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, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Department of Obstetrics and Department of 'Experimental Obstetrics', 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 – University Medicine Berlin
Departments of Obstetrics and Division of Experimental Obstetrics
Charité Campus Virchow, Berlin
Germany
Tel: +49-30-664309
Fax: +49-30-564901
Email: thorsten.braun@charite.de

Summary

Shifts in the gut microbiome have been implicated in mediating maternal metabolic adaptations to pregnancy. We investigated the impact of pre-pregnancy BMI (pBMI) and gestational weight gain (GWG) on pregnancy-associated remodeling of the maternal gut microbiome and infant colonization, and whether previous pregnancies modulated observed associations. We found 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 microbiotal adaptations to pregnancy. This ecological memory appears to be passed to the next generation, as parity impacted infant microbial composition of first-pass meconium and 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 parity as a key consideration for studies of the microbiome in pregnancy and infants.

Background

Pregnancy requires physiological, metabolic, and immune adaptations to support fetal growth and development (Tan and Tan, 2013). Pregnancy-induced metabolic adaptations mirror those seen in non-pregnant metabolic syndrome; including hyperglycemia and hyperinsulinemia and increased adiposity. Although some data suggest that these adaptations may influence (Nuriel-Ohayon *et al.*, 2019) and/or be influenced by (Koren *et al.*, 2012) shifts in the gut microbiota over the course of pregnancy, most studies have investigated only one (sometimes two) timepoints during gestation (Aatsinki et al., 2018; Crusell et al., 2018; Koren et al., 2012; Zacarías et al., 2018). An evaluation of whether gut microbiota change temporally across pregnancy, birth and the postnatal period are 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.

The impact of pregnancy on the gut microbiome in humans is challenging to assess due to the large effect of interpersonal variation (Turnbaugh *et al.*, 2007). One of the first studies of the gut microbiome during pregnancy reported an increase in differences in overall community composition between samples (beta diversity) and a decrease in community richness within samples (alpha diversity) in otherwise normal pregnant individuals during the third trimester of pregnancy compared to the first (Koren *et al.*, 2012). In contrast, others have found the maternal gut microbiota to be stable over the course of pregnancy

(DiGiulio *et al.*, 2015; Faucher *et al.*, 2020; Kumbhare *et al.*, 2020). Even fewer studies have investigated pregnancy-associated maternal microbial shifts in the context of pregnancy complications like excessive pre-pregnancy body mass index (pBMI) or gestational weight gain (GWG) (Collado *et al.*, 2008; Santacruz *et al.*, 2010; Stanislawski *et al.*, 2017). If microbial shifts do occur, no studies to date have investigated whether these shifts are influenced by previous pregnancies in humans.

Both high maternal pBMI and excess GWG are significantly associated with pregnancy complications (Triunfo and Lanzone, 2014) and impaired offspring metabolism (Ludwig et al., 2013; Retnakaran et al., 2012). Reports suggest that some of these maternal and infant associations may be mediated by obesity-associated "dysbiosis" of the maternal gut microbiome during pregnancy (Santacruz *et al.*, 2010; Zacarías *et al.*, 2018). As humans are sterile before birth (Kennedy *et al.*, 2021) the maternal microbiome may influence offspring development both via changes to the in utero environment (e.g. altered maternal metabolic adaptations to pregnancy) and via altered seeding of the infant microbiome during and after birth. Indeed, shortly after birth, maternal vaginal, skin, and gut microbes can be detected in the infant gut microbiota (Dominguez-Bello *et al.*, 2010), but only specific gut-adapted microbes such as *Bifidobacterium* and *Bacteroides* persist at least up to 1 year (Korpela *et al.*, 2018). As temporary shifts in the microbiota during critical developmental windows can have long-term effects on offspring (Cox *et al.*, 2014) and priority effects influence gut microbiota community composition (Martínez *et al.*, 2018), the initial seeding of the neonate gut by maternal microbes can have long-term effects even in the absence of persistent colonization. Thus, we set out to determine whether temporal changes in maternal gut microbial composition occur with pregnancy and whether maternal BMI and/or excess GWG and parity impact maternal microbiotal adaptations to pregnancy in a prospective study. We also investigated how these contexts influence infant microbiome at 6 months of age.

Results

Study cohort

A total of 89 participants were recruited in Berlin, Germany. After excluding participants who reported nicotine or antibiotic use during pregnancy, maternal samples from 65 participants were analyzed (primiparous n=40; multiparous n=25). Pre-pregnancy BMI (pBMI) was used as a continuous variable or by BMI category as defined by the World Health Organization(1995): optimal (18.5-24.9, n=39), overweight (25-29.9, n=12), or obese (>30, n=14). GWG was categorized into below (n=9), within

(n=18), or above (n=38) the recommended range by pBMI category according to Institute of Medicine guidelines(2010). The majority of participants with pre-pregnancy overweight (10 of 12; 83%) or obesity (12 of 14; 86%) had GWG above the recommended range, compared to less than half of participants with an optimal pBMI (12 of 39; 33%; Table S1). Participants predominantly delivered vaginally overall (51/65; 78%) and within each pBMI category: optimal (30/39; 81%), overweight (10/12; 83%), obese (10/14; 71%). Multiparous participants (n=25) were older than primiparous participants (n=40; 32.8±3.1 years of age vs 29.6±4.4, p=0.002), but there was no significant effect of parity on pBMI, GWG, post-partum weight retention, length of gestation, or delivery mode.

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

Maternal stool samples were collected 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 a total of 7577 ASVs after removal of host-associated reads (Kingdom Eukaryota, Order Chloroplast, Family Mitochondria, or no assigned Phylum). The median sample read count was 62,853 (minimum 14,872; maximum 136,000).

To assess differences in diversity within samples, the Shannon Index alpha diversity metric was used as a measure of richness and evenness (Whittaker, 1972). Consistent with previous studies (DiGiulio *et al.*, 2015; Faucher *et al.*, 2020; Kumbhare *et al.*, 2020), alpha diversity was similar throughout pregnancy (p=0.60) and across all sample time points including 6 months post-partum (p=0.057; Fig. S1). Alpha diversity was decreased by GWG outside the recommended range in multiparous (p=0.013), but not primiparous participants (p=0.78; Fig. 1a) and was positively correlated with pBMI (R^2 = 0.11, p=0.010), particularly in participants with GWG above the recommended range (R^2 = 0.12, p=0.0069; Fig. 1b). This correlation was significant within each pregnancy time point (R^2 = 0.14-0.21, p=0.012-0.046) but not at 6 months post-partum (R^2=0.014, p=0.57; Fig. S1). This suggests that GWG may have a greater influence on microbial diversity within individuals experiencing their second (or third) pregnancies compared to those in their first pregnancy (multiparous vs primiparous) and that the effects of pBMI on alpha diversity do not persist post-partum.

Differences in overall microbiota community composition between samples were assessed by the Bray-Curtis dissimilarity beta-diversity metric and visualized by principal coordinate analysis (PCoA; Fig 1c, d). Interindividual variability explained the majority of variation between samples (PERMANOVA;

124 R^2=62.1%, p=0.0001), with comparatively small effects of GWG category (R^2 = 2.5%, p=0.0001; Fig.
125 1c), pBMI category (R^2 = 2.8%, p=0.0001; Fig. 1d), parity (R^2 = 1.4%, p=0.0001; Fig. S2a), and sample
126 time point (R^2 = 1.0%, p=0.0001; Fig. S2b). Within sample time points, the effect of pBMI was
127 significant in the first trimester (R^2=2.6%, p=0.0052) and third trimester (R^2=2.5%, p=0.030; Fig. S2c)
128 and the effect of GWG category was significant at 6 months post-partum (R^2=6.0%, p=0.026; Fig. S2d).
129 This indicates that the impact of pBMI is greatest during pregnancy and does not persist post-partum,
130 when the impact of GWG is greatest. The contributions of pBMI and GWG to overall beta-diversity were
131 modest relative to the large effect of inter-individual variation. High inter-individual variation prior to
132 pregnancy may lead to inter-individual variation in microbiotal adaptations to pregnancy, and in the
133 impacts of pBMI and GWG on those adaptations.

134 To investigate whether pBMI impacts beta-diversity within individual participants, we calculated the
135 mean distance to the centroid within each participant (beta dispersion) using the Bray-Curtis dissimilarity
136 metric. We found that pBMI is negatively correlated with mean beta dispersion in primiparous (R^2 = 0.26,
137 p=0.016) but not multiparous participants (R^2 = 0.0013, p=0.90; Fig. S3). We further assessed the effect
138 of pBMI on sample beta-diversity within each participant by mixed linear model with pBMI and sample
139 time point as interacting fixed effects and participant ID as a random effect. Overall, beta-diversity within
140 participants is negatively correlated with pBMI across all sample time points in primiparous (R^2 = 0.083,
141 p=0.0017) but not multiparous participants (R^2 = 0.0026, p=0.70; Fig. 1e). These data suggest that over
142 the course of primiparous pregnancy overall gut microbiota community composition changes less in
143 participants with a higher pBMI and may reflect a reduced requirement for microbiotal adaptations to
144 pregnancy. Together these data highlight the impact of pre-gravid BMI on microbiota diversity and
145 suggest that this impact exists not only during pregnancy but also up to 6 months postpartum. These data
146 also highlight a novel observation: gut microbial diversity during pregnancy is influenced by previous
147 pregnancies. Parity therefore is a key confounder that must be considered in all future studies
148 investigating the maternal microbiome.

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

150 Because of their importance in metabolic signalling pathways (Koh *et al.*, 2016), we assessed SCFA level
151 associations with pBMI and GWG. We analyzed relative SCFA concentrations in a subset of maternal
152 fecal samples (primiparous, n=16; multiparous, n=16) across all timepoints by gas-chromatography mass
153 spectroscopy (GC-MS). To account for variability in recovery between samples, the relative concentration
154 of each SCFA was assessed as a percentage of total SCFAs (Fig. 2). Fecal acetate was decreased by GWG

155 above the recommended range (p=0.0011), particularly in primiparous participants (p=0.00055). This
156 decrease was accompanied by corresponding increases in propionate (p=0.019) and butyrate (p=0.015)
157 with GWG above the recommended range in primiparous participants. Lactate was also decreased in
158 participants with GWG above the recommended range (p=0.0074), particularly in multiparous
159 participants (p=0.028). In participants with GWG above the recommended range, the relative abundance
160 of the branched SCFAs isobutyrate and isovalerate were decreased by pBMI>25 (p=0.020, p=0.051
161 respectively), particularly in multiparous participants (p=0.014, p=0.020). These data suggest that parity
162 not only modulates the impact of pBMI and GWG on the gut microbiota community composition but may
163 also modulate the relative abundance of key microbial metabolites.

164 Maternal taxonomic associations with pBMI, GWG, and parity

165 Taxa that were differentially abundant during pregnancy between GWG and pBMI categories and by
166 parity were identified using DESeq2 (RRID: SCR_015687). The relative abundances of 9 genera (mean
167 abundance >150) differed by parity across all participants (Table S2). Primiparous participants had
168 increased abundances of several butyrate-producing genera within the family Lachnospiraceae, including
169 *Roseburia* and *NK4A136 group*, compared to multiparous participants (Fig. 3a-b, Table S2). Conversely,
170 compared to primiparous participants, multiparous participants have an increased relative abundance of
171 *Bifidobacterium* and *Romboutsia* (Table S1), both of which have been linked to improved glucose control
172 in obesity (Schellekens *et al.*, 2021; Therdtatha *et al.*, 2021).

173 All but 4 of the 26 participants with a pBMI > 25 experienced excess GWG (Table S1). As pBMI and
174 GWG did not vary independently, and we were not able to assess interacting effects of pBMI and GWG
175 and therefore these 4 participants were excluded from analysis. The impact of pBMI category was
176 assessed within participants who experienced excess GWG (pBMI <25, n=12; pBMI 25-30, n=10; pBMI
177 > 30, n=12; Fig. 2a-c) and the impact of excess GWG was assessed within participants with a pBMI < 25
178 (below, n=7; within, n=17; above, n=12; Fig 3a-c). The relative abundances of 6 and 11 genera (mean
179 abundance >150) were significantly impacted by a pBMI of 25-30 and >30 respectively, compared to
180 pBMI <25 overall (Fig. 2c). An additional 7 genera differed significantly by pBMI only in multiparous
181 participants (Fig. 2c; Table S2). *Bacteroides* and *Anaerostipes* were decreased in participants with pBMI
182 >30 (Fig. 2a-c). *Blautia* was also decreased in participants with a pBMI 25-30 and > 30 (Fig. 2a-c) and
183 increased with excess GWG (vs. GWG within the recommended range; Fig. 3a-c), particularly in
184 primiparous participants (Table S2). In contrast, *Prevotella 9* was markedly increased in participants with
185 pBMI > 25 or > 30 but decreased by excess GWG. Excess GWG was also associated with decreased

186 relative abundances of 6 genera of the SCFA-producing family Ruminococcaceae (Fig. 3c), including
187 uncultured genus (UCG) 005 which has previously been reported to decrease with gestational diabetes
188 mellitus (Ma *et al.*, 2020) .

189 Maternal taxonomic associations with diet during pregnancy

190 As diet plays a role not only gut microbial composition but also the production of bacterial metabolites,
191 we assessed maternal diet during pregnancy. Dietary data was collected in the first, second, and third
192 trimester using a semiquantitative 25-item food frequency questionnaire (FFQ) adapted from the validated
193 PrimeScreen FFQ (Rifas-Shiman *et al.*, 2001). There was no significant effect of pBMI, GWG, or parity
194 on overall diet quality score or average weekly intake of any one FFQ item in any trimester after
195 correction for multiple comparisons (FDR correction). FFQ data were further analyzed by principal
196 component analysis (PCA) to identify dietary patterns. The first three principal components (PCs)
197 cumulatively describe 31.4% of the FFQ data set variance (13.1%, 9.9%, and 8.4%). Each PC was
198 divided into tertiles and genera that were differentially abundant between the high and low tertiles were
199 identified by DESeq2. The first principal component (PC1) was characterized by high factor loadings for
200 fried foods, processed meat, and refined grains and negative loadings for vegetables, nuts, and legumes
201 (Fig. 5a). Participants with low PC1 scores had increases in *Roseburia* and *Prevotella* (Fig. 5b), consistent
202 with their ability to degrade plant-derived polysaccharides (Fehlner-Peach *et al.*, 2019; La Rosa *et al.*,
203 2019). The second principal component (PC2) was characterized by high factor loadings for meats, fruits,
204 and vegetables and had few associations with differentially abundant taxa. The third principal component
205 (PC3) was characterized by high loadings for meats and dairy, and negative loadings for vegetables,
206 grains, and legumes. As with PC1, participants with low PC3 scores had increases in *Roseburia* and
207 *Prevotella*, while participants with high PC3 scores had increases in *Bifidobacterium* and
208 *Catenibacterium*, both of which have previously been associated with fermented dairy-product
209 consumption (Volokh *et al.*, 2019). These data indicate that diet likely contributes to interindividual
210 variability in gut microbiome community composition, but does not differ by pBMI, GWG or parity.

211 Impact of delivery mode, parity, and sex on first-pass meconium

212 Of 70 total first-pass meconium samples, only 44 (62.9%) produced any sequencing reads. This
213 proportion varied by delivery mode: primary C-section neonates had the lowest proportion of samples
214 with any reads (17%; 1 of 6 samples), while other delivery modes had similar proportions of samples with

215 any reads: secondary C-section, 62.5% (5 of 8 samples); normal spontaneous vaginal delivery, 68.9% (31
216 of 45 samples); vacuum extraction, 63.6% (7 of 11 samples). Of samples with any reads, the number of
217 reads also varied by delivery mode: the single remaining primary C-section neonate had the fewest reads
218 per sample (172 reads, n=1), followed by vacuum extraction (median, 397; mean, 14,681; n=7), normal
219 spontaneous vaginal delivery neonates (median, 11353; mean, 31848.23.18; n=28), and secondary C-
220 section (median, 21,906; mean, 27,744; n=5). To the extent that read counts reflect microbial load, these
221 data are consistent with colonization during delivery as neonates born without labour (primary C-section)
222 are not exposed to the densely populated maternal vaginal and gut microbiomes. These data support our
223 previous finding(Kennedy *et al.*, 2021) that microbial colonization begins during birth.

224 Meconium samples with fewer than 100 reads were removed from further analyses, leaving 30 samples
225 (primary C-section, n=1; secondary C-section; n=4, normal spontaneous vaginal delivery, n=21; vacuum
226 extraction, n=4). Consistent with out previous study(Kennedy *et al.*, 2021), the microbiota of first-pass
227 meconium samples was highly variable. A large proportion of samples (14 of 30 total) were dominated by
228 one or two genera, including *Escherichia/Shigella*, *Enterococcus*, *Lactobacillus*, and *Streptococcus*. The
229 most prevalent genus was *Bacteroides*, which was detected in 21 of 30 samples (70%), followed by
230 *Faecalibacterium* (20 of 30, 67%), *Blautia* (19 of 30, 63%) and *Escherichia*/*Shigella* (19 of 30, 63%). Of
231 244 genera detected, only 20 were present in more than half of first-pass meconium samples. Among
232 infants who were not exposed to perinatal antibiotics (n=22 total; vaginal, n=21; C-section, n=1), no
233 samples were dominated by *Enterococcus*, and *Bacteroides* (18 of 22; 82%) and *Streptococcus* (16 of 21;
234 73%) were the most prevalent genera.

235 To eliminate potential confounding effects of delivery mode and antibiotics further analysis was
236 performed using only first-pass meconium from spontaneous vaginal deliveries without antibiotic
237 exposure during delivery (n=17). Due to limited sample size, we performed descriptive analysis and
238 found that, consistent with a previous report (Cong *et al.*, 2016), most females were dominated by
239 Clostridiales (7 of 10 with abundance greater than 65%), while males were more commonly dominated by
240 Enterobacteriales (4 of 7 with abundance greater than 41%), which was rare among females (detected in 3
241 of 10 females with a maximum abundance of 2.1%; Fig. S4). The mean abundance of *Bifidobacterium*
242 was greater in neonates of multiparous participants (9.2%) than those of primiparous participants (0.30%;
243 Fig. S5). This was also true for *Bacteroides* (multiparous, 20.9%; primiparous, 6.1%), particularly in
244 females (multiparous, 33.7%; primiparous, 7.8%), who also had a greater mean abundance compared to
245 males (female, 20.8%; male 4.3%). There was also an effect of neonate sex on *Escherichia/Shigella*,
246 which was detected in only 3 of 10 females (mean abundance, 0.34%; maximum abundance, 2.1%) but
247 was detected in 5 of 7 males (mean abundance, 40.6%), and in 4 of males was the dominant genus

248 (minimum abundance, 41.3%; maximum abundance, 98.4%). These data suggest that parity and infant
249 sex may impact the initial colonization of the infant gut microbiota, although this should be confirmed in
250 a larger sample size.

251 Impact of perinatal antibiotic exposure on the infant gut microbiota

252 Of the 63 total mother-infant dyads who provided an infant fecal sample at 6 months of age, we excluded
253 3 for nicotine exposure during pregnancy, 1 for pre-term birth, and 1 for low sequencing read count,
254 which left 58 infants for full analysis. As perinatal antibiotic exposure has previously been shown to
255 perturb the infant gut microbiota (Mueller *et al.*, 2015), we assessed the impact of perinatal antibiotics
256 (between 33 weeks gestation and birth) on the infant gut microbiota in this cohort. The majority of infants
257 delivered by C-section were exposed to perinatal antibiotics (12/14; 86%;) while relatively few vaginally
258 delivered infants were exposed (9/44; 20%). While there was no significant impact of antibiotics on alpha
259 diversity (Observed p=0.70, Shannon p=0.39; Fig. S5a), infant gut microbiota beta diversity differed by
260 antibiotic exposure in primiparous participants (PERMANOVA, R^2=0.068, p=0.019), but not in
261 multiparous participants (R^2=0.043, p=0.56; Fig. S5b). Differentially abundant taxa were identified by
262 DESeq2 (RRID: SCR_015687) and showed that antibiotic exposure was associated with a decreased
263 abundance of 8 genera, including *Bifidobacterium*, *Streptococcus*, *Blautia*, and *Parabacteroides* (Fig. S6).
264 Three additional genera (*Lachnoclostridium*, *Flavonifractor*, *Intestinibacter*) were significantly decreased
265 by antibiotic exposure only in primiparous participants. *Akkermansia* and an unidentified genus of the
266 family Lachnospiraceae were the only genera that increased with antibiotic exposure, although again only
267 in primiparous participants, and both were decreased by antibiotic exposure in multiparous participants.
268 These data show that perinatal antibiotic exposure significantly perturbs the infant gut microbiota, and
269 that parity may influence these effects.

270 Infant participant characteristics

271 We excluded 21 infants due to perinatal antibiotic use, leaving 37 infants included in our analyses
272 (female, n=19; male, n=18). Compared to our study cohort overall, a greater proportion of participants
273 with infant samples delivered vaginally overall (35/37; 95%) and within each pBMI category—optimal
274 (21/22; 95%), overweight (7/8; 88%), obese (7/7; 100%)—likely due to the exclusion of infants exposed
275 to antibiotics during delivery. Infant characteristics at birth did not differ by maternal pBMI category
276 overall or within primiparous and multiparous participants. Although infant birth weight did not differ by

277 GWG overall, excess GWG was associated with higher birth weight in multiparous participants (p=0.028;
278 Table S6), although this was not significant after adjusting for multiple testing. Among infants with
279 available feeding data, at 6 months of age the majority were both breastfed (27/30; 90%) and eating solid
280 foods (23/28; 82%). At 6 months of age, infant weight did not differ significantly by maternal pBMI or
281 GWG.

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

283 We evaluated the impacts of maternal pBMI and GWG on the gut microbiota of infants at 6 months of
284 age. We identified a total of 1063 ASVs after removal of host-associated reads (Kingdom Eukaryota,
285 Order Chloroplast, Family Mitochondria, or no assigned Phylum). The median sample read count was
286 59,561 (minimum 6,045; maximum 124,303). There were only 3 participants (1 male, 2 female) with
287 maternal pre-pregnancy overweight/obesity who did not experience excess GWG. Therefore, pBMI and
288 GWG did not vary independently, and we were not able to assess interacting effects of pBMI and GWG.
289 The impact of pre-pregnancy BMI was assessed within participants who experienced excess GWG (pBMI
290 <25, n=8; >25, n=12) and the impact of excess GWG was assessed within participants with an optimal
291 pre-pregnancy BMI (GWG below, n=4; within, n=12; above, n=8).

292 Infant microbiota alpha diversity (number of observed ASVs) was increased by GWG above the
293 recommended range (p=0.0342; main effect of GWG category, p=0.0336; Fig. 5a) but did not differ by
294 maternal pBMI (p=0.255), mode of delivery (0.30), or infant sex (p=0.78). There was a significant effect
295 of maternal GWG on beta diversity in infants of multiparous (R^2=0.378, p=0.0011), but not primiparous,
296 mothers (R^2=0.0950, p=0.377; Fig. 5b). Infant beta diversity did not differ by maternal pBMI category
297 (R^2=0.0511, p=0.565) or infant sex (R^2 = 0.0126, p=0.914). Differentially abundant taxa were identified
298 by DESeq2 (RRID: SCR_015687). Excess maternal GWG was associated with an increase in
299 *Bifidobacterium* in the infant gut microbiota (Fig. 6a-c), echoing increased levels of this genus in the
300 maternal gut microbiota during pregnancy (Fig. 3c). Excess GWG was also associated with a decreased
301 relative abundance of *Bacteroides* in infants of multiparous, but not, primiparous mothers (Fig. 6c).
302 Maternal pBMI >25 was associated with a decreased relative abundance of Clostridiales genera *Blautia*
303 and *Intestinibacter* in the infant gut microbiota, which were also decreased by pBMI >25 in the maternal
304 gut microbiota. Members of the order Clostridiales typically increase in abundance with the introduction
305 of solid food in association with increased levels of propionate and butyrate (Tsukuda *et al.*, 2021), and
306 have previously been found to protect against colonization by enteric pathogens (Kim *et al.*, 2017).
307 Depleted *Blautia* in obese children has been linked to intestinal inflammation (Benítez-Páez *et al.*, 2020)

308 which is consistent with an observed association between maternal excess adiposity with childhood
309 obesity and inflammation (Singer and Lumeng, 2017). The overlap in differentially abundant taxa
310 between the maternal and infant gut microbiota is consistent with vertical transmission of the maternal gut
311 microbiota(Ferretti *et al.*, 2018). In addition to previous reports linking maternal GWG and BMI to shifts
312 in the infant gut microbiota (Baumann-Dudenhoeffer et al., 2018; Collado et al., 2010; Galley et al., 2014;
313 Mueller et al., 2016; Robinson et al., 2017; Stanislawski et al., 2017; Tun et al., 2018), we now show that
314 that parity modulates the impact of maternal GWG on the infant gut microbiota, and that this observation
315 is likely driven by the effect of parity on the maternal gut microbiota.

316 Discussion

317 Maternal metabolic adaptations to pregnancy mirror changes seen in non-pregnant obesity and the gut
318 microbiota have been implicated in mediating these adaptations (Koren *et al.*, 2012), but few studies have
319 investigated the gut microbiota longitudinally over the course of pregnancy (DiGiulio *et al.*, 2015; Ruebel
320 *et al.*, 2021). In this study, we investigated the independent and combined impacts of maternal pBMI and
321 GWG on the maternal gut microbiota over the course of pregnancy and during lactation, and the infant
322 gut microbiota at 6 months of age. Overall, our study suggests that parity modulates the impact of pBMI
323 on pregnancy-associated remodeling of the maternal gut microbiota, colonization of the neonatal gut, and
324 the impact of GWG and antibiotic exposure on the infant gut microbiota.

325 We found that the gut microbiota of participants with higher pBMI changed less over the course of
326 pregnancy in primiparous, but not multiparous, participants. Due to the large effect of intraindividual
327 variation on beta diversity across all samples, the effects of sample time point and pBMI were relatively
328 small. Previous studies of the gut microbiota have found little-to-no overall effect of pBMI on overall
329 beta diversity in the first trimester (Gomez-Arango *et al.*, 2016) and across pregnancy (Faucher *et al.*,
330 2020; Ruebel *et al.*, 2021) but have not investigated beta diversity within individuals over the course of
331 pregnancy. Shifts in the gut microbiome due to diet or antibiotic perturbation are highly individual-
332 specific (Fassarella *et al.*, 2021) and our data suggest pregnancy-associated remodeling of the gut
333 microbiota is also individual-specific. Our observed decrease in intra-individual beta diversity with
334 increasing pBMI in primiparous participants is consistent with a decreased magnitude of metabolic
335 adaptation to pregnancy with increasing pBMI (Forbes *et al.*, 2015; Mouzon and Lassance, 2015; Tinius
336 *et al.*, 2020), and highlights the necessity of longitudinal sampling for investigations of pregnancy-
337 associated microbiota remodeling.

338 As this remodeling was not associated with pBMI in multiparous participants, microbiotal adaptations to
339 pregnancy appear to be modulated by previous pregnancies. First-pregnancies are associated with greater
340 maternal constraint (Gluckman and Hanson, 2004; Juntunen, 1997), which may include reduced or
341 delayed metabolic adaptations to pregnancy (Abu-Heija et al., 2017). The impact of parity on the gut
342 microbiome and its contribution to metabolic adaptations to pregnancy has not previously been
343 investigated in human pregnancy. Recently, Berry et al. (2021) found that maternal microbiotal
344 adaptations to pregnancy occurred more rapidly with increasing parity in pigs. This may reflect increased
345 maternal metabolic flexibility resulting from persistent effects of adaptations to first pregnancy
346 (Deslypere et al., 1990). Whether the same is true in humans is still unclear, but our data support the
347 notion that parity influences microbial community composition and must be taken into account when
348 investigating human pregnancy-related microbial (and intestinal) adaptations.

349 Maternal adaptations to pregnancy include increased intestinal surface area and increased transit time,
350 allowing increased nutrient uptake to provide the energy required by fetal development and lactation
351 (Astbury et al., 2015). SCFAs may support these intestinal adaptations: butyrate promotes proliferation of
352 the intestinal epithelium and propionate promotes migration of intestinal epithelial cells (IECs) up the
353 crypt-villus axis (Bilotta et al., 2021). Both butyrate (Wang et al., 2012) and propionate (Tong et al.,
354 2016) contribute intestinal barrier maintenance. Although human data on intestinal adaptations to
355 pregnancy are limited, animal models have found excess adiposity during pregnancy is associated with
356 decreased intestinal length and increased intestinal permeability (Ribeiro et al., 2022). We found that
357 parity modulated the impact of pBMI and GWG on the relative abundance of maternal fecal SCFAs. In
358 primiparous participants, GWG above the recommended range was associated with increased butyrate
359 and propionate. This is consistent with a study that found increased fecal propionate in pregnant
360 individuals with a pBMI>25 compared to pBMI<25, although parity and GWG were not reported
361 (Szczuko et al., 2020). As SCFAs are a fuel source for IECs (Morrison and Preston, 2016), increased fecal
362 SCFAs could be due to decreased uptake by IECs resulting from reduced intestinal adaptations to
363 pregnancy, but we did not test this hypothesis.

364 Alternatively, differences in fecal SCFA levels may result from shifts in microbial production due to
365 altered microbiota community composition. *Prevotella*-dominant microbiota produce more propionate—
366 and more SCFAs overall—than *Bacteroides*-dominant microbiota (Chen et al., 2017). This is consistent
367 with our finding that both *Prevotella* and propionate were increased in participants with a pBMI>25,
368 while *Bacteroides* was decreased, particularly in primiparous participants. A previous study also found
369 decreased *Bacteroides* in the first and third trimester with pre-pregnancy overweight (Santacruz et al.,
370 2010), although others have reported increased levels in the third trimester with pre-pregnancy

371 overweight but not obesity (Collado et al., 2008; Sugino et al., 2019). Crusell et al. (2018) found
372 *Prevotella* abundance was positively correlated with oral glucose-stimulated plasma glucose in the third
373 trimester of pregnancy, but did not differ by pBMI, potentially due to the low prevalence of this taxon (23
374 of 184 participants). In independent studies, *Prevotella* has been associated with improved glucose
375 tolerance (Kovatcheva-Datchary et al., 2015), impaired glucose tolerance and insulin resistance (Pedersen
376 et al., 2016), and increases susceptibility to colitis (Scher et al., 2013). This functional diversity mirrors
377 that of *Bacteroides* and suggests the impacts of both genera depend on host physiology including
378 pregnancy history.

379 Shifts in the maternal gut microbiota can impact the infant gut microbiome by (1) altering fetal intestinal
380 development through the *in-utero* environment and (2) direct transmission of maternal microbes to the
381 infant during and after birth. Our finding that the proportion of meconium samples with any sequencing
382 reads varied by delivery mode is consistent with our previous report that fetal meconium does not have a
383 microbiome (Kennedy et al., 2021). The greatest proportion of meconium samples without any reads (i.e.
384 low microbial presence)—and the lowest number of reads per sample—came from neonates delivered by
385 primary C-section and therefore not exposed to the maternal vaginal or fecal microbiota during labour or
386 delivery. Delivery mode has been shown to impact the mother-to-infant transmission of key bacterial
387 taxa, including the genus *Bifidobacterium* (Makino et al., 2013). We found that *Bifidobacterium* was
388 increased not only in all multiparous participants, but also in the meconium of their infants born via
389 spontaneous vaginal delivery, compared to primiparous participants. Disruption of this vertical
390 transmission may have persistent effects on the infant gut microbiota, as we found perinatal antibiotic
391 exposure was associated with a decrease in *Bifidobacterium* in the infant gut microbiota at 6 months of
392 age. Unexpectedly, we found that infant sex also impacted the bacterial taxa present in meconium of
393 vaginally delivered infants; females were dominated by Clostridiales and males by Enterobacteriales.
394 These data are consistent with one previous report (Cong et al., 2016). Although the mechanism behind
395 this sex-effect is unclear, it is possible this is related to sex-specific differences in intestinal development.
396 Other corroborative data include higher rates of necrotizing enterocolitis in preterm males (Boghossian et
397 al., 2018). These data suggest that the impacts of parity on the maternal microbiome may be transmitted
398 to the next generation and could be sex-specific. Future studies should continue to combine parity with
399 infant sex in determining impacts on offspring microbial communities.

400 Parity modulated the impact of maternal GWG on infant characteristics at birth and the infant gut
401 microbiota at 6 months of age. GWG above the recommended range is often associated with a reduced
402 risk of small for gestational age (SGA) birth weight and an increased risk of large for gestational age
403 (LGA) birth weight (Goldstein et al., 2017), although some data suggest that maternal obesity can also be

404 associated with intrauterine growth restriction (IUGR) (Radulescu et al., 2013). Little to no data exist on
405 the modulating effect of parity. We found excess GWG was associated with higher birth weight in
406 multiparous participants, and a trend towards lower birthweight in primiparous participants. Others have
407 shown that excess GWG is associated with increased intrapartum weight retention (Nehring et al., 2011) –
408 which may then predispose multiparous pregnant individuals to excess adiposity. These impacts of excess
409 GWG were also associated with a shift in microbiota beta diversity at 6 months of age in infants of
410 multiparous—but not primiparous—participants, who also had a decrease in *Bacteroides* and increase in
411 *Bifidobacterium* reflecting similar shifts in the maternal gut microbiota. Therefore, health behaviours both
412 during pregnancy and the postpartum/intrapartum interval should be supported not only for maternal
413 health but also infant microbiotal health.

414 Our study has both strengths and limitations. Our longitudinal sampling of a prospective pregnancy
415 cohort allowed us to explore beta-diversity within participants to overcome the overwhelming effect of
416 inter-individual variation. However, as we recruited participants in the first trimester of pregnancy, we are
417 not able to address pregnancy-associated shifts in the gut microbiota that may occur early in gestation.
418 We also show functional outcomes of observed microbial shifts in our analysis of fecal SCFA levels, but
419 did not quantify circulating SCFA levels, which more directly modulate host metabolism (Müller et al.,
420 2019). Finally, although our study is unique among studies of the pregnancy microbiome in its design and
421 sample size, we were unable to examine any interacting effects of pBMI and GWG as the vast majority of
422 participants with high pBMI had GWG above the recommended range.

423 In conclusion we show that parity modulates impact of pBMI on pregnancy-associated remodeling of the
424 maternal gut microbiota, colonization of the neonatal gut, and the impact of GWG on the infant gut
425 microbiota. We also show that high pBMI is associated with diminished remodeling of the gut
426 microbiome during pregnancy, which may reflect a reduced requirement for metabolic adaptations to
427 pregnancy. Overall, these data support a role for the gut microbiota in modulating maternal metabolic
428 adaptations to pregnancy and suggest the gut microbiome may retain an "ecological memory"(Letourneau
429 et al., 2022) of prior pregnancies (Berry et al., 2021).

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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.

Figure Legends

Figure 1. Parity modulates impact of pBMI and GWG on pregnant gut microbiota. a, Boxplots of Shannon alpha diversity by gestational weight gain (GWG) category in primiparous participants (circles; n=23) and multiparous participants (stars; n=13) with a pBMI <25. Alpha diversity differed by GWG in multiparous participants (p=-0.013) but not primiparous participants (p=0.76). Significance assessed by a linear mixed model (Kenward–Roger d.f.), with GWG category as a fixed effect and participant ID as a random 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**, Shannon alpha diversity is positively correlated with pBMI across all participants with excess GWG (R^2=0.12, p=0.0074), particularly in multiparous participants (n=14, R^2=0.21, p=0.036) but not primiparous participants (n=20, R^2=0.073, p=0.11). Significance assessed by linear mixed model (Kenward–Roger d.f.), with pBMI as a fixed effect and participant ID as a random effect. **c-d**, PCoA of Bray-Curtis distances in primiparous (n=39) and multiparous (n=23) participants shows modest clustering by **c**, GWG category (primiparous, R^2=0.039, p<0.0001; multiparous R^2=0.068, p<0.0001) and **d**, pBMI category (primiparous, R^2=0.041, p<0.0001; multiparous R^2=0.071, p<0.0001). Significance was assessed by PERMANOVA. **e**, Bray-Curtis dissimilarity at each timepoint to all other timepoints within individual participants are negatively associated with pBMI in primiparous participants, but not in multiparous participants.

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). 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, GWG category, and timepoint as fixed effects and participant ID as a random effect.

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), 25-30 (red), and >30 (yellow) 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 by pBMI category overall (filled dots) and in primiparous (no fill dots) and multiparous

476 (stars) participants (dot size based on mean abundance, colours correspond to genus colour in a where a
477 differentially abundant genus is among the 20 most abundant genera).

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

487 **Figure 5. Taxonomic shifts associated with maternal diet. a,** Principal Component Analysis (PCA)
488 dimensionality reduction of the Food Frequency Questionnaire (FFQ) factors was performed and the
489 relative contributions (loadings) of these factors to Principle Component 1 (PC1), PC2, and PC3 are
490 shown. **b,** Dot plots showing DESeq2 results of differentially abundant genera between individuals who
491 score in the top tertile for each PC versus individuals who scored in the bottom tertile (dot size based on
492 mean abundance, colours correspond to genus colour in a where a differentially abundant genus is among
493 the 20 most abundant genera).

494 **Figure 6. Parity modulates impact of GWG on infant gut microbiota. a**, Box plots of alpha diversity
495 by gestational weight gain (GWG) category in primiparous participants (n=26) and multiparous
496 participants (n=14). Alpha diversity (observed ASVs) was significantly increased by GWG above the
497 recommended range (p=0.034) in infants of participants with pBMI <25. Significance assessed by a linear
498 model (Kenward–Roger d.f.), with GWG category as a fixed effect. The box plot centre line represents
499 the median; the box limits represent the upper and lower quartiles; the whiskers represent the 1.5×
500 interquartile range; the points represent the outliers. **b**, PCoA of Bray-Curtis distances shows modest
501 clustering by GWG category (primiparous, R^2=0.039, p<0.0001; multiparous R^2=0.068, p<0.0001).
502 Significance was assessed by PERMANOVA.

503 **Figure 7. Taxonomic shifts with maternal GWG in infant gut microbiota. a-b**, The relative
504 abundances of the 20 most abundant genera in the infant gut microbiota by GWG below (orange), within
505 (purple), and above (dark blue) the recommended range are shown as **a**, mean relative abundance in taxa
506 bar plots and **b**, mean ± standard error of the mean for primiparous (below, n=4; within, n=7; above,

n=12) and multiparous (within, n=5; above, n=8) participant with pBMI <25. **c**, Dot plots showing DESeq2 results of differentially abundant genera 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).

Star Methods

Resource availability

Lead contact

Further information and requests should be directed to the lead contact, Deborah Sloboda (sloboda@mcmaster.ca)

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Sequencing data have been deposited at [repository] and are publicly available as of the data of publication. Accession numbers are listed in the key resources table.
- All original code has be deposited at [repository] 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

526 # Experimental model and subject details

527 ## Study design

528 The study protocol was reviewed and approved by the Charité ethics committee (EA 4/059/16). All
529 participants provided written informed consent. Study participants were part of an ongoing research
530 program at the Charité University Berlin. Healthy females >18 years of age with singleton pregnancies
531 were prospectively recruited between 9-17 weeks of gestation. We excluded participants who (1) had
532 severe chronic gastrointestinal diseases or conditions, (2) had any significant heart, kidney, liver, or
533 pancreatic diseases, (3) had pre-existing diabetes, (4) had depression, or (5) delivered infants with severe
534 malformations noted at birth. A total of 89 participants were included in the study. We additionally
535 excluded maternal and infant samples from participants who smoked during pregnancy and excluded
536 maternal samples from participants who took antibiotics during pregnancy. After these exclusions, this
537 study included first-pass meconium samples from 70 participants, infant samples from 58 participants,
538 and maternal samples from 65 participants.

539 ## Sample collection

540 Participants were evaluated at the end of the first trimester (9-17 weeks gestation), second trimester (26-
541 28 weeks), and third trimester (>33 weeks) of pregnancy, at delivery, and at 6 months postpartum. Pre-
542 pregnancy body mass index (pBMI) was obtained from measured height recruitment and recalled pre-
543 pregnancy weight. BMI was categorized into normal weight (18.5-24.9kg/m2), overweight (25-29.9
544 kg/m2), and obese (>30<40 kg/m2). At each study visit, participant weight was measured and participants
545 completed a 25-item food frequency questionnaire (FFQ) (Atkinson *et al.*, 2022) adapted from the
546 validated PrimeScreen FFQ (Rifas-Shiman *et al.*, 2001). Participant collected fecal samples at home prior
547 to each study visit following a standardized sterile stool collection protocol. Additionally, offspring fecal
548 samples were obtained after delivery (meconium) and at six months of age. Stool was frozen at -20°C
549 immediately after collection in provided sample collection bags and stored at -80°C upon arrival until
550 further use. None had taken prebiotics, laxatives, or diarrhea inhibitors in the days before the sampling.

551 # Method details

552 ## DNA extraction and amplification

553 Genomic DNA (gDNA) was extracted from fecal samples as described previously (Whelan *et al.*, 2014)
554 with the addition of a mechanical lysis step using 0.2 g of 2.8 mm ceramic beads to improve extraction
555 efficiency and without mutanolysin. PCR amplification of the variable 3 and 4 (V3-V4) regions of the
556 16S rRNA gene was performed on the extracted DNA from each sample using methods previously
557 described(Bartram *et al.*, 2011). Each reaction contained 5 pmol of primer (341F –
558 CCTACGGGNGGCWGCAG, 806R – GGACTACNVGGGTWTC-TAAT), 200 mM of dNTPs, 1.5µl 50
559 mM MgCl2, 2 µl of 10 mg/ml bovine serum albumin (irradiated with a transilluminator to eliminate
560 contaminating DNA) and 0.25µl Taq polymerase (Life Technologies, Canada) for a total reaction volume
561 of 50 µl. 341F and 806R rRNA gene primers were modified to include adapter sequences specific to the
562 Illumina technology and 6-base pair barcodes were used to allow multiplexing of samples.

563 ## 16S rRNA gene sequencing

564 16S DNA products of the PCR amplification were sequenced using the Illumina MiSeq platform
565 (2x300bp) at the Farncombe Genomics Facility (McMaster University, Hamilton ON, Canada). Primers
566 were trimmed from FASTQ files using Cutadapt (Martin, 2011) (RRID: SCR_011841) and
567 DADA2(Callahan *et al.*, 2016) was used to derive amplicon sequence variants (ASVs). Taxonomy was
568 assigned using the Silva 132 reference database (Quast *et al.*, 2013). Non-bacterial ASVs were culled
569 (kingdom Eukaryota, family Mitochondria, order Chloroplast, or no assigned phylum), as was any ASV
570 to which only 1 sequence was assigned.

571 ## Quantification of short-chain fatty acids

572 SCFA levels were measured in a subset of fecal samples by the McMaster Regional Centre of Mass
573 Spectrometry. A weight equivalent amount of 3.7% HCl, 10 µL of internal standard, and 500 µL of
574 diethyl ether was added to each fecal sample and vortexed for 15 min. After vortexing, 400 µL of diethyl
575 ether fecal extract was transferred to a clean 1.5 mL Eppendorf tube. In a chromatographic vial containing
576 an insert, 20 µL of N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide was added, after which 60 µL of
577 diethyl ether fecal extract was added. The mixture was incubated at room temperature for 1 hour and
578 analyzed using gas chromatography-mass spectrometry (6890N GC, coupled to 5873N Mass Selective

579 Detector; Agilent Technologies, Santa Clara, CA, USA). Statistical significance was assessed by mixed
580 linear model with pBMI, GWG category, and parity as fixed effects and participant ID as a random effect,
581 and multiple comparisons by pBMI and GWG category were performed within primiparous and
582 multiparous participants (Kenward-Rogers degrees of freedom with Tukey adjustment for multiple
583 comparisons).

584 Quantification and statistical analysis

585 Participant characteristics

586 Participant characteristic variables shown in Tables S1 and S6 were analyzed in R using the gtsummary
587 package (RRID: SCR_021319). Normality was determined by Shapiro-Wilk test (rstatix; RRID:
588 SCR_021240) and significance was assessed by Kruskal-Wallis test and false discovery rate correction
589 for multiple testing.

590 16S analysis

591 We performed alpha and beta-diversity analyses in R using phyloseq (McMurdie and Holmes, 2013)
592 (RRID: SCR_013080) and tested for whole community differences across groups using vegan's(Oksanen
593 *et al.*, 2013) (RRID: SCR_011950) implementation of permutational multivariate analysis of variance
594 (PERMANOVA) in the adonis command. These results were visualized via Principal Coordinate
595 Analysis (PCoA) ordination using R's ggplot2 package (RRID: SCR_014601) (Wickham, 2016).
596 Significance of alpha diversity was analyzed by linear mixed model with pBMI and GWG categories as
597 fixed effects and participant ID as a random effect. Significance of Bray-Curtis dissimilarity was analyzed
598 within primiparous and multiparous participants by mixed linear model with pBMI, GWG category, and
599 sample time point as fixed effects and participant ID as a random effect. Differential abundance analysis
600 was performed using DESeq2 (RRID: SCR_015687) across all pregnancy time points. Due to the
601 interdependence of pBMI and GWG, taxa differing by pBMI category were analyzed within participants
602 with excess GWG and taxa differing by GWG category were assessed within participants with a pBMI
603 <25.

604 SCFA analysis

605 Statistical significance was assessed by mixed linear model with pBMI, GWG category, and parity as
606 fixed effects and participant ID as a random effect, and multiple comparisons by pBMI and GWG

607 category were performed within primiparous and multiparous participants (Kenward-Rogers degrees of
608 freedom with Tukey adjustment for multiple comparisons). All statistical details, including statistical tests
609 used, sample sizes, and definition of center and dispersion can be found in the figure legends.

610

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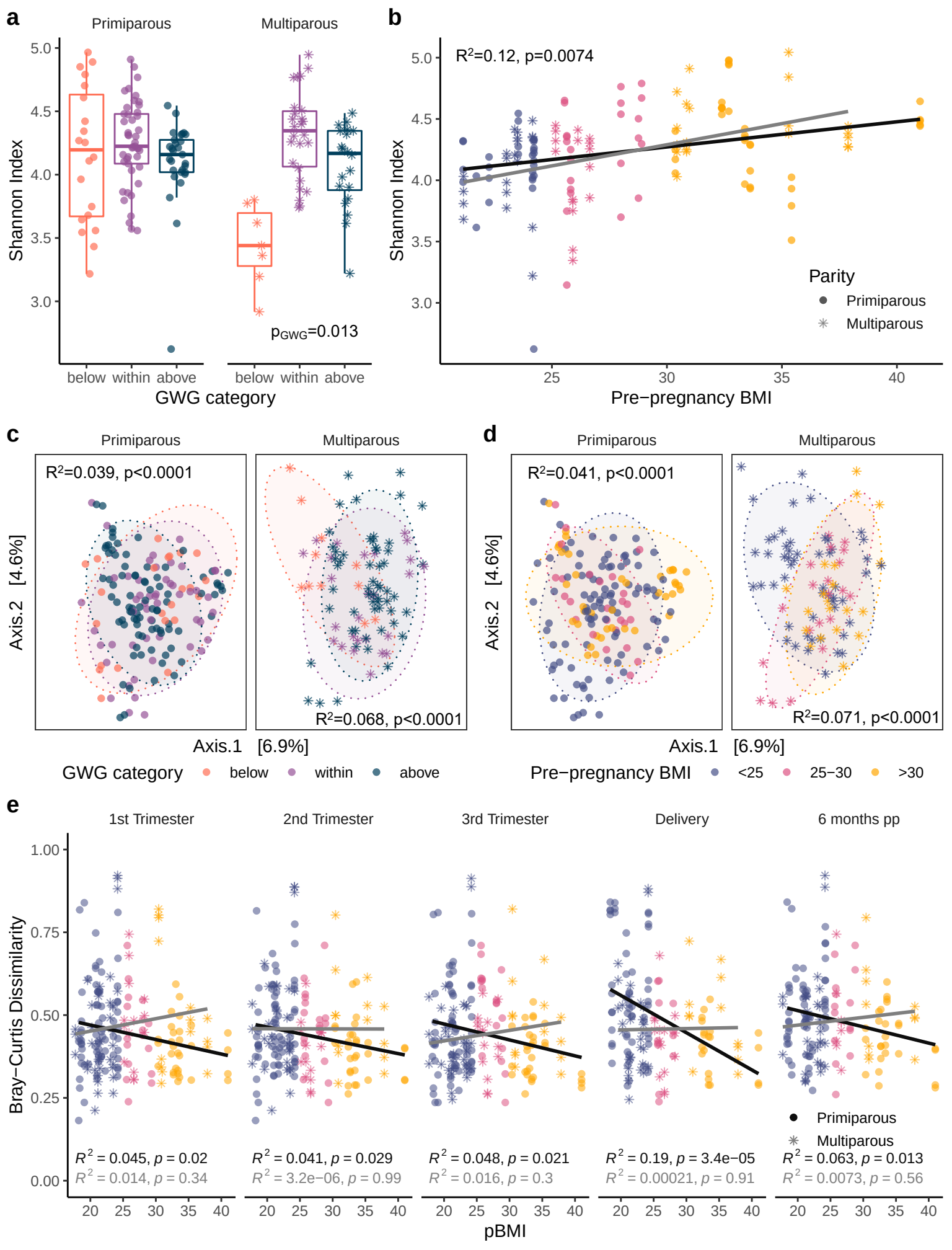
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Primiparous
Multiparous
Shannon Index
5.0
4.5
4.0
3.5
3.0
p_{GWG}=0.013
below within above
below within above
GWG category
b
R^2=0.12, p=0.0074
Shannon Index
Pre−pregnancy BMI
25
30
35
40
Parity
Primiparous
Multiparous
c
Primiparous
Multiparous
R^2=0.039, p<0.0001
R^2=0.068, p<0.0001
Axis.2 [4.6%]
Axis.1 [6.9%]
GWG category
below
within
above
d
Primiparous
Multiparous
R^2=0.041, p<0.0001
R^2=0.071, p<0.0001
Axis.2 [4.6%]
Axis.1 [6.9%]
Pre−pregnancy BMI
<25
25−30
>30
e
1st Trimester
2nd Trimester
3rd Trimester
Delivery
6 months pp
Bray−Curtis Dissimilarity
1.00
0.75
0.50
0.25
0.00
R^2 = 0.045, p = 0.02
R^2 = 0.014, p = 0.34
R^2 = 0.041, p = 0.029
R^2 = 3.2e−06, p = 0.99
R^2 = 0.048, p = 0.021
R^2 = 0.016, p = 0.3
R^2 = 0.19, p = 3.4e−05
R^2 = 0.00021, p = 0.91
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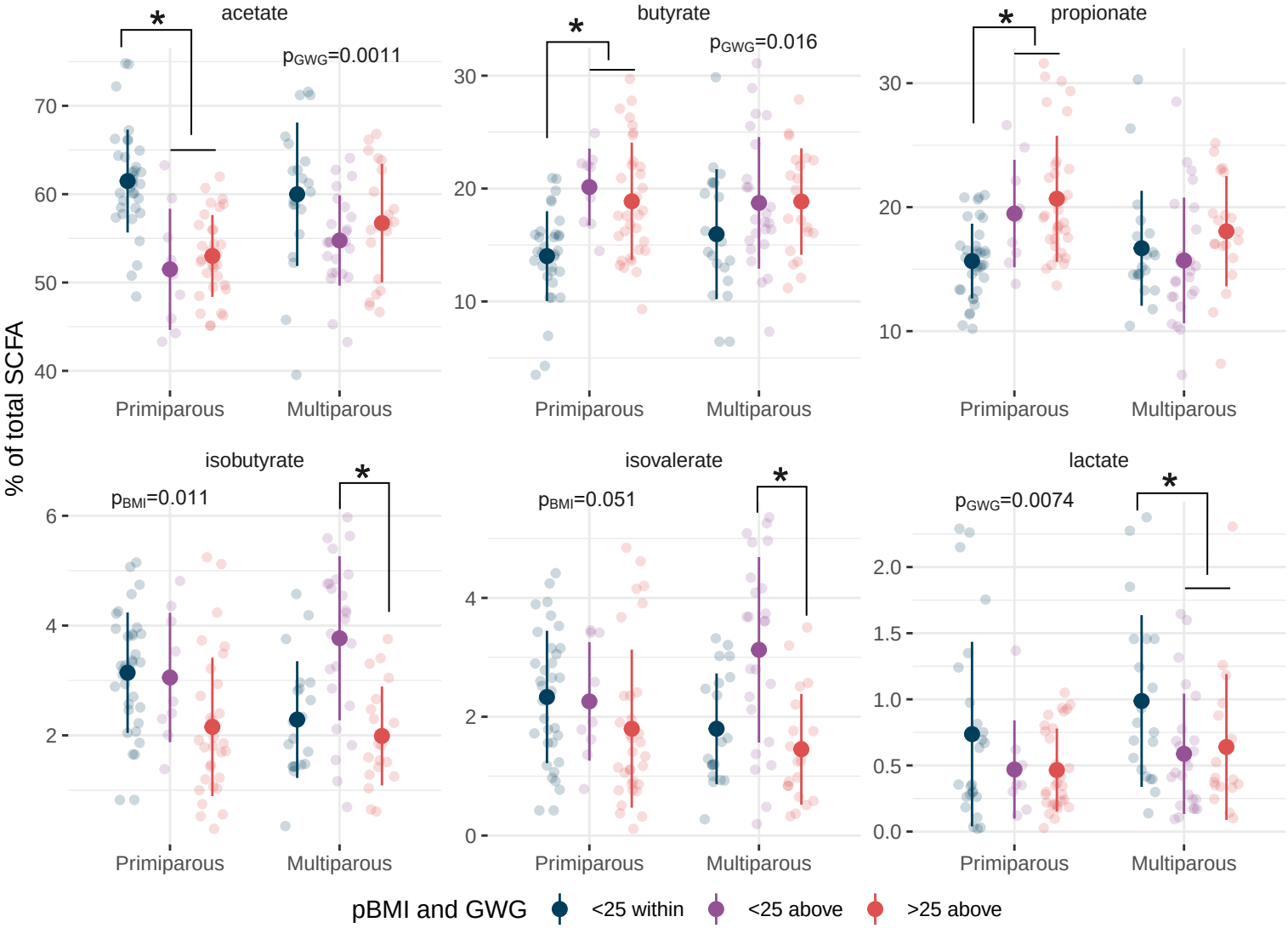
Primiparous
Multiparous
20 25 30 35 40
pBMI


acetate
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butyrate
p_{GWG}=0.016
propionate
isobutyrate
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isovalerate
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% of total SCFA
Primiparous
Multiparous
pBMI and GWG
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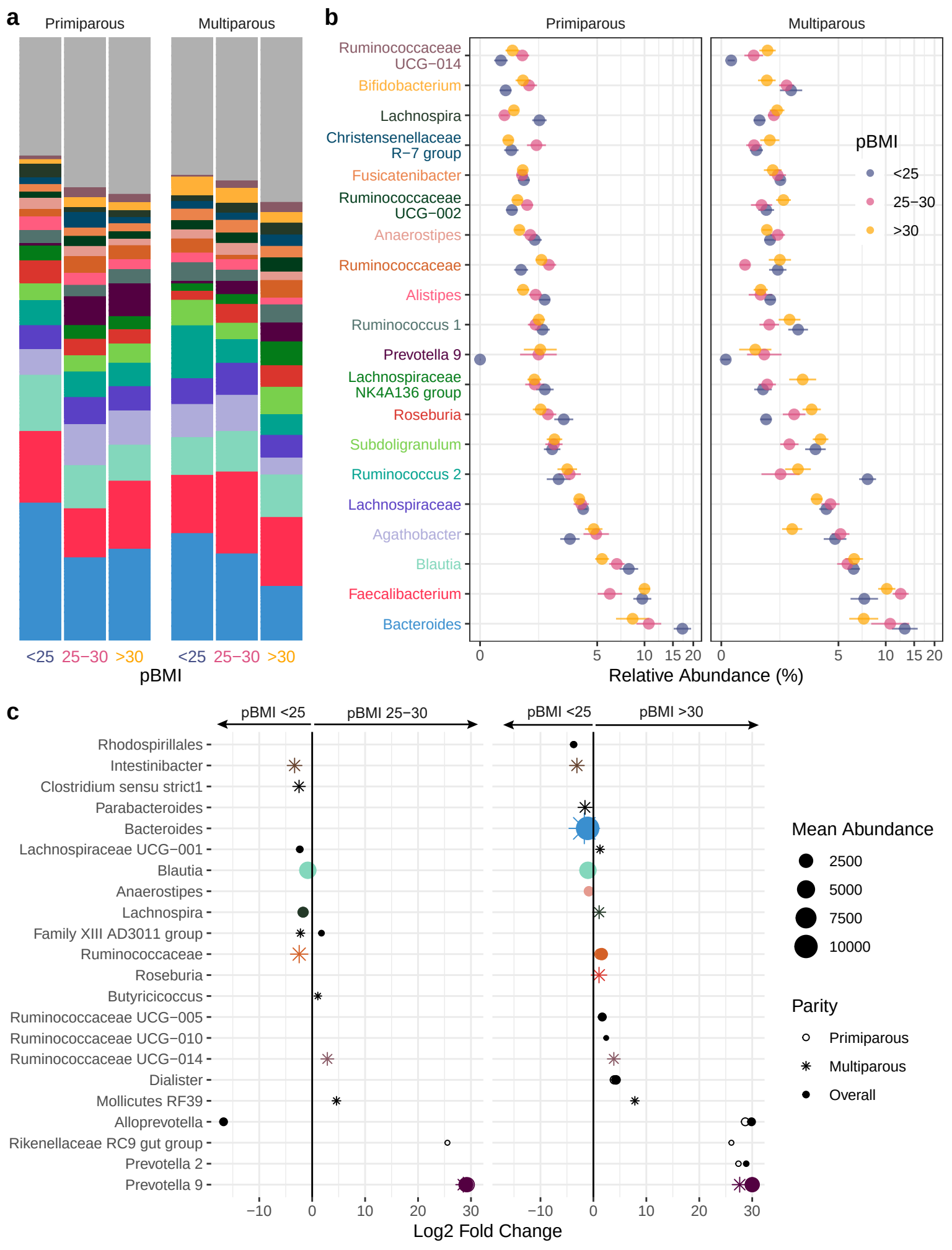
<25 above
>25 above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pBMI
b
Primiparous
Multiparous
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Bifidobacterium
Lachnospira
Christensenellaceae R−7 group
Fusicatenibacter
Ruminococcaceae UCG−002
Anaerostipes
Ruminococcaceae
Alistipes
Ruminococcus 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
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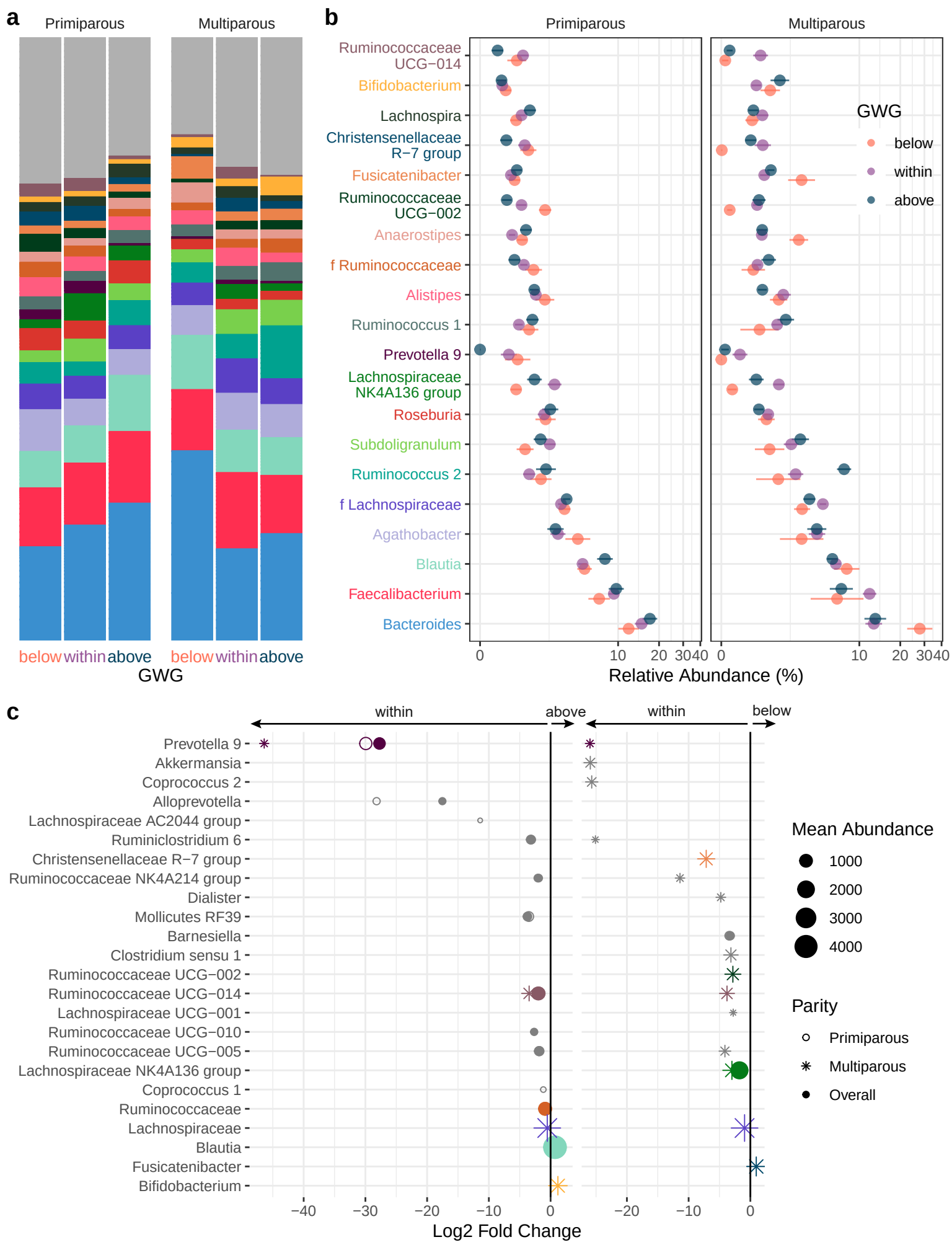
5000
7500
10000
Parity
Primiparous
Multiparous
Overall


a
Primiparous
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below within above
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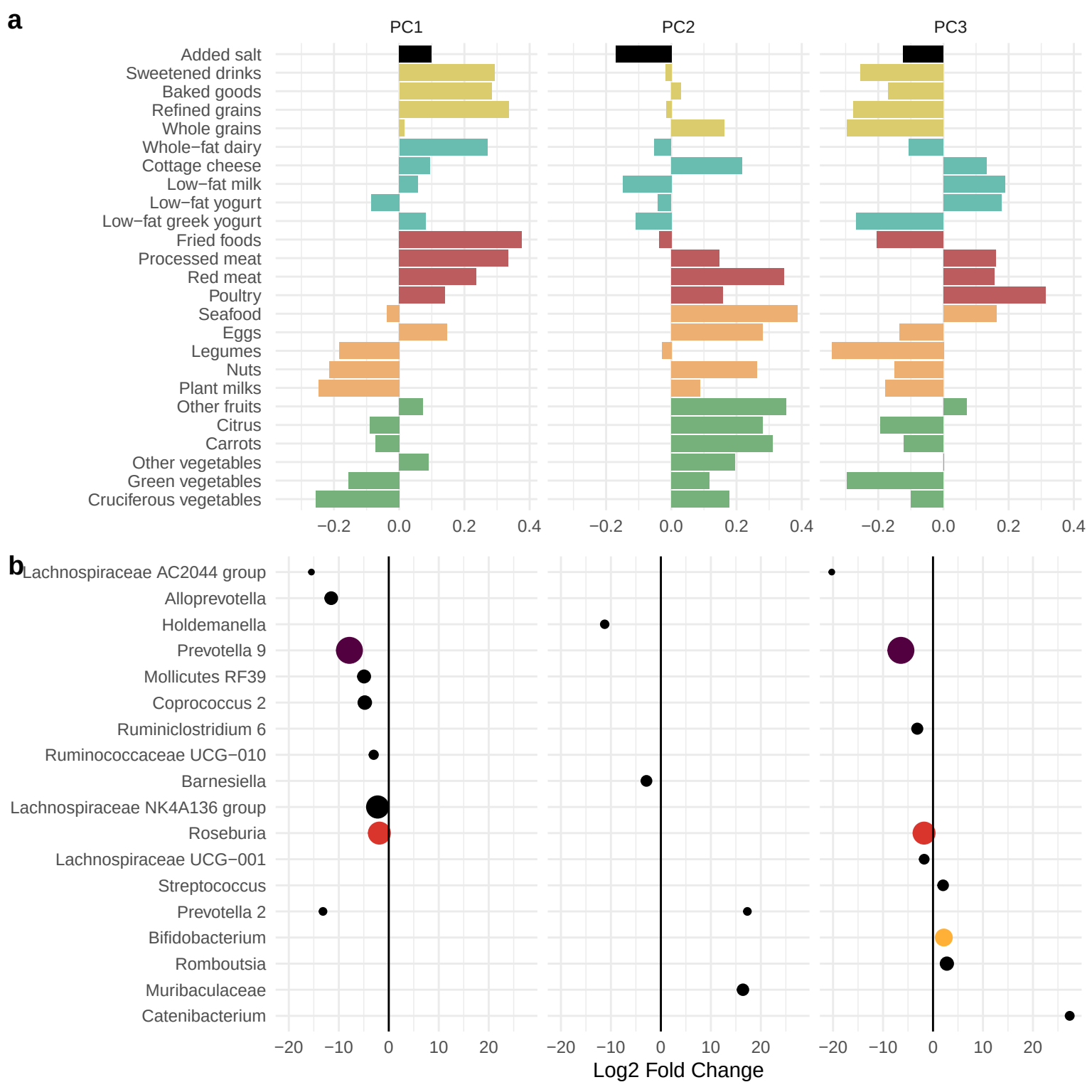
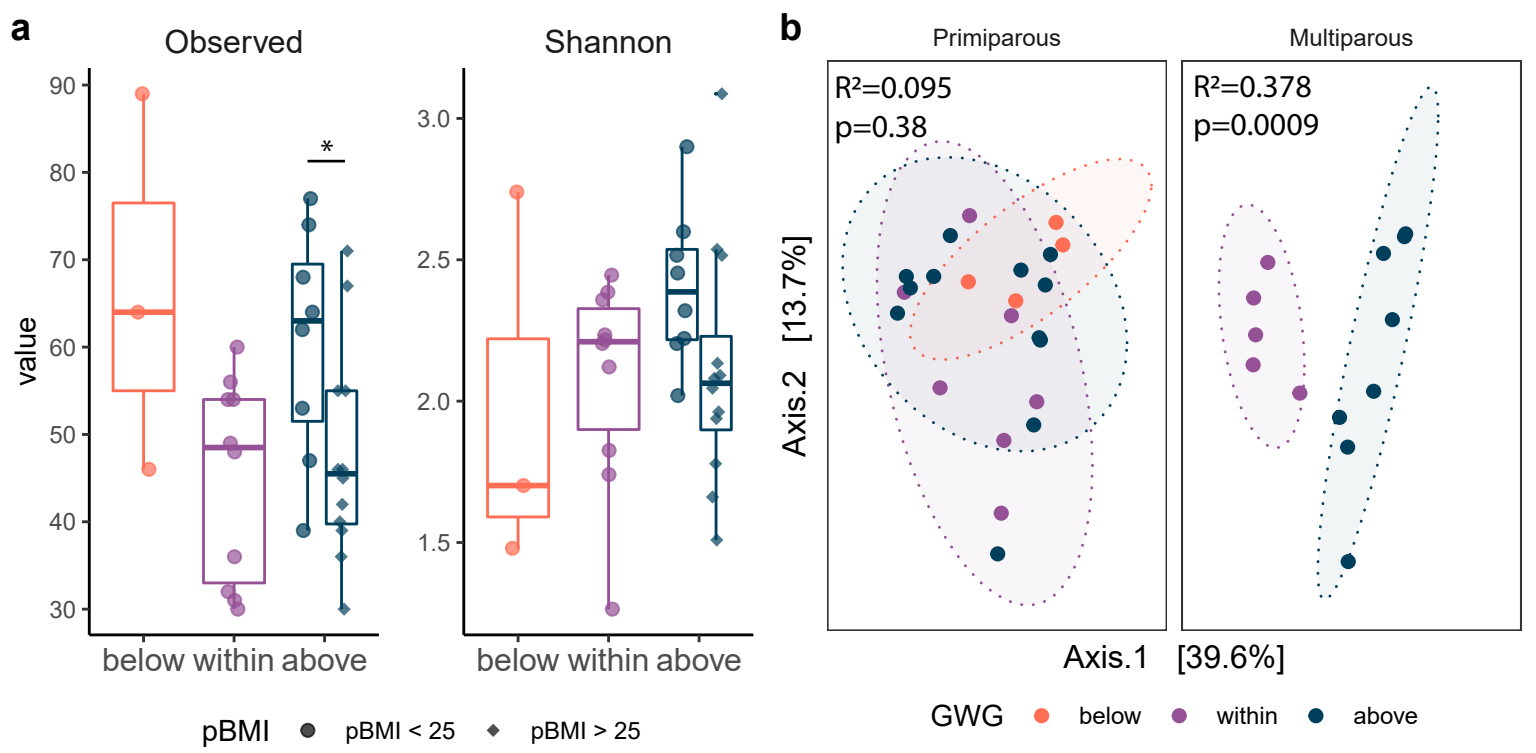
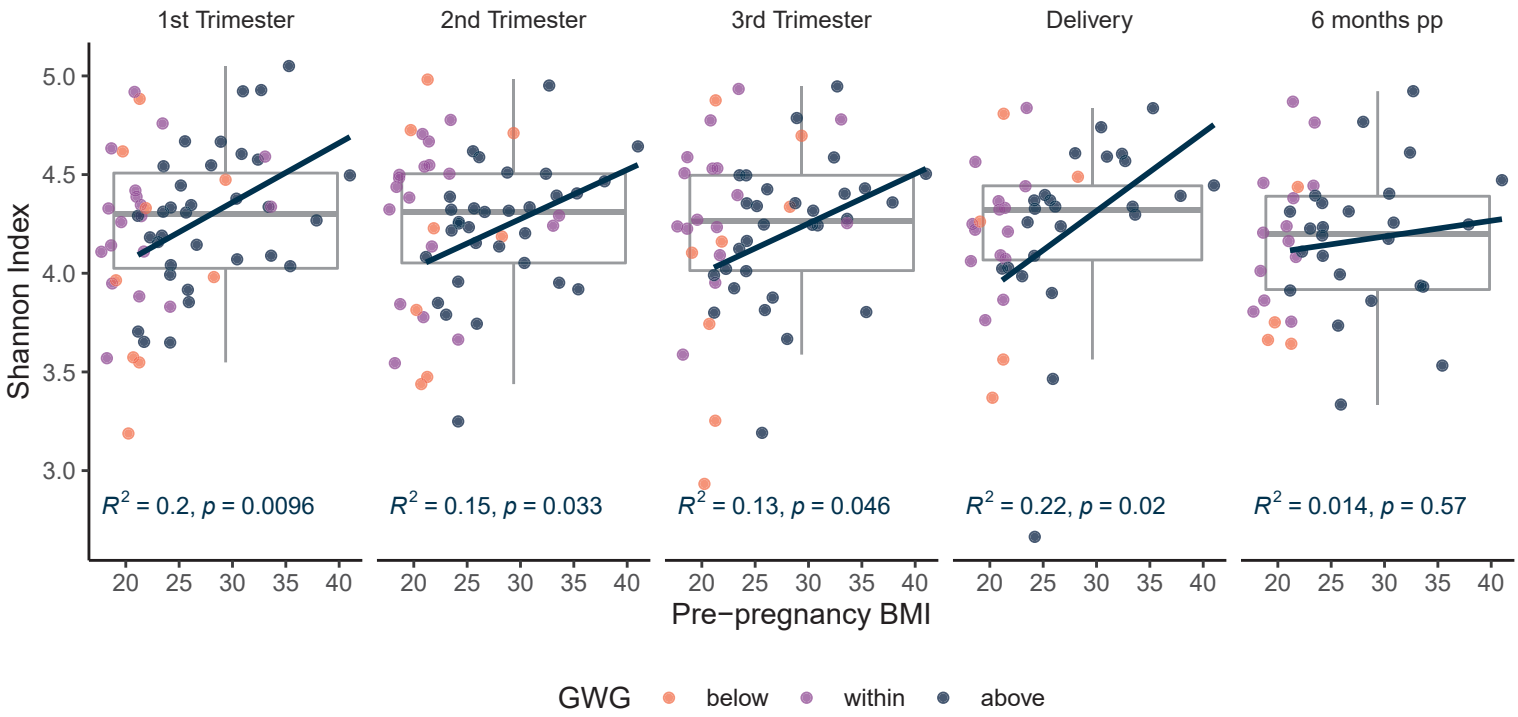
Multiparous
Ruminococcaceae UCG−014
Bifidobacterium
Lachnospira
Christensenellaceae R−7 group
Fusicatenibacter
Ruminococcaceae UCG−002
Anaerostipes
f Ruminococcaceae
Alistipes
Ruminococcus 1
Prevotella 9
Lachnospiraceae NK4A136 group
Roseburia
Subdoligranulum
Ruminococcus 2
f Lachnospiraceae
Agathobacter
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

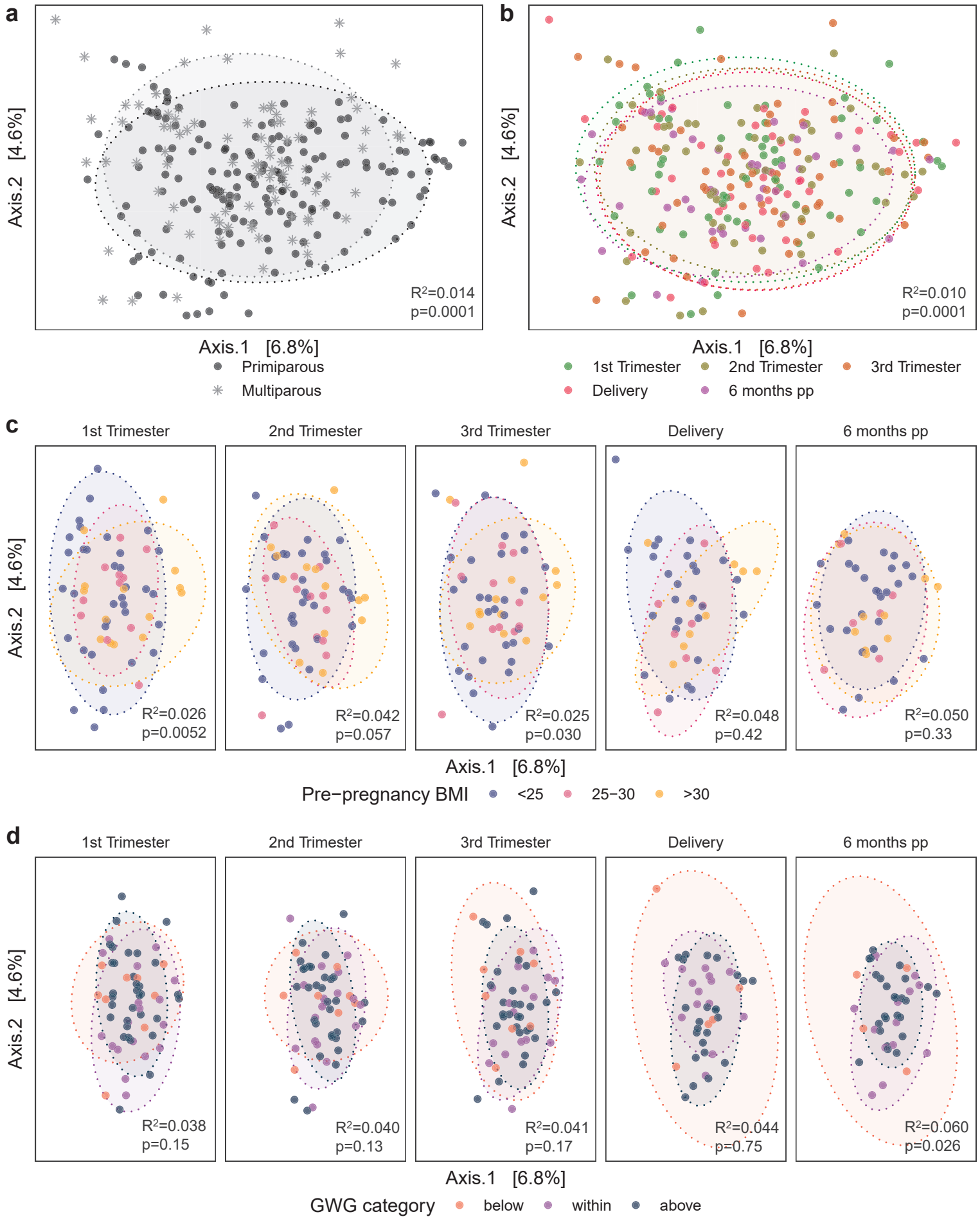

a
PC1
PC2
PC3
Added salt
Sweetened drinks
Baked goods
Refined grains
Whole grains
Whole−fat dairy
Cottage cheese
Low−fat milk
Low−fat yogurt
Low−fat greek yogurt
Fried foods
Processed meat
Red meat
Poultry
Seafood
Eggs
Legumes
Nuts
Plant milks
Other fruits
Citrus
Carrots
Other vegetables
Green vegetables
Cruciferous vegetables
−0.2
0.0
0.2
0.4
b
Lachnospiraceae AC2044 group
Alloprevotella
Holdemanella
Prevotella 9
Mollicutes RF39
Coprococcus 2
Ruminiclostridium 6
Ruminococcaceae UCG−010
Barnesiella
Lachnospiraceae NK4A136 group
Roseburia
Lachnospiraceae UCG−001
Streptococcus
Prevotella 2
Bifidobacterium
Romboutsia
Muribaculaceae
Catenibacterium
−20
−10
0
10
20
Log2 Fold Change


a
Observed
Shannon
value
90
80
70
60
50
40
30
3.0
2.5
2.0
1.5
*
below within above
below within above
pBMI
pBMI < 25
pBMI > 25
b
Primiparous
Multiparous
R²=0.095
p=0.38
R²=0.378
p=0.0009
Axis.2 [13.7%]
Axis.1 [39.6%]
GWG
below
within
above

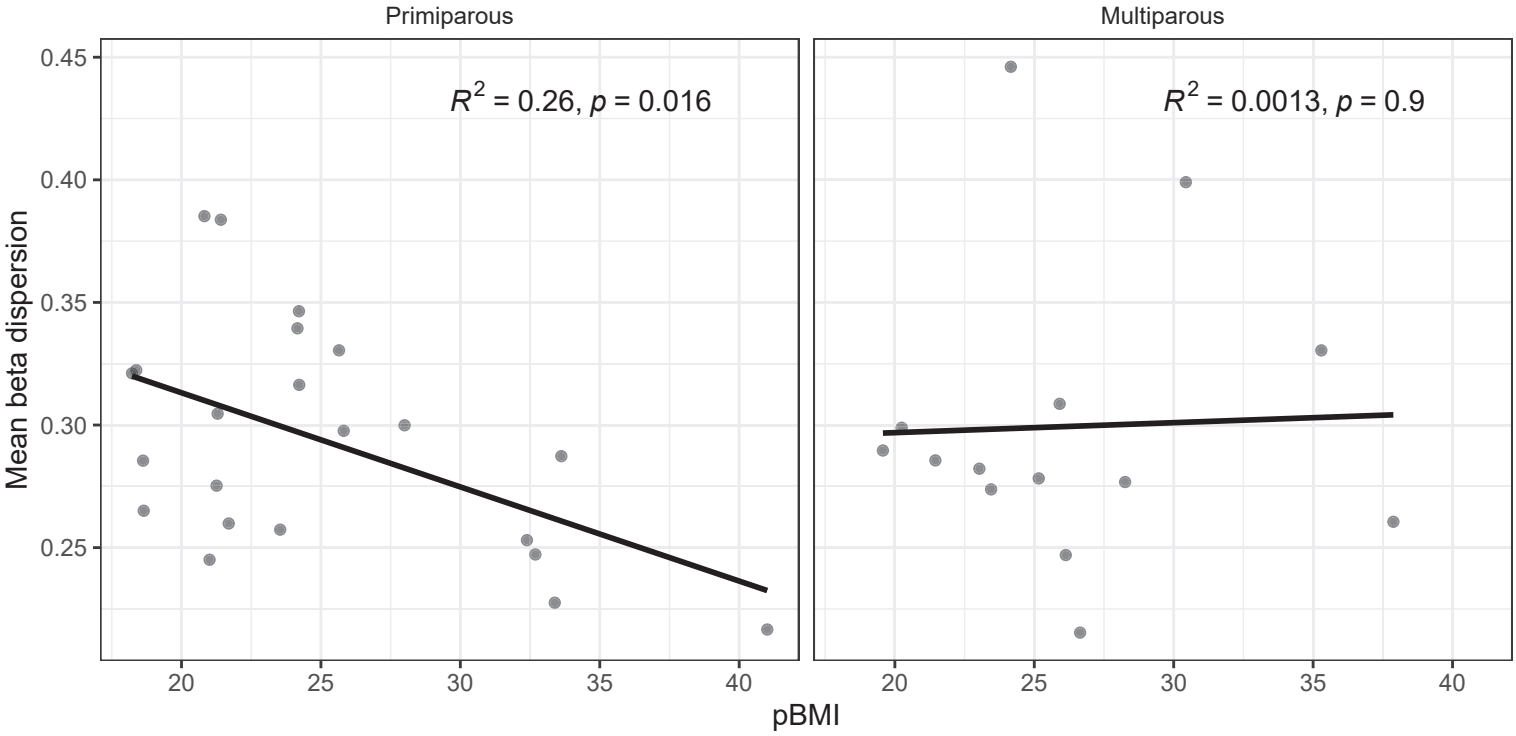
a
Primiparous
Multiparous
below
within
above
within
above
GWG
b
Primiparous
Multiparous
Sutterella
Intestinibacter
Enterobacter
Lactobacillus
Lachnoclostridium
Faecalibacterium
Akkermansia
Blautia
Enterobacteriaceae
Lachnospiraceae
Parabacteroides
Klebsiella
Streptococcus
Enterococcus
Veillonella
Haemophilus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



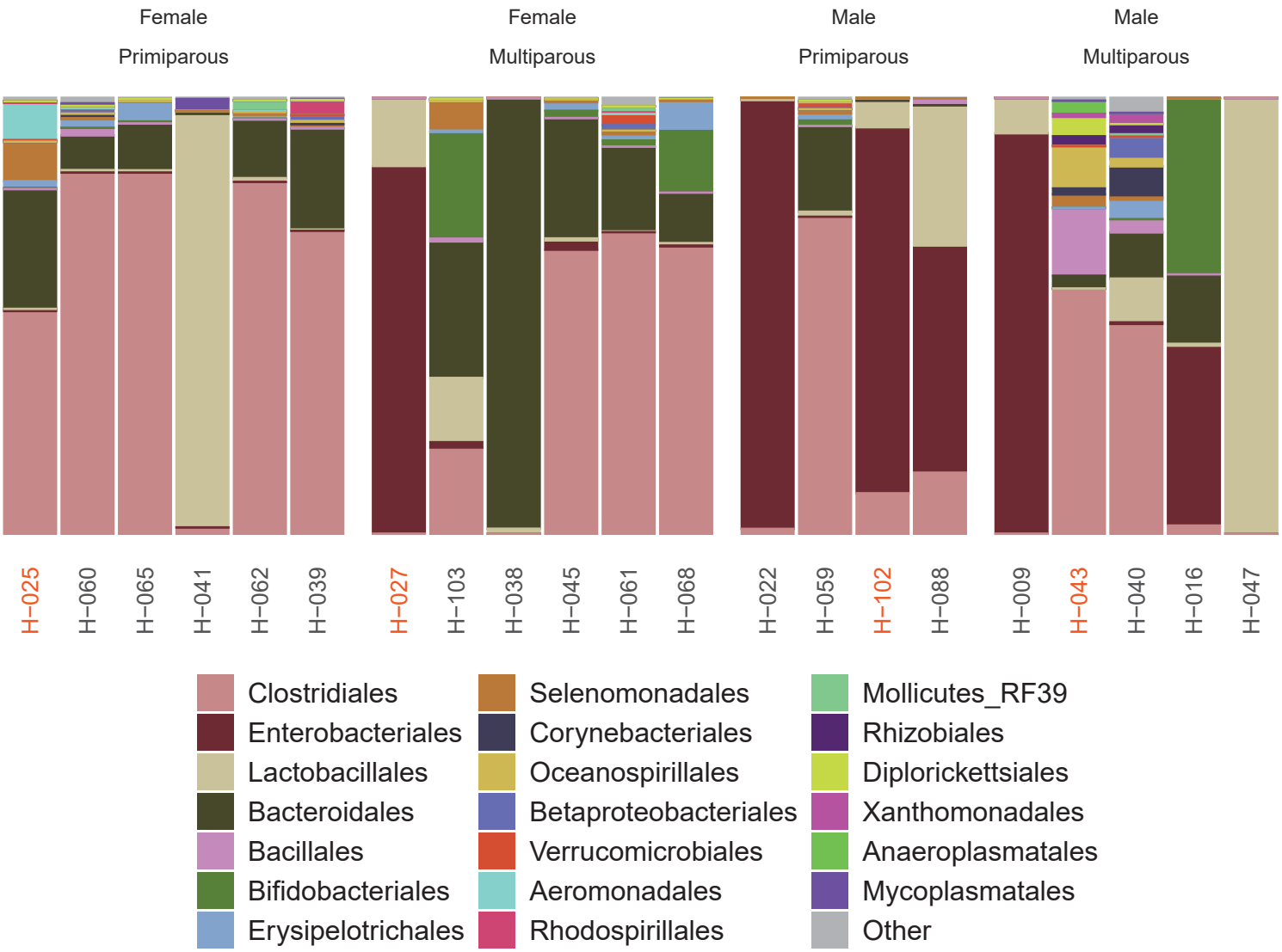
Supplementary Figure 1. Alpha diversity correlated with pBMI during pregnancy. Boxplots of Shannon alpha diversity overlayed with dot plots show a positive correlation with pBMI at each pregnancy timepoint (p=0.012-0.044) but not at 6 months postpartum (p=0.58) in participants with excess GWG and no 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

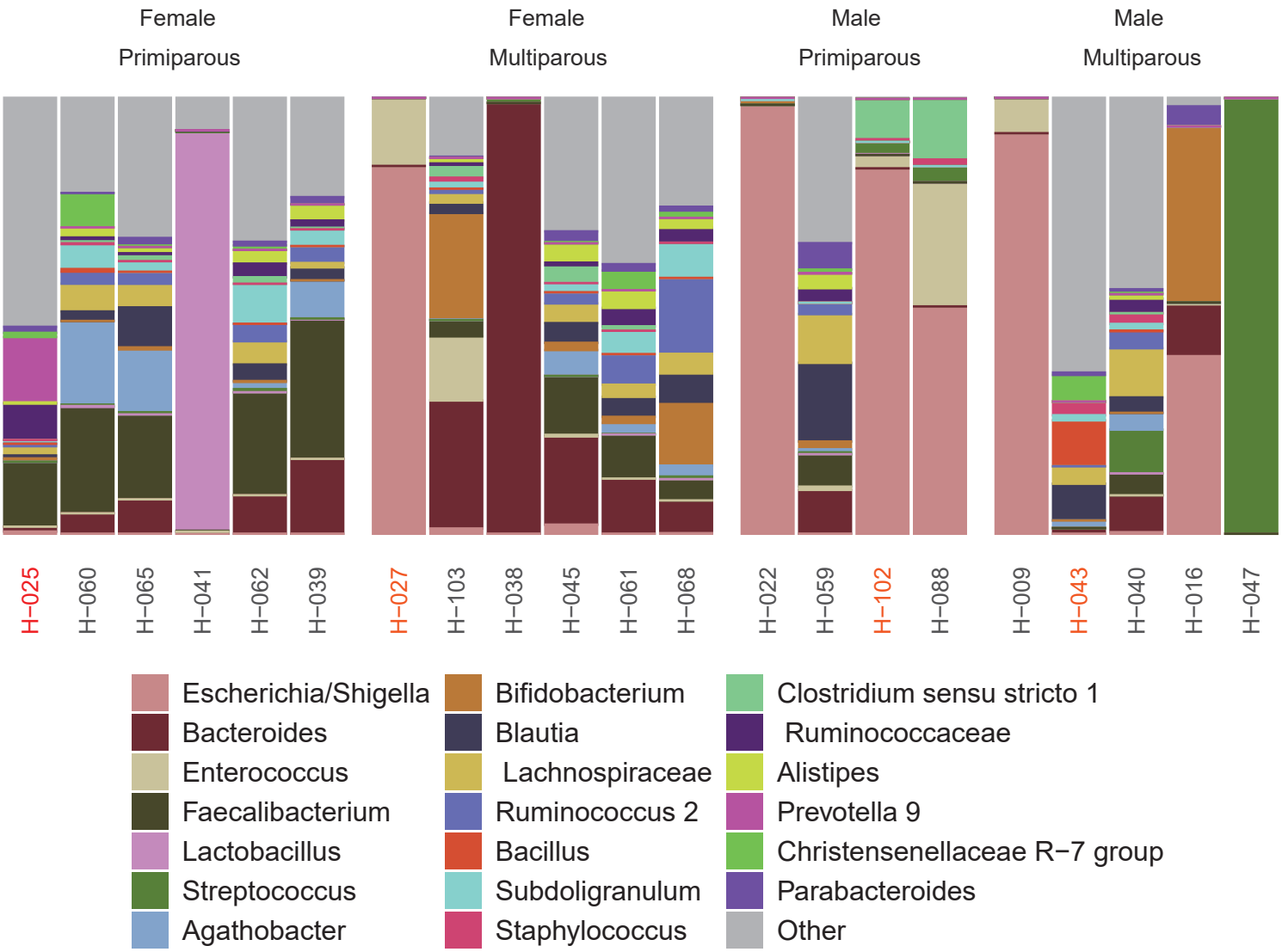
Supplementary Figure 2. Impacts of pBMI, GWG, parity, and sample timepoint on maternal gut microbiome beta diversity. PCoA of Bray-Curtis distances shows modest clustering by **a**, parity (R^2=0.014, p<0.0001) and **b**, timepoint (R^2=0.010, p<0.0001). **c**, Clustering by pBMI category within timepoints 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.



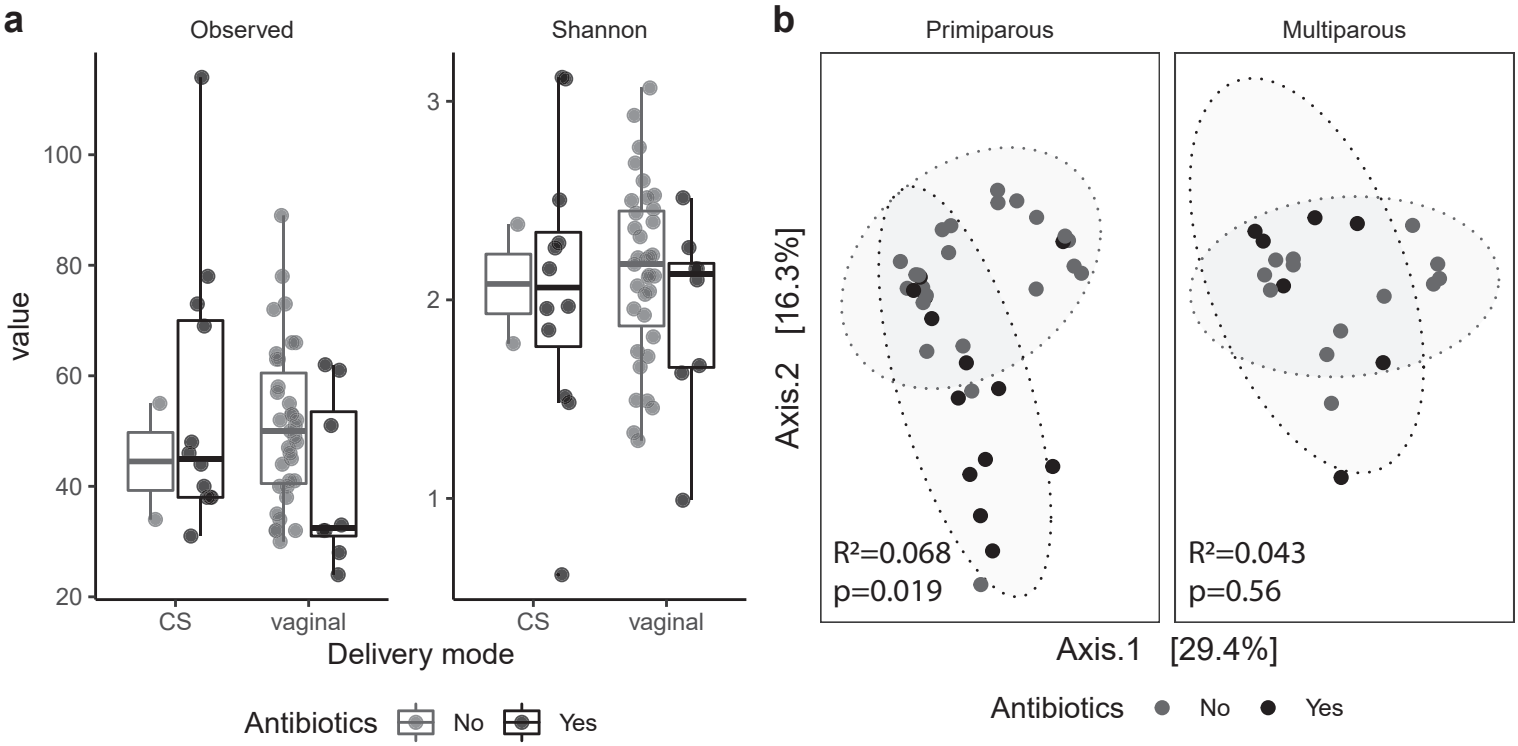
Supplementary Figure 3. Beta dispersion is negatively correlated with pBMI in primiparous participants. Mean beta-dispersion (Bray-Curtis dissimilarity) within individual participants is negatively associated with pBMI in primiparous participants (R2=0.26, p=0.016), but not in multiparous participants (R2=0.0013, p=0.9).



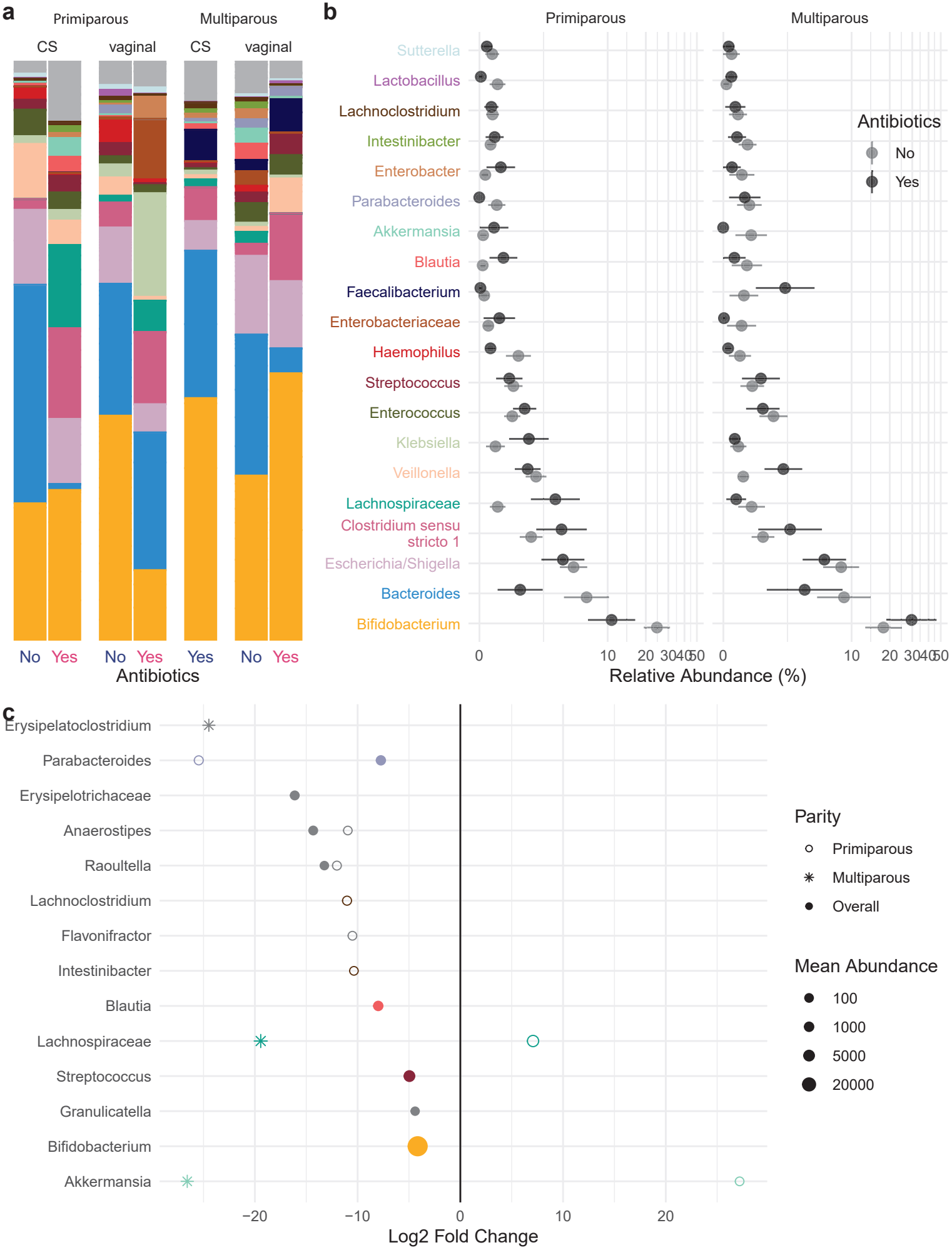
Supplementary Figure 4. Relative abundance of bacterial orders in first-pass meconium of vaginally-delivered neonates differ by parity and sex. The relative abundances of the 20 most abundant orders in first-pass meconium by infant sex and parity show female neonates tend to be dominated by the order Clostridiales, while males are more commonly dominated by Enterobacteriales. Neonates with peripartum antibiotic exposure are indicated by red text.



Supplementary Figure 5. Relative abundance of bacterial genera in first-pass meconium of vaginally-delivered neonates differ by parity and sex. The relative abundances of the 20 most abundant genera in first-pass meconium by infant sex and parity. Neonates with peripartum antibiotic exposure are indicated by red text.



Supplementary Figure 6. 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

Supplementary Figure 7. 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).

Table S1. 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

Table S3. 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