


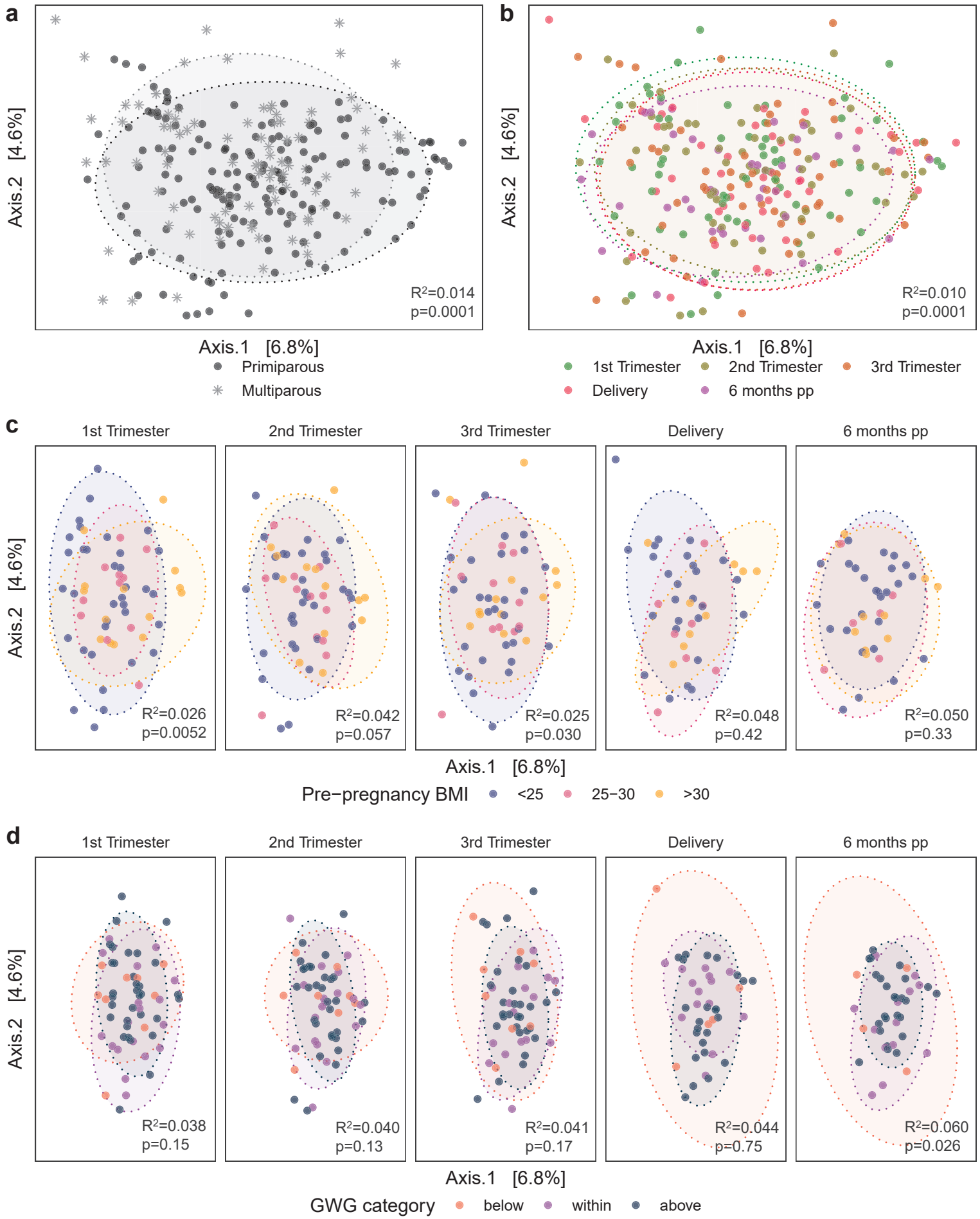
Figure 5. Parity modulates impact of GWG on infant gut microbiota. a, Box plots of alpha diversity by gestational weight gain (GWG) category in primiparous participants (n=24) and multiparous participants (n=13). Alpha diversity (observed ASVs) was significantly increased by GWG above the recommended range (p=0.034) in infants of participants with pBMI <25. Significance assessed by a linear model (Kenward–Roger d.f.), with GWG category as a fixed effect. The box plot centre line represents the median; the box limits represent the upper and lower quartiles; the whiskers represent the 1.5× interquartile range; the points represent the outliers. **b**, PCoA of Bray-Curtis distances shows modest clustering by GWG category (primiparous, R2=0.039, p<0.0001; multiparous R2=0.068, p<0.0001). Primiparous (below, n=4; within, n=7; above=12); Multiparous (within, n=5; above, n=8). Significance was assessed by PERMANOVA.

a
Primiparous
Multiparous
below
within
above
within
above
GWG
b
Primiparous
Multiparous
Sutterella
Intestinibacter
Enterobacter
Lactobacillus
Lachnoclostridium
Faecalibacterium
Akkermansia
Blautia
Enterobacteriaceae
Lachnospiraceae
Parabacteroides
Klebsiella
Streptococcus
Enterococcus
Veillonella
Haemophilus
Clostridium sensu stricto 1
Escherichia/Shigella
Bacteroides
Bifidobacterium
GWG
below
within
above
0
20
40 60
0
20
40 60
Relative Abundance (%)
c
GWG within
GWG above
Bacteroides
Citrobacter
Bifidobacterium
Anaerostipes
Flavonifractor
pBMI < 25
pBMI > 25
Akkermansia
Intestinibacter
Blautia
Primiparous
Multiparous
Akkermansia
Raoultella
Blautia
Citrobacter
Enterococcus
Enterobacter
Clostridioides
−20
−10
10
20
30
Log2 Fold Change
Parity
Primiparous
Multiparous
Overall
Mean Abundance
100
1000
5000
20000

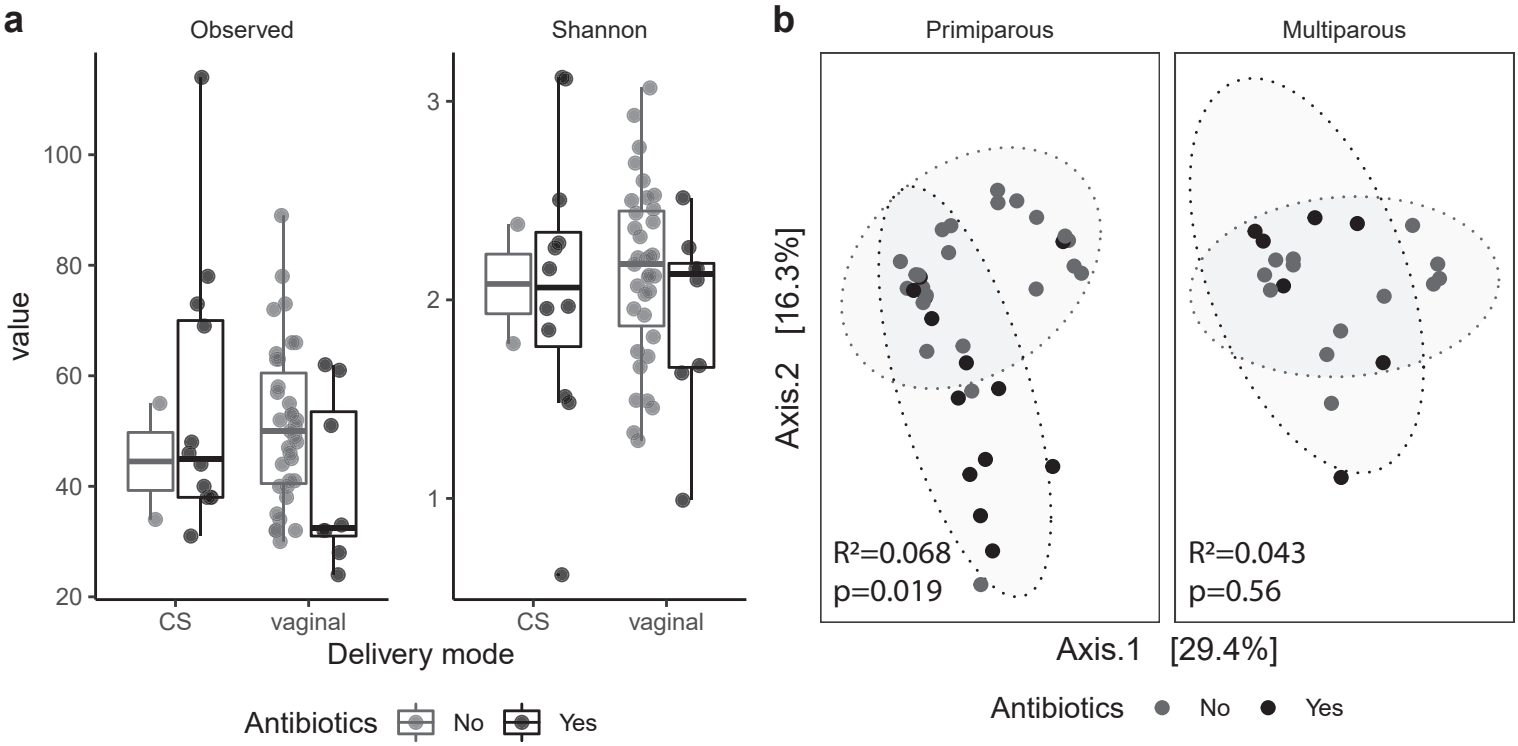
Figure 6. Taxonomic shifts with maternal GWG in infant gut microbiota. a-b, The relative abundances of the 20 most abundant genera in the infant gut microbiota by GWG below (orange), within (purple), and above (dark blue) the recommended range are shown as a, mean relative abundance in taxa bar plots and b, mean ± standard error of the mean for infants of primiparous (below, n=4; within, n=7; above, n=12) and multiparous (within, n=5; above, n=8) participants. **c**, Dot plots showing DESeq2 results of differentially abundant genera overall (filled dots) and in primiparous (no fill dots) and multiparous (stars) participants by maternal GWG, BMI, and parity. Dot size based on mean abundance, colours correspond to genus colour in a where a differentially abundant genus is among the 20 most abundant genera.



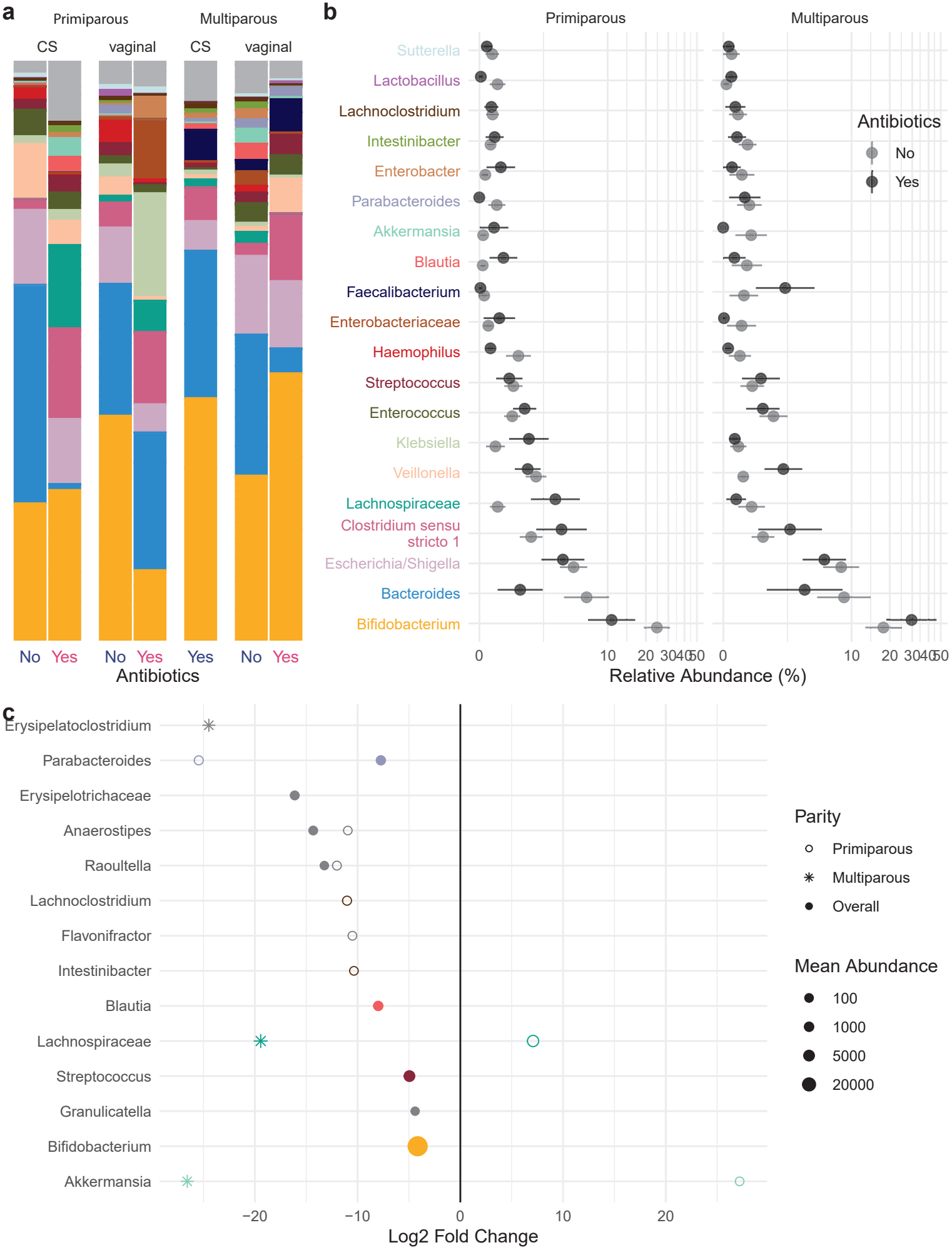
Extended Data Figure 1. Alpha diversity correlated with pBMI during pregnancy. Boxplots of Shannon alpha diversity overlayed with dot plots show a positive correlation with pBMI at each pregnancy timepoint (p=0.012-0.046) but not at 6 months postpartum (p=0.57) in participants with excess GWG and no overall difference in alpha diversity between timepoints across all participants. Significance assessed by linear model (Kenward–Roger d.f.), with pBMI as a fixed effect. The box plot centre line represents the median; the box limits represent the upper and lower quartiles; the whiskers represent the 1.5× interquartile range; the points represent the outliers.


a
Axis.2 [4.6%]
R²=0.014
p=0.0001
Axis.1 [6.8%]
Primiparous
Multiparous
b
Axis.2 [4.6%]
R²=0.010
p=0.0001
Axis.1 [6.8%]
1st Trimester
2nd Trimester
3rd Trimester
Delivery
6 months pp
c
1st Trimester
2nd Trimester
3rd Trimester
Delivery
6 months pp
Axis.2 [4.6%]
R²=0.026
p=0.0052
R²=0.042
p=0.057
R²=0.025
p=0.030
R²=0.048
p=0.42
R²=0.050
p=0.33
Axis.1 [6.8%]
Pre−pregnancy BMI
<25
25−30
>30
d
1st Trimester
2nd Trimester
3rd Trimester
Delivery
6 months pp
Axis.2 [4.6%]
R²=0.038
p=0.15
R²=0.040
p=0.13
R²=0.041
p=0.17
R²=0.044
p=0.75
R²=0.060
p=0.026
Axis.1 [6.8%]
GWG category
below
within
above

Extended Data Figure 2. Impacts of pBMI, GWG, parity, and sample timepoint on maternal gut microbiome beta diversity. PCoA of Bray-Curtis distances shows modest clustering by **a**, parity (R^2=0.014, p<0.0001) and **b**, timepoint (R^2=0.010, p<0.0001). **c**, Clustering by pBMI category within timepoints is significant in the first trimester (R^2=0.026, p=0.0052) and the third trimester (R^2=0.025, p=0.033). **d**, Clustering by GWG category was significant at 6 months post-partum (R^2=0.060, p=0.026). First trimester, n=62; second trimester, n=58; third trimester, n=56; delivery, n=41; 6 months post-partum, n=43. Significance was assessed by PERMANOVA.



Extended Data Figure 3. Parity modulates impact of peripartum antibiotics on infant gut microbiota. a, Box plots of alpha diversity at 6 months of age shows no effect of peripartum antibiotic exposure. Significance assessed by a linear model (Kenward–Roger d.f.), with antibiotic exposure as a fixed effect. The box plot centre line represents the median; the box limits represent the upper and lower quartiles; the whiskers represent the 1.5× interquartile range; the points represent the outliers. **b,** PCoA of Bray-Curtis distances in primiparous (n=38) and multiparous (n=20) participants shows modest clustering by GWG category (primiparous, R2=0.039, p<0.0001; multiparous R2=0.068, p<0.0001). Significance was assessed by PERMANOVA.


a
Primiparous
Multiparous
CS
vaginal
CS
vaginal
No
Yes
No
Yes
Yes
No
Yes
Antibiotics
b
Primiparous
Multiparous
Sutterella
Lactobacillus
Lachnoclostridium
Intestinibacter
Enterobacter
Parabacteroides
Akkermansia
Blautia
Faecalibacterium
Enterobacteriaceae
Haemophilus
Streptococcus
Enterococcus
Klebsiella
Veillonella
Lachnospiraceae
Clostridium sensu stricto 1
Escherichia/Shigella
Bacteroides
Bifidobacterium
Antibiotics
No
Yes
0
10
20
30
40
50
Relative Abundance (%)
c
Erysipelatoclostridium
Parabacteroides
Erysipelotrichaceae
Anaerostipes
Raoultella
Lachnoclostridium
Flavonifractor
Intestinibacter
Blautia
Lachnospiraceae
Streptococcus
Granulicatella
Bifidobacterium
Akkermansia
−20
−10
0
10
20
Log2 Fold Change
Parity
Primiparous
Multiparous
Overall
Mean Abundance
100
1000
5000
20000

Extended Data Figure 4. Taxonomic shifts with peripartum antibiotics in infant gut microbiota. a-b, The relative abundances of the 20 most abundant genera in the infant gut microbiota by peripartum antibiotic exposure are shown as a, mean relative abundance in taxa bar plots and b, mean ± standard error of the mean for primiparous (n=38) and multiparous (n=20) participant with pBMI <25. **c**, Dot plots showing DESeq2 results of differentially abundant genera by perinatal antibiotic exposure (dot size based on mean abundance, colours correspond to genus colour in a where a differentially abundant genus is among the 20 most abundant genera).

Extended Data Table 1. Participant characteristics

	Parity				Overall pBMI					Primiparous pBMI					Multiparous pBMI				
Characteristic	**primiparous**[1]	**multiparous**[1]	**p**[2]	**q**[3]	**<25**[1]	**25-30**[1]	**>30**[1]	**p**[2]	**q**[3]	**<25**[1]	**25-30**[1]	**>30**[1]	**p**[2]	**q**[3]	**<25**[1]	**25-30**[1]	**>30**[1]	**p**[2]	**q**[3]
n	40	25			39	12	14			24	7	9			15	5	5		
Age	29.6 (4.4)	32.8 (3.1)	0.002	**0.009**	30.9 (4.3)	31.3 (4.6)	30.3 (3.9)	0.8	0.8	29.9 (4.6)	29.6 (5.3)	29.1 (3.5)	>0.9	>0.9	32.60 (3.20)	33.80 (2.17)	32.40 (4.04)	0.8	>0.9
Primigravida	27 (68%)	0 (0%)	<0.001	**<0.001**	17 (44%)	3 (25%)	7 (50%)	0.4	0.8	17 (71%)	3 (43%)	7 (78%)	0.3	0.6	0 (0%)	0 (0%)	0 (0%)		
pBMI	23.8 (21.0, 29.0)	23.5 (21.2, 26.6)	>0.9	>0.9	21.3 (19.6, 23.2)	26.4 (25.8, 28.4)	33.2 (31.3, 34.9)	<0.001	**<0.001**	21.2 (19.4, 22.0)	28.0 (25.7, 28.8)	33.4 (32.7, 33.6)	<0.001	**<0.001**	21.3 (20.2, 23.4)	26.1 (25.9, 26.6)	31.0 (30.9, 35.3)	<0.001	**<0.001**
GWG (kg)	15.0 (12.0, 19.0)	15.0 (13.0, 20.0)	0.8	>0.9	15.0 (13.0, 18.3)	16.5 (14.6, 21.1)	13.5 (10.0, 19.4)	0.6	0.8	14.9 (13.0, 17.3)	19.0 (15.5, 21.8)	12.0 (10.0, 18.0)	0.4	0.6	15.0 (13.0, 20.0)	15.0 (14.8, 16.0)	16.0 (10.0, 19.9)	>0.9	>0.9
GWG category			0.5	0.8				0.001	**0.004**				0.014	0.056				0.069	0.2
below	6 (15%)	3 (13%)			7 (19%)	2 (17%)	0 (0%)			5 (22%)	1 (14%)	0 (0%)			2 (15%)	1 (20%)	0 (0%)		
within	13 (33%)	6 (26%)			17 (47%)	0 (0%)	2 (14%)			11 (48%)	0 (0%)	2 (22%)			6 (46%)	0 (0%)	0 (0%)		
above	20 (51%)	14 (61%)			12 (33%)	10 (83%)	12 (86%)			7 (30%)	6 (86%)	7 (78%)			5 (38%)	4 (80%)	5 (100%)		
pp Weight Retained	3.1 (-0.2, 7.6)	3.2 (2.0, 8.8)	0.3	0.6	1.8 (0.3, 5.0)	4.7 (1.4, 8.1)	7.6 (3.1, 11.9)	0.053	0.14	1.6 (-1.3, 3.8)	4.4 (1.0, 6.5)	7.8 (3.1, 12.4)	0.083	0.2	2.5 (1.3, 7.4)	8.0 (2.7, 8.7)	6.0 (3.2, 9.8)	0.5	>0.9
Length of gestation (days)	282 (274, 284)	277 (273, 282)	0.3	0.6	280 (273, 284)	276 (272, 284)	279 (275, 288)	0.7	0.8	282 (276, 284)	273 (270, 283)	282 (277, 289)	0.5	0.6	277 (270, 282)	276 (275, 284)	278 (274, 279)	0.9	>0.9
Delivery mode = vaginal	32 (80%)	18 (78%)	0.9	>0.9	30 (81%)	10 (83%)	10 (71%)	0.7	0.8	20 (83%)	5 (71%)	7 (78%)	0.8	0.9	10 (77%)	5 (100%)	3 (60%)	0.3	0.7

[1] n; Mean (SD); n (%); Median (IQR)
[2] Kruskal-Wallis rank sum test
[3] False discovery rate correction for multiple testing

Extended Data Table 2. Infant characteristics

	Parity				Overall GWG				Primiparous GWG				Multiparous GWG			
Characteristic	**primiparous**[1]	**multiparous**[1]	**p**[2]	**q**[3]	**within**[1]	**above**[1]	**p**[2]	**q-value**[3]	**within**[1]	**above**[1]	**p**[2]	**q-value**[3]	**within**[1]	**above**[1]	**p**[2]	**q-value**[3]
n	24	13			12	20			7	12			5	8		
Age	30.4 (4.3)	32.7 (3.5)	0.073	0.3	32.0 (3.6)	31.1 (4.0)	0.5	>0.9	30.6 (3.7)	30.7 (4.2)	0.9	>0.9	34.0 (2.4)	31.9 (3.9)	0.3	0.5
pBMI	24.2 (20.7, 29.1)	23.5 (21.5, 25.9)	0.6	0.7	21.4 (18.6, 23.6)	25.8 (24.2, 30.5)	0.002	**0.011**	18.7 (18.6, 25.4)	25.8 (24.2, 31.1)	0.031	0.2	21.5 (21.3, 23.3)	25.0 (23.9, 27.7)	0.019	0.076
GWG (kg)	15.0 (11.5, 19.0)	16.0 (14.0, 20.0)	0.4	0.7	13.5 (13.0, 15.0)	19.0 (17.0, 20.2)	<0.001	**0.002**	13.0 (12.0, 15.0)	19.0 (17.8, 20.2)	0.003	**0.036**	14.0 (13.0, 15.0)	18.8 (16.8, 21.2)	0.027	0.076
Length of gestation (days)	281 (276, 284)	280 (277, 284)	0.8	0.8	282 (275, 282)	281 (278, 288)	0.4	>0.9	282 (282, 283)	280 (276, 287)	0.7	0.9	277.0 (270.0, 280.0)	283.0 (279.0, 289.0)	0.078	0.14
Delivery mode = vaginal	22 (92%)	13 (100%)	0.3	0.6	11 (92%)	19 (95%)	0.7	>0.9	6 (86%)	11 (92%)	0.7	0.9	5 (100%)	8 (100%)		
Infant sex = female	14 (58%)	5 (38%)	0.3	0.6	5 (42%)	12 (60%)	0.3	>0.9	4 (57%)	8 (67%)	0.7	0.9	1 (20%)	4 (50%)	0.3	0.5
Birth weight (g)	3,431 (447)	3,655 (353)	0.11	0.3	3,540 (306)	3,526 (490)	>0.9	>0.9	3,665 (246)	3,320 (510)	0.069	0.3	3,366 (318)	3,835 (245)	0.028	0.076
Birth length (cm)	51.83 (2.62)	51.38 (2.26)	0.5	0.7	52.17 (2.48)	51.60 (2.58)	0.6	>0.9	53.29 (2.75)	51.42 (2.54)	0.2	0.3	50.60 (0.55)	51.88 (2.80)	0.4	0.5
Birth weight:length	65 (61, 70)	72 (70, 74)	0.024	0.3	68 (64, 71)	71 (61, 74)	0.8	>0.9	69.0 (65.5, 71.6)	61.7 (59.9, 66.7)	0.10	0.3	66.6 (64.7, 69.6)	72.9 (72.2, 74.9)	0.023	0.076
Birth weight percentile	50 (20, 60)	61 (50, 80)	0.073	0.3	57 (51, 62)	54 (20, 80)	0.8	>0.9	59 (52, 62)	26 (12, 60)	0.11	0.3	46 (28, 58)	72 (57, 82)	0.062	0.14
Birth length percentile	46 (18, 74)	34 (23, 46)	0.4	0.7	41 (30, 78)	46 (18, 62)	0.5	>0.9	72 (42, 89)	49 (18, 68)	0.3	0.5	31 (24, 38)	38 (20, 52)	0.6	0.6
Infant weight at 6 months (g)	7,730 (6,960, 8,250)	7,738 (7,632, 8,154)	0.5	0.7	7,800 (7,305, 8,280)	7,815 (7,611, 8,200)	>0.9	>0.9	7,730 (6,960, 8,450)	7,940 (7,634, 8,200)	>0.9	>0.9	7,959 (7,752, 8,154)	7,662 (7,598, 8,342)	0.6	0.6

[1] n; Mean (SD); Median (IQR); n (%)
[2] Kruskal-Wallis rank sum test
[3] False discovery rate correction for multiple testing