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1 **Antibiotics and the developing intestinal microbiome, metabolome and inflammatory**
2 **environment: a randomized trial of preterm infants**

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16 **Abstract**

17 Antibiotic use in neonates can have detrimental effects on the developing gut
18 microbiome, increasing the risk of morbidity. A majority of preterm neonates receive antibiotics
19 after birth without clear evidence to guide this practice. Here microbiome, metabolomic, and
20 immune marker results from the Routine Early Antibiotic use in SymptOmatic preterm Neonates
21 (REASON) study are presented. The REASON study is the first trial to randomize symptomatic
22 preterm neonates to receive or not receive antibiotics in the first 48 hours after birth. Using 16S
23 rRNA sequencing of stool samples collected longitudinally for 91 neonates, the effect of such
24 antibiotic use on microbiome diversity is assessed. The results illustrate that type of nutrition
25 shapes the early infant gut microbiome. By integrating data for the gut microbiome, stool
26 metabolites, stool immune markers, and inferred metabolic pathways, an association was
27 discovered between *Veillonella* and the neurotransmitter gamma-aminobutyric acid (GABA).
28 These results suggest early antibiotic use may impact the gut-brain axis with the potential for
29 consequences in early life development, a finding that needs to be validated in a larger cohort.

30 **Main**

31 Premature infants are particularly susceptible to infections secondary to increased need
32 for invasive procedures and immaturity of the immune system, skin, and gastrointestinal tract[1–3].
33 Increasingly, there is growing concern that risk factors for mortality may originate from
34 underlying pathologies that could also be responsible for premature birth[4]. Symptoms of
35 prematurity are difficult to discern from symptoms of infection which, compounded by the
36 increased risk of infection, have led to most premature infants being exposed to antibiotics early

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37 in life[5–7]. Despite high mortality rates, the incidence of culture positive early onset sepsis (EOS)
38 is relatively low, between 0.2-0.6%[8]. In the absence of a positive culture, a majority of preterm
39 infants receive antibiotics immediately after birth based on maternal risk factors (e.g. intra-
40 amniotic infection) or laboratory abnormalities (e.g. elevated serum C-reactive protein (CRP))
41 because of the risk of mortality[8]. Given the low incidence of culture-positive EOS in this
42 population, it is possible that such high rates of antibiotic use are unnecessary and may increase
43 morbidity in these infants[9]. Other morbidities in the neonatal intensive care unit (NICU) such as
44 necrotizing enterocolitis (NEC) and late onset sepsis (LOS) also have high mortality rates and
45 have been associated with prolonged antibiotic exposure.[10,11]. Nevertheless, antibiotics remain
46 the most commonly prescribed medication in the NICU[12,13].

47 The gut microbiome comprises a highly volatile community structure early in life[14].
48 Microbial colonization is influenced as early as birth by mode of delivery, and perhaps even in
49 the uterine environment by maternal factors[15,16]. Not surprisingly, antibiotic use has been shown
50 to also change the composition of the preterm gut community[17–20]. Furthermore, antibiotic use
51 early in life has increasingly been associated with adverse outcomes both short- and long-
52 term[21,22]. One possible consequence is the disruption of the gut-brain axis (GBA), which
53 involves bi-directional transmission of bio-molecular signals between the gut microbiota and the
54 nervous system[23]. Aberrations in the GBA have been associated with altered immune
55 homeostasis, as well as psychiatric, behavioral and metabolic conditions in adulthood[24]. It is
56 therefore imperative to determine if such high rates of antibiotic use in preterm infants is
57 necessary, as it could have lifelong consequences on future health.

58 Randomized clinical trials have the advantage of controlling for many of the numerous
59 covariates that could interfere with answering whether preemptive antibiotic use in preterm

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60 infants affects outcomes. The Routine Early Antibiotic use in SymptOmatic preterm Neonates
61 (REASON) study is the first to randomize symptomatic premature infants to either receive or not
62 receive antibiotics soon after birth. Previously reported results from this study demonstrate the
63 feasibility of such a trial and that withholding antibiotics did not lead to a significant increase in
64 neonatal mortality or morbidity[25]. By employing a multi-omic approach, this cohort also
65 provides the unique opportunity to understand how antibiotic intervention perturbs the early life
66 gut microbiome, metabolome, and inflammatory environment in ways that may be consequential
67 to health and development.

68 **Results**

69 **Cohort and study description**

70 Ninety-one of the total 98 enrolled infants had stool samples collected. Seven infants had
71 no samples due to early mortality. Eligible infants were enrolled into groups based on previously
72 described enrollment criteria[25]: group A – antibiotics indicated (n=28), group B – antibiotics not
73 indicated (n=11), and group C – eligible for randomization (n=52). Twenty-six infants were from
74 group C1 (antibiotics in first 48 hours) and 14 infants were from group C2 and did not receive
75 antibiotics 48 hours after birth. For 12 infants (46%) randomized to group C2, antibiotics were
76 prescribed in the first 48 hours after birth upon clinical assessment, and these infants were placed
77 in a separate analysis group C2Bailed. One infant in group B were changed (bailed) to receive
78 antibiotics within 48 hours after birth and was excluded from this analysis. Therefore, there are a
79 total of 90 infants with stool samples analyzed across 5 enrollment groups, 2 of which did not
80 receive antibiotics within 48 hours after birth (groups B and C2). Neither sex (p=0.352) nor
81 mode of delivery (p=0.227) were significantly different between groups using the chi-square test.
82 Both weight (p-value=0.005) and gestational age (GA) (p-value=0.002) were significantly

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83 different between groups overall, with group A infants on average with lower GA and at lower
84 birth weights. Neither birth weight nor GA were significantly different between the randomized
85 subgroups (C1, C2, C2Bailed) by the Kruskal-Wallis test ($p>0.05$). A summary of the infants in
86 this analysis is provided (Table 1). Likewise, a summary of the types of antibiotics and the
87 number of times antibiotics were prescribed by group (Supplementary Table S1). A full
88 description of enrollment has been described previously[25].

89 Six hundred ninety-three stool samples were collected longitudinally for 91 of the total
90 98 enrolled infants. Stool data were not available for 7 infants due to early mortality. Sequencing
91 data for 16S rRNA were obtained for 656 of those samples. After rarefying to an even
92 sequencing depth of 10,000 reads per sample, 642 samples remained. Since GA is significantly
93 different between groups, we chose to focus on corrected GA between weeks 28 to 39 because
94 there were not enough samples among groups at younger and older timepoints. Therefore, 522
95 stool samples remained within this corrected GA window (Supplementary Table S2). The aim
96 for this analysis is to test the effects of randomization to antibiotics vs. no antibiotics on the
97 developing gut microbiome, metabolome and inflammatory environment using high-throughput
98 16S rRNA sequencing, quantitative PCR (qPCR), metabolomics, pathway inference, immune
99 marker analysis and open-source statistical tools.

100 **Antibiotic use and trends in early gut microbiome diversity development**

101 Using amplicon sequencing variants (ASVs), the richness and Shannon alpha diversity
102 were not significantly different between enrollment groups at each corrected GA timepoint using
103 the Kruskal-Wallis test (Fig. 1A, B). Furthermore, the number of copies of 16S rRNA were not
104 significantly different between groups at any timepoint (Fig. 1C). Interestingly, group B infants

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105 who did not receive antibiotics and were typically older and had an increasing trend in copies of
106 16S rRNA over time, but the same trend was weaker for group C2 infants who also did not
107 receive antibiotics but were typically younger. Using a linear mixed-effects model (LME)
108 through Qiime2[26–28], neither richness nor Shannon diversity changed significantly over the time
109 frame of corrected GA between 28 – 39 weeks (p=0.407, p=0.861, respectively) (Fig. 1D, E).
110 Notably, groups C1 and C2Bailed had significant positive trends in richness over time (p=0.019,
111 p=0.002, respectively). Groups B and C2 had negative trends in richness that were not significant
112 (p>0.05). All groups had positive trends in Shannon diversity development over time. However
113 none were significant (p>0.05). Surprisingly, there was no significant difference in diversity
114 between groups C1 (or similarly, C2Bailed) and C2, which are the informative groups for
115 comparing effects of antibiotics or no antibiotics 48 hours after birth. Certain considerations need
116 to be made when comparing infants by enrollment group. For example, group A infants had
117 significantly lower GA and birth weights, particularly compared to group B infants who on
118 average had the highest GAs and birth weights, and the shortest stays in the NICU. Comparison
119 between group B and the other enrollment groups is limited in scope because of shorter stays, i.e.
120 fewer longitudinal samples. Furthermore, although the randomized groups C1 and C2 had similar
121 number of enrolled infants in the beginning, nearly half of group C2 infants were bailed to
122 receive antibiotics 48 hours after birth. Therefore, the power to compare the randomized groups
123 by antibiotic use with 48 hours after birth is limited.

124 Similar to alpha diversity, there were no apparent changes in overall bacterial community
125 structure between groups when assessing beta-diversity over time and at each timepoint. Both the

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126 Bray-Curtis and Jaccard distance indices were used to assess community structure, taking into
127 account quantitative ASV abundance and qualitative ASV presence/absence information,
128 respectively. Principle coordinates analysis (PCoA) did not reveal any immediately obvious
129 clustering differences between groups for either metric (Fig. 2), which may suggest little or no
130 persistent effect of antibiotic use beyond 48 hours after birth. Beta dispersion was not
131 significantly different between groups using the Bray-Curtis metric (ANOVA; Df = 4, Sum of
132 Squares = 0.011, Mean Squares = 0.003, F = 1.088, p = 0.362) but was significant using Jaccard
133 (ANOVA: Df = 4, Sum of Squares = 0.048, Mean Squares = 0.012, F = 9.574, p = 1.79E-07),
134 specifically group A versus all other groups (TukeyHSD, A vs. B: p = 0.010, A vs. C1: p-value =
135 0.0003, A vs. C2: p = 0.004, A vs. C2Bailed: p=0.00001). This might suggest differences in
136 dispersion heterogeneity (i.e. greater spread in variance) between group A infants and infants in
137 other groups, which could be explained by group A infants often receiving antibiotics beyond 48
138 hours after birth. However, when the non-parametric permutational analysis of variance
139 (PERMANOVA) test was applied to each timepoint across groups, there were no significant
140 differences in Bray-Curtis or Jaccard distances among all groups at any given corrected GA
141 timepoint (Fig. 2).

142 **Feeding patterns drive changes in gut diversity and bacterial load**

143 For preterm infants, diet generally consists of mother's breast milk (MBM), pasteurized
144 donor breast milk (DBM), formula, or some combination of these sources. Some infants also
145 experienced periods of no enteral feeding (NPO: nil per os). To investigate effects of feeding
146 while still considering effects from antibiotic use, feeding types were compared within each

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147 respective analysis group. In addition, for purposes of comparing feeding types at each corrected
148 GA timepoint, feeding types with only a single sample at each timepoint (n=1) were removed.
149 This reduced the total number of stool samples from 522 to 461. The number of samples in each
150 group at each timepoint, and also by feeding type, is summarized in Supplementary Table S3.
151 Using the calculated alpha diversity metrics described previously, feeding type was significantly
152 different in bacterial richness only at corrected GA week 32 in group A infants (Kruskal-Wallis,
153 p= 0.0069), where samples collected during feeding with all or partial mother's milk tended to
154 have higher bacterial richness (Fig. 3A). Furthermore, Shannon diversity was significantly lower
155 in infants not fed orally at corrected GA week 36 in group A infants (p=0.042) (Fig. 3B). The
156 log_{10}-transformed number of 16S rRNA copies were not significant at any timepoint in any
157 group, although notably feeding with MBM alone typically had higher 16S rRNA copies
158 compared with formula in all groups except for group A, which might be explained by continued
159 antibiotic use beyond 48 hours (Fig. 3C).

160 Beta diversity between feeding types within groups was likewise only significant at few
161 specific timepoints. For group A infants, Bray-Curtis distances between formula and MBM
162 feeding were significantly different at corrected GA week 34 using PERMANOVA (R^2=0.093,
163 p=0.030). For group C1, Bray-Curtis distances were different between formula, MBM and
164 MBM+formula at corrected GA week 36 (R^2=0.370, p=0.005). Also in group C1, Jaccard
165 distances were significantly different between formula, MBM and MBM+formula at corrected
166 GA week 34 (R^2=0.141, p=0.024), corrected GA week 35 (R^2=0.158, p=0.011) and corrected
167 GA week 36 (R^2=0.296, p=0.007).

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168 Applying LME modelling by feeding type according to analysis group allows the
169 observation of feeding effect over time, focusing again on the 12 weeks of corrected GA after
170 removal of feeding type singletons at each timepoint (Fig. 4). Perhaps not surprisingly, among all
171 groups, periods of NPO led to a lower trend in Shannon diversity over time (p=0.003) (Fig. 4G).
172 While bacterial richness appeared to trend lower, the trend was not significant (p=0.341) (Fig.
173 4A). For group A infants, which received antibiotics in 48 hours after birth and often beyond,
174 MBM was associated with a slight increase in richness (p=0.009) (Fig. 4B), and periods of NPO
175 led to a lower trend in Shannon diversity (p=0.031) (Fig. 4H). in Group B infants, who never
176 received antibiotics and tended to be older, larger and healthier, all feeding types including
177 formula (p=0.004), MBM (p<0.001) and MBM+formula (p=0.018) led to increasing trends in
178 Shannon diversity (Fig. 4I). However, no significant trends could be identified in richness (Fig.
179 4C). It is difficult to evaluate group B infants due to lower enrollment size, shorter NICU stays,
180 and fewer samples overall. For the randomized infants that received antibiotics 48 hours after
181 birth, MBM and formula were associated with positive trends in richness (C1 and C2Bailed
182 MBM: p<0.001; C1 formula: p=0.004; C2Bailed formula: p=0.016) (Figs. 4D, 4F). Only group
183 C2Bailed infants saw increased trends in Shannon diversity for feeding MBM (MBM only:
184 p=0.018; MBM+DBM: p=0.004; MBM_formula: p=0.002) (Fig. 4L). Finally, group C2 infants
185 randomized to not receive antibiotics 48 hours after birth saw a lower trend in both richness and
186 Shannon diversity during feeding with DBM (Figs. 4E, 4K). However, neither trend was
187 significant, likely due to the few numbers of samples. The power for detecting trends in group
188 C2 is likely hampered because half of the infants randomized were bailed within 48 hours after
189 birth.

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190 **Gut microbial community development is highly variable and unique to each infant**

191 Although the infants in this study were analyzed among 5 groups, each infant's stay in
192 the NICU is highly personalized, by type, frequency, number or length of antibiotic use, type and
193 length of feeding patterns, and adverse clinical events over time. To aid in visual identification of
194 patterns throughout the NICU course, detailed charts were created for each infant that depict
195 both clinical and laboratory data over time (Days Post Birth) integrated into a single graphic per
196 infant (Fig. 5 and Supplementary Figure S1). This includes results from the 16S rRNA analysis
197 as pie charts for each stool sample, color coded by bacterial taxonomy and sized based on the
198 $\log_{10}$-transformed number of 16S rRNA copies per gram of stool, as well as adverse clinical
199 events coded by a single letter code (Fig. 5). With these visualizations, patterns are more easily
200 observed between antibiotic treatments, feeding types, and the gut microbiome. Typically, the
201 microbiome composition after birth is homogenous in composition and diversifies, as well as
202 increases in size over time, as might be expected. One interesting association involved the
203 administration of the anti-fungal fluconazole and a resultant lowering of bacterial load and
204 diversity. However, fluconazole was often administered in conjunction with antibiotics and was
205 administered frequently to group A infants.

206 The more subtle effects of antibiotic use and feeding patterns become visually apparent
207 by taking this individualized approach. To illustrate, infant 5 was exclusively fed mother's milk
208 from day 14 through day 71 post birth. At day 31, antibiotics vancomycin and piperacillin were
209 administered (Supplementary Figure S1). During treatment, *Veillonella* was entirely removed
210 from the stool microbiome, falling from 3.23E+06 cells per gram (73%) from the pre-treatment

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211 time point to an undetectable level while antibiotics were used. At day 47, 9 days after antibiotic
212 treatment ceased, *Veillonella* again dominated the stool at 3.83E+06 cells per gram (80%),
213 almost a complete replacement of levels before treatment. Also, the proportions of the other 2
214 genera found in the stool, *Escherichia* and an unclassified Enterobacteriaceae spp., were nearly
215 identical after treatment and continuation of mother's milk as before treatment (pre-treatment:
216 *Escherichia* – 21%, Enterobacteriaceae spp. – 4.7%; post-treatment: *Escherichia* – 15%,
217 Enterobacteriaceae spp. – 3.3%). Thus, either that mother's milk effectively restored the stool
218 microbiome to its pre-treatment state or this effect occurred due to removal of antibiotic selective
219 pressure, or both. A similar effect can be seen in infant 12 between 25 and 50 DOL where the
220 microbiome is restored post-antibiotics. In this case, the restoration is observed with MBM,
221 DBM, and formula. In some cases, antibiotic use had no effect on the microbiome composition
222 (e.g. infant 42, 84), suggesting the presence of resistance mechanisms in the dominant gut
223 microbes (in these 2 cases, members of Enterobacteriaceae). In fact, Enterobacteriaceae presence
224 followed administration of ampicillin and gentamicin, the 2 most commonly prescribed
225 antibiotics immediately after birth. This occurred in 24 of the 91 infants. Other times antibiotic
226 use appears to dramatically and irreversibly change microbiome composition (e.g. infant 25).

227 **Bacterial genera correlate with stool metabolites and inferred metabolic pathways**

228 In addition to 16S rRNA profiling, 90 stool samples from 10 infants were analyzed for
229 metabolomic profiling (Table 1). Four of the 5 groups were included in these samples for
230 comparison (group B samples not included). Peak height responses were recorded for 454
231 identifiable metabolites. To determine if gut bacteria were associated with relative concentrations

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232 of metabolites in stool, the top 10 most abundant bacterial genera associated with identified
233 metabolites were determined. Repeated measures correlation values were plotted using a
234 heatmap, which indicated numerous significant, positive and negative, associations between
235 bacteria and metabolites (Fig. 6A). Interestingly, *Veillonella* were positively associated with the
236 neurotransmitter 4-aminobutanoate (GABA) (R = 0.27, p = 0.013) and *Veillonella* counts were
237 significantly different between groups A and C2 (p =0.0475), C1 and C2 (p=0.029), and C2 and
238 C2Bailed (p=0.042) using the Wilcoxon paired test (Fig. 6C). Also, *Veillonella* counts were not
239 significantly different between samples of infants that received antibiotics, i.e. A and C1
240 (p=0.57) or C1 and C2Bailed (p=0.17). GABA peak height responses followed similar trends as
241 *Veillonella* counts, that is, responses were significantly different between groups that received
242 and did not receive antibiotics (A vs. C2, C1 vs. C2, C2 vs. C2Bailed) but not between groups
243 that both received antibiotics (A vs. C1, C1 vs. C2Bailed) (Fig. 6B).

244 Furthermore, using PICRUSt2, functional pathway abundances were inferred based on
245 the rarefied 16S rRNA data[29]. The *Veillonella* counts of predicted pathways were strongly
246 correlated with biosynthesis of the GABA precursor L-glutamate (R = 0.88, p = 3.02E-27)
247 (Supplementary Figure S2). Thus, it may be that *Veillonella* could be at least partially
248 responsible for GABA neurotransmitter production and that this function is negatively impacted
249 by antibiotic use early in life. Alternatively, *Veillonella* may instead be involved in biosynthesis
250 and export of L-glutamate in the gut, which is then converted to GABA by the host glutamate
251 decarboxylase. However, PICRUSt2 results are based on inferred pathways from reference

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252 genomes closely related to the 16S rRNA data used here and are, at best, predictions in the
253 absence of functional data specific to this cohort.

254 A negative correlation between *Bifidobacterium* counts and glycocholic acid was
255 observed (R = -0.39, p = 0.0098), which was also impacted by antibiotic use between groups. In
256 addition, bifidobacteria were negatively associated with other conjugated bile acids including
257 taurocholic (R = -0.22, p = 0.045) and glycocholic acids (R = -0.21, p = 0.048), but positively
258 associated with deconjugated cholic (R = 0.25, p = 0.027) (Fig. 6A). Thus, gut microbiota
259 affected by antibiotic use may be responsible for modification of neuroactive metabolites (i.e.
260 deconjugated bile salts) in addition to production of neurotransmitters.

261 **Immune markers in stool correlate with bacterial abundance**

262 Antibiotic use was examined for its correlation with inflammatory marker levels in stool.
263 These levels were also correlated with gut bacterial abundances. Twelve immune markers were
264 measured in 110 stool samples across 18 of the first enrolled infants. A summary of immune
265 marker data samples including infants per group and number of samples per infant is given in
266 Table 1. Ten bacterial genera had at least one significant correlation with an immune marker ($p <$
267 0.05) (Fig. 7A). Significant correlations between the bacterial genera and stool immune markers
268 were classified as either inflammatory or anti-inflammatory based on the known function of the
269 marker (Fig. 7B). Interestingly, *Enterococcus* counts were negatively correlated with levels of
270 TNF-alpha and macrophage inflammatory protein 1-alpha (MIP1α). *Citrobacter* were positively
271 correlated with MIP1α and IL6 (R = 0.21, p = 3.74E-05), and were significantly higher in group
272 C1 compared to group C2 (p=7.7E-07) and group C2 compared to C2Bailed (p=0.00022) by the

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273 Wilcoxon test (Fig. 7C). Lastly, counts of *Escherichia*/*Shigella* were significantly negatively
274 correlated with levels of epidermal growth factor (EGF), which was the strongest correlation
275 within the dataset. *Escherichia*/*Shigella* counts were highest among group A samples, but not
276 significantly higher compared to other groups (Wilcoxon, p>0.05).

277 **Discussion**

278 There is an urgent need for evidence supporting or refuting the widespread practice of
279 routine antibiotic use after birth in symptomatic preterm neonates. The REASON study
280 represents a significant step as it is the first randomized controlled trial to test the feasibility of
281 randomizing symptomatic preterm infants to antibiotics versus no antibiotics, evaluating the
282 effect of antibiotic treatment on the developing gut microbiome, metabolome, and inflammatory
283 environment. Our results expand upon previous reports that early routine antibiotic use leads to
284 alterations in the early life gut microbiome, even after discontinuation of antibiotics[17,30,31]. The
285 results presented here suggest that antibiotic use 48 hours after birth did not tend to have a
286 lasting effect on the development of gut microbiome diversity over time, and that the gut
287 microbiota diversity was recoverable. However, use of antibiotics extending beyond 48 hours
288 after birth often did have significant impacts on the microbiome over time, as evidenced in group
289 A infants compared to the other enrollment groups. The power to detect significant associations
290 in this study was hampered however, mainly because many of the infants randomized to not
291 receive antibiotics were changed to antibiotic administration. Furthermore, there were few
292 infants who were enrolled in group B (an important antibiotic-free control group) and those
293 enrolled in group B had few samples due to short stays in the NICU. A larger multi-center
294 randomized study is needed to validate and expand upon the extended effect of antibiotics on the
295 developing gut microbiome.

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296 Our results support the notion that feeding types likely also have a significant influence
297 on gut microbiome richness and diversity, though in this case only at specific timepoints[32–34].
298 Exclusive or partial feeding with mother's own milk appeared to have higher bacterial load
299 compared to formula and NPO, though not significantly. This observation is backed by previous
300 evidence that breast milk harbors maternal-originating bacteria, as well as nutritional
301 components (prebiotics) that support bacterial proliferation in the intestinal tract[35,36].
302 Interestingly, formula-fed infants had comparable levels of richness and diversity as mother's
303 milk. This supports the idea that mother's milk drives early colonization of a limited set of
304 dominant microbes through nutrient and antimicrobial-mediated selection[37–39]. Feeding trends
305 over time were able to be assessed for the main feeding types such as MBM, DBM and formula
306 however again the ability to detect meaningful results for rarer feeding types (particularly
307 combinations of sources) and group B infants was hampered by small sample size and will
308 require a larger cohort.

309 Integrating detailed and personalized records of clinical and laboratory data led us to
310 identify overlooked patterns in the data. One such peculiar pattern was that stool samples taken
311 during administration of the anti-fungal fluconazole had lowered copies of 16S rRNA,
312 suggesting lower bacterial load. A previous study reported that fluconazole, though not
313 inherently bactericidal, increased the bactericidal activity of neutrophils[40]. Immune marker data
314 were collected for 18 of the first enrolled infants, and one or more of those markers, such as
315 calprotectin which is secreted by neutrophils, may help explain this pattern[41]. However, only 3 of
316 the 18 infants that had immune marker data received fluconazole. Therefore more data are
317 needed to test this hypothesis. Interestingly, counts of *Enterococcus* were negatively correlated
318 with levels of pro-inflammatory markers such as TNFα and MIP1α, an odd finding considering

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319 Enterococci have been associated with risk for infection in preterm neonates[42,43]. On the other
320 hand, *Citrobacter* counts were associated with increased levels of the macrophage chemokine
321 MIP1α and counts were significantly higher in infants randomized to receive or were bailed to
322 receive antibiotics. Increased levels of MIP1α are likely related to recruitment of intestinal
323 macrophages leading to a heightened inflammatory environment, suggesting that antibiotic use
324 may select for bacteria which lead to intestinal inflammation[44]. Finally, *Escherichia/Shigella*
325 counts had a relatively strong negative correlation with EGF levels. Previous studies have found
326 that reduced concentrations of maternally derived EGF in mice correlated with *E. coli* gut
327 translocation, and that supplementation with EGF protected the gut from colonization by
328 enteropathogenic *E. coli* in a young rabbit model[45,46]. Perhaps such factors as EGF concentration
329 could be important in ameliorating the effect of antibiotics on pathogen colonization in the
330 preterm gut. Further work including a larger sample size will be needed to understand how
331 changes in the preterm infant gut caused by routine antibiotics impacts the gut inflammatory
332 environment.

333 Neurological development can be impaired in infants born very prematurely compared to
334 their full-term counterparts; a trend that extends into delayed cognitive and behavioral
335 development through childhood[47–49]. Could routine early antibiotic use, or prolonged antibiotic
336 use, in preterm neonates play a role in this association? Intestinal microbes produce a plethora of
337 metabolites and bio-active compounds that can be absorbed by the host[50]. Some of these
338 compounds have direct neurologic implications including neurotransmitters such as GABA,
339 which is reduced in preterm infants, is critical for early brain development, and possesses
340 immunomodulatory properties[51,52]. Antibiotic use was negatively affected the abundance of
341 *Veillonella* and that *Veillonella* were positively correlated with GABA concentrations in the gut.

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342 Furthermore, *Veillonella* correlated strongly with the L-glutamine biosynthesis pathway, the
343 precursor to GABA production. Aside from production of neurotransmitters, negative
344 correlations were identified between *Bifidobacterium* abundance and concentrations of
345 conjugated bile acids, particularly glyco- and taurocholic acid. Conjugated bile acids were also
346 significantly different based on antibiotic use. Bifidobacteria, which were more abundant in
347 infants that did not receive antibiotics, are known to deconjugate bile acids to primary forms
348 including cholic acid[53,54]. Cholic acid can passively diffuse into the brain where it blocks
349 signaling in the $GABA_A$ receptor[55]. Bifidobacteria may therefore be essential in regulating
350 GABA signaling in the developing brain. These are significant findings, for they suggest routine
351 antibiotic use could be disrupting processes involved in the gut-brain axis and
352 immunomodulatory pathways critical for neonatal and future childhood development.

353 Evidence-based antibiotic use to prevent infection in preterm neonates is critical in
354 preventing unnecessary treatment that may be doing more harm than good. Overuse of
355 antibiotics can change the developmental trajectory of the infant gut microbiome during a time of
356 critical establishment and interaction. However, antibiotics remain a critical treatment for a
357 population at greater risk for infection, and there naturally exists a delicate balance between
358 when antibiotics are truly necessary for treatment or not. Given the potential for extensive
359 crosstalk between gut microbiota and the host, changes in microbiome composition could have
360 both short- and long-term effects on outcomes and overall health and development. Future
361 randomized studies with greater infant enrollment will be crucial in our understanding of the
362 effects current neonatal practice has on health which will allow for the reevaluation of practices.
363 Such trials will need to expand on the findings from this pilot study from a multi-omic standpoint
364 to identify direct links between antibiotic-induced dysbiosis and health outcomes.

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365 **Materials and Methods**

366 **Experimental design, enrollment, and clinical sample and data collection**

367 The REASON study was conducted from January 2017 - January 2019 at the University
368 of Florida and was approved by the institutional review board (IRB201501045). This study is
369 funded by the NIH (R21HD088005). A detailed description of the study design including
370 enrollment, group selection, randomization, and collection of clinical samples and data including
371 outcomes has been previously described[25]. Briefly, 98 premature infants were enrolled in the
372 study and placed into one of three groups according to previously described criteria: group A
373 with indication for antibiotic use, group B without indication for antibiotic use, and group C
374 eligible for randomization to antibiotics (C1) or no antibiotics (C2) in the first 48 hours after
375 birth. Infants not receiving antibiotics in the first 48 hours after birth (group B, C2) could be
376 changed to receive antibiotics at any time at the medical team's discretion. Clinical samples
377 relevant to this analysis include weekly fecal collection starting with meconium when possible
378 (all stored at -80°C) and results of bacterial and fungal cultures (blood, urine, sputum, and
379 cerebrospinal fluid - when available) and laboratory measurements of CRP, white blood cell
380 count and immature to neutrophil ratio. Clinical metadata from the mothers such as antepartum
381 antibiotic use, type, duration, and proximity to delivery were recorded. Pertinent clinical
382 metadata from the infants include group placement, antibiotic use status, antibiotics and
383 antifungal use including type and duration throughout NICU course, feeding type and duration,
384 GA at birth, sex, mode of delivery and any serious adverse events (SAEs) including NEC, late
385 onset sepsis, spontaneous intestinal perforations, bronchopulmonary dysplasia, and death.

386 **Stool DNA extraction, 16S rRNA PCR and Sequencing Analysis**

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387 DNA extraction and 16S rRNA barcoded PCR was carried out exactly as described
388 previously[56]. Approximately 60 gigabases of nucleotide sequencing data was generated across 5
389 Illumina Miseq flowcells for stool samples collected from 91 (of the 98 total) study participants
390 where samples were collected (ICBR, Gainesville, FL, USA). The resulting sequencing reads
391 were merged, demultiplexed, trimmed, filtered for quality and processed into amplicon
392 sequencing variants (ASVs) as previously described with no alterations in method[56]. Briefly,
393 sequences were processed to ASVs using the DADA2 package in R (https://www.R-project.org)
394 and assigned taxonomy using the SILVA_v132 training dataset[57–61]. Samples were rarefied to
395 10,000 reads per sample, leaving 642 of the total 656 individual longitudinal stool samples for
396 analysis.

397 **Total bacterial quantification by universal 16S qPCR**

398 Total bacterial load per gram of stool was determined by universal 16S rRNA qPCR
399 using the same primer set used for amplicon sequencing (341F and 806R). QPCR assays were
400 performed on a QuantStudio 3 system (Applied Biosystems, Life Technologies, USA). The
401 reaction mixture contained 12.5 µl PowerUp SYBR Green 2X Master Mix (Applied
402 Biosystems), 1 µl each of forward (341F) and reverse (806R) primer (10 µM), 1 µl of DNA
403 template, 0.1 µg/µl BSA and brought to a final volume of 25 µl with nuclease free water.
404 Cycling conditions were identical to those of the endpoint PCR used for sequencing. However,
405 with a total of 40 cycles and replacing the final elongation step with a melt curve. Each sample
406 reaction was performed in triplicate and these values were averaged for each sample copy
407 calculation. A standard curve was generated for copy quantification using known concentrations
408 of the expected PCR product amplified from a similar stool sample. Copies of 16S rRNA per
409 gram of stool was calculated by multiplying the average copy number per replicate reaction (i.e.

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410 1 µl DNA template) by the total DNA extraction volume (75 µl) and dividing this value by the
411 mass of stool extracted in grams.

412 **Absolute bacterial abundance by copy number correction**

413 Absolute bacterial abundance was calculated on a per gram of stool basis by correcting
414 the relative sequencing abundance by the variable number of copies of the 16S rRNA gene in
415 each observed organism. This correction was done using the "Estimate" tool provided as part of
416 the rrnDB copy number database[62]. Briefly, after rarefying each sample to an even sequencing
417 depth, the ASV sequences were submitted through the rrnDB online portal where they were
418 classified down to the genus level using the RDP classifier version 2.12 and copy number
419 adjusted using rrnDB copy number data version 5.6[62,63]. The copy number adjusted relative
420 abundance for each observed taxon was multiplied by the total number of 16S rRNA copies
421 obtained by qPCR, resulting in the absolute abundance of each taxon per gram of stool.

422 **Fecal inflammatory markers**

423 Inflammatory markers were analyzed using a combination of multiplex technologies
424 using the Bio-Rad Bio-Plex platform (Bio-Rad, California, USA). The markers evaluated include
425 common markers of intestinal inflammation including calprotectin and S100A12, in addition to
426 other markers such as IL-6, TNF, IL-10 and other cytokines and chemokines that may play a role
427 in inflammatory or anti-inflammatory processes. The data were analyzed using direct
428 comparisons of all infant groups using ANOVA and subsequent individual comparisons. Fecal
429 calprotectin and S100A12 levels were measured using the fCal ELISA kit from BUHLMANN
430 Laboratories AG (Schonenbuch, Switzerland) and the Inflamark S100A12 kit from Cisbio
431 Bioassays (Codolet, France), respectively, according to the manufacturer's instructions. Samples

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432 were then analyzed for the presence of both pro-inflammatory and anti-inflammatory
433 cytokines/chemokines using Multiplex Human Cytokine Magnetic kit, Milliplex MAP Kit
434 (Millipore, Billerica, MA, USA). Twelve cytokines/chemokines, including EGF, IL-10, IL-1RA,
435 IL-B, IL-4, IL-6, IL-8, IP-10, MCP-1, MIP-1a, TNFα, and VEGF were analyzed according to the
436 manufacturer's instructions. Plates were read using the MILLIPLEX Analyzer 3.1 xPONENT™
437 System (Luminex 200). Cytokine concentrations were determined using BeadView software
438 (Millipore, Billerica, MA, USA).

439 **Metabolomics**

440 The infant stool samples were suspended in 400 µl 5 mM ammonium acetate.
441 Homogenization was done 3 times for 30 seconds each time using a cell disruptor. Protein
442 concentrations of the homogenates were measured using Qubit Protein Assay. Samples were
443 normalized to 500 µg/ml protein at 25 µl for extraction. Each normalized sample was spiked
444 with 5 µl of internal standards solution. Extraction of metabolites was performed by protein
445 precipitation by adding 200 µl of extraction solution consisting of 8:1:1 acetonitrile: methanol:
446 acetone to each sample. Samples were mixed thoroughly, incubated at 4°C to allow protein
447 precipitation, and centrifuged at 20,000 x g to pellet the protein. 190 µl supernatant was
448 transferred into clean tube and dried using nitrogen. Samples were reconstituted with 25 µl of
449 reconstitution solution consisting of injection standards, mixed, and incubated at 4° C for 10-15
450 min. Samples were centrifuged at 20000 x g. Supernatants were transferred into LC-vials.
451 Global metabolomics profiling was performed as previously described using a Thermo Q-
452 Exactive Orbitrap mass spectrometer with Dionex UHPLC and autosampler[64]. Briefly, samples
453 were analyzed in positive and negative heated electrospray ionization with a mass resolution of
454 35,000 at m/z 200 as separate injections. Separation was achieved on an ACE 18-pfp 100 x 2.1

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455 mm, 2 μm column with mobile phase A as 0.1% formic acid in water and mobile phase B as
456 acetonitrile. The flow rate was 350 μl/min with a column temperature of 25°C. 4 μl was injected
457 for negative ions and 2 μl for positive ions.

458 Data from positive and negative ions modes were processed separately. LC-MS files
459 were first converted to open-format files (i.e. mzXML) using MSConvert from Proteowizard[65].
460 Mzmine was used to identify features, deisotope, align features and perform gap filling to fill in
461 any features that may have been missed in the first alignment algorithm[66]. Features were
462 matched with SECIM internal compound database to identify metabolites. All adducts and
463 complexes were identified and removed from the data set prior to statistical analysis.

464 **Statistical Analysis**

465 The ASV and taxonomy tables resulting from DADA2 were manipulated using the
466 phyloseq R package v1.30.0[67]. Inferred metabolic pathway abundances were determined from
467 the rarefied 16S rRNA data using PICRUSt2[29]. Alpha diversity measures, including the observed
468 number of ASVs and the Shannon diversity index were calculated using the microbiome R
469 package v1.8.0 (https://bioconductor.org/packages/devel/bioc/html/microbiome.html). Box plots
470 (including statistical testing where applicable) were generated using the ggpubr R package v0.2.4
471 (https://github.com/kassambara/ggpubr), which serves as a wrapper for ggplot2[68]. The linear
472 mixed-effects modeling and associated plots were done using the longitudinal plugin “q2-
473 longitudinal” offered in Qiime2 v2019.4[26–28]. The biomformat R package (https://biom-
474 format.org) was used to convert data in phyloseq format to BIOM format for import into
475 Qiime2[69]. Bray-Curtis and Jaccard distance dissimilarities were calculated using the vegan R
476 package v2.5.6 (https://github.com/vegandevs/vegan) and PCoA plots were made using ggplot2
477 v3.3.0[68]. Individual infant charts were also generated using ggplot2. Non-parametric statistical

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478 tests including the Wilcoxon and Kruskal-Wallis tests were used for pairwise and overall
479 comparisons of 3 or more factors, respectively[70,71]. The permutational analysis of variance
480 (PERMANOVA) test was used in the vegan package to compare overall microbiome
481 dissimilarities between antibiotic use, feeding type, and enrollment groups. P-values were
482 adjusted for false discovery rate (FDR) via the Benjamin-Hochberg method[72]. Repeated
483 measures correlation values (for non-independent repeated samples for multiple subjects) were
484 calculated using the rmcorr R package[73].

485 **Data availability**

486 The demultiplexed 16S rRNA sequencing data generated in this study is deposited in the
487 NCBI Sequence Read Archive (SRA) under BioProject PRJNA515272.

488 **Trial Registration**

489 This project is registered at clinicaltrials.gov under the name "Antibiotic 'Dysbiosis' in
490 Preterm Infants" with trial number NCT02784821.

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661

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685 **Author contributions**

686 J.N. designed and oversaw the study. J.L.R. aided in implementation in the NICU, review of
687 clinical events for the cohort, preparation for the DSMB and IRB reviews, and analyzed clinical
688 data. J.N, J.L.R., D.C., and C.B. carried out patient enrollment/consent/group allocation,
689 provided care for the infants in the NICU during the study. J.L.R, C.B. L.P. recorded clinical
690 data. N.L. maintained/distributed the samples and performed the stool immune marker assays.

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691 T.J.G performed the stool metabolomics assays and analysis. K.L.M. assisted with stool DNA
692 extraction, 16S PCR/qPCR, and figure generation. J.T.R. performed the stool microbiome
693 analysis, data integration, figure generation and wrote most of the manuscript. E.W.T. helped
694 with manuscript writing and designed the data figures for each infant which were then prepared
695 by K.L.M. R.A.P. and E.W.T. assisted with data interpretation. All authors reviewed the
696 manuscript before submission.

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699

700 **Ethics Declarations**

701 **Competing Interests**

702 Dr. Josef Neu is the principal investigator of a study with Infant Bacterial Therapeutics and
703 on the Scientific Advisory Boards of Medela and Astarte. No other authors have conflicts of
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705

706 **Tables and Figures**

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707 **Table 1 – Summary of infant enrollment, covariates, and samples**

708 Summary of the number of enrolled infants per group used in this analysis and the number of
709 infants changed from group C2 to C2Bailed. Enrollment groups are also summarized by infant
710 sex (male::female), mode of delivery (vaginal::caesarean), gestational ages, maternal antibiotic
711 exposure (yes::no) and birth weight ranges in grams. The number of infants and number of stool
712 samples used in the metabolomics and immune marker analyses are listed.

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713

	Group A	Group B	Group C1	Group C2	Group C2Bailed
Total infants	28	11	26	14	12
Sex (M::F)	14::14	4::7	11::15	7::7	9::3
Delivery mode (V::C)	13::15	7::4	10::16	6::8	2::10
Gestational age range (median)	24 – 32 (28)	29 – 32 (32)	25 – 32 (29)	23 – 32 (29)	24 – 32 (28)
Maternal antibiotic exposure (Yes::No)	20::8	6::5	20::6	8::6	11::1
Birth weight range in grams (median)	695 – 2132 (1015)	1100 – 2770 (1820)	525 – 2425 (1240)	630 – 2116 (1223)	605 – 1667 (888)
Samples post-normalization	232	42	171	99	98
Average number of samples/infant (median, 1st quartile, 3rd quartile)	8.3 (9, 5, 11)	3.8 (3, 2, 5)	6.6 (6, 4, 8.75)	7.1 (6, 4.5, 10)	8.2 (9.5, 4.25, 11.25)
Number of infants with metabolomic samples	4	0	2	2	2
Number of metabolomic samples (samples per infant)	32 (8, 10, 5, 9)	0	17 (12, 5)	16 (6, 10)	25 (14, 11)
Number of infants with immune marker samples	7	0	5	3	3
Number of immune marker samples (samples per infant)	40 (7, 7, 1, 3, 5, 7, 10)	0	22 (12, 2, 2, 5, 1)	21 (5, 10, 6)	26 (13, 11, 2)

714

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715 **Figure 1 – Antibiotic use 48 hours after birth does not significantly affect alpha diversity**
716 **development**

717 Boxplots displaying **(A)** the observed ASV richness **(B)** the Shannon diversity and **(C)** log_{10}-
718 transformed copies of 16S rRNA by enrollment group across corrected gestational ages between
719 weeks 28 and 39. P-values were calculated at each corrected GA timepoint between enrollment
720 groups using the non-parametric Kruskal-Wallis test. Linear mixed-effects modeling of the **(D)**
721 observed ASV richness and **(E)** Shannon diversity over time between enrollment groups. Time
722 scale on the x-axis is days of life (DOL) for corrected GA weeks 28 – 39. Greyed areas around
723 each regression line represent 95% confidence intervals upper and lower around the coefficients.

724

725 **Figure 2 – Antibiotic use explains little effect on beta diversity**

726 PCoA ordination of stool samples using the **(A)** abundance-based Bray-Curtis and **(B)**
727 presence/absence-based Jaccard distance metric among enrollment groups of corrected GA
728 between 28 -39. Ellipses are calculated based on a 95% confidence interval of a multivariate t-
729 distribution.

730

731 **Figure 3 – Effects of feeding patterns on the gut microbiome are transient over time**

732 Effect of various feeding patterns on the **(A)** observed ASV richness **(B)** Shannon diversity and
733 **(C)** log_{10}-transformed copies of 16S rRNA by enrollment group over corrected GA from weeks
734 28 – 39. Only feeding patterns that have at least 2 samples at each timepoint were kept.

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735 Statistical comparison of feeding patterns at each corrected GA timepoint was performed using
736 the non-parametric Kruskal-Wallis test.

737

738 **Figure 4 – Linear mixed effects modelling identifies feeding effects by group over time**

739 Linear mixed-effects results plotted as observed bacterial richness over time by group and
740 feeding types **(A – F)** and the Shannon diversity over time by group and feeding types **(G – L)**.
741 The number of samples used in this analysis by group and by feeding type are listed in
742 Supplementary Table S3.

743

744 **Figure 5 – Integration of clinical and laboratory data gives detailed view of infant stay in**
745 **NICU**

746 Extensive clinical and laboratory data, when combined, provide a detailed summary of each
747 infant's stay in the NICU. Data included in each chart from top to bottom include: the infant ID,
748 group assignment, antibiotic change status (bail), gestational age, any adverse clinical events
749 (which are further described in Supplementary Figure S1), the type and duration of antibiotic use
750 (if any), the copy-number corrected absolute composition of each weekly stool sample and it's
751 $\log_{10}$-scale number of bacterial 16S rRNA copies, the type and duration of each feeding
752 including administration of human milk fortifier, the relative levels of C-reactive protein
753 measured from blood, and relative concentrations of measured stool immune markers (for infants
754 where these measurements were performed). DBM: donor breast milk, MBM: mother's breast
755 milk, NPO: no enteral nutrition, CRP: C-reactive protein, EGF: epidermal growth factor. Listed
756 below is a key for the color-coded bacterial taxa used in the stool 16S rRNA copy-number

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757 corrected composition pie chart for each infant chart. A key for the bacterial color codes, adverse
758 clinical events (including infections by body site) and the administration of human milk fortifier
759 for each infant chart is given below.

760

761 **Figure 6 – Metabolites in stool correlate with abundance of bacterial genera**

762 **(A)** Heatmap of repeated measures correlation coefficients between peak response heights of
763 identified metabolites in stool and the top 10 bacterial genera from the same samples (n=90 stool
764 samples). Significant correlations are indicated by a '+' with FDR-corrected p-values < 0.05. **(B)**
765 Boxplot comparing the peak response heights for 4-aminobutyric acid (GABA) between
766 enrollment groups. Statistical comparisons were made using the Wilcoxon test. **(C)** Boxplot
767 comparing the number of rarefied *Veillonella* counts between the enrollment groups. Statistical
768 comparisons were made using the Wilcoxon test. A summary of the number of infants and
769 samples by group for metabolomics is given in Table 1.

770

771 **Figure 7 – Stool immune marker levels show modest correlation with gut microbiota**

772 **(A)** Heatmap of repeated measures correlation coefficients between immune markers measured
773 from stool and the most abundant bacterial genera from the same samples (n=110 stool samples).
774 Only the bacterial genera with at least one significant correlation with an immune marker are
775 displayed (10 genera). Significant correlations are marked with an '*' by the coefficient, with
776 FDR-adjusted p-values < 0.05. **(B)** Table listing the immune markers used for correlation
777 analysis and their commonly known general functions. **(C)** Comparison of $\log_{10}$-transformed
778 number of *Citrobacter* counts by enrollment group and their significance by the Wilcoxon test. A

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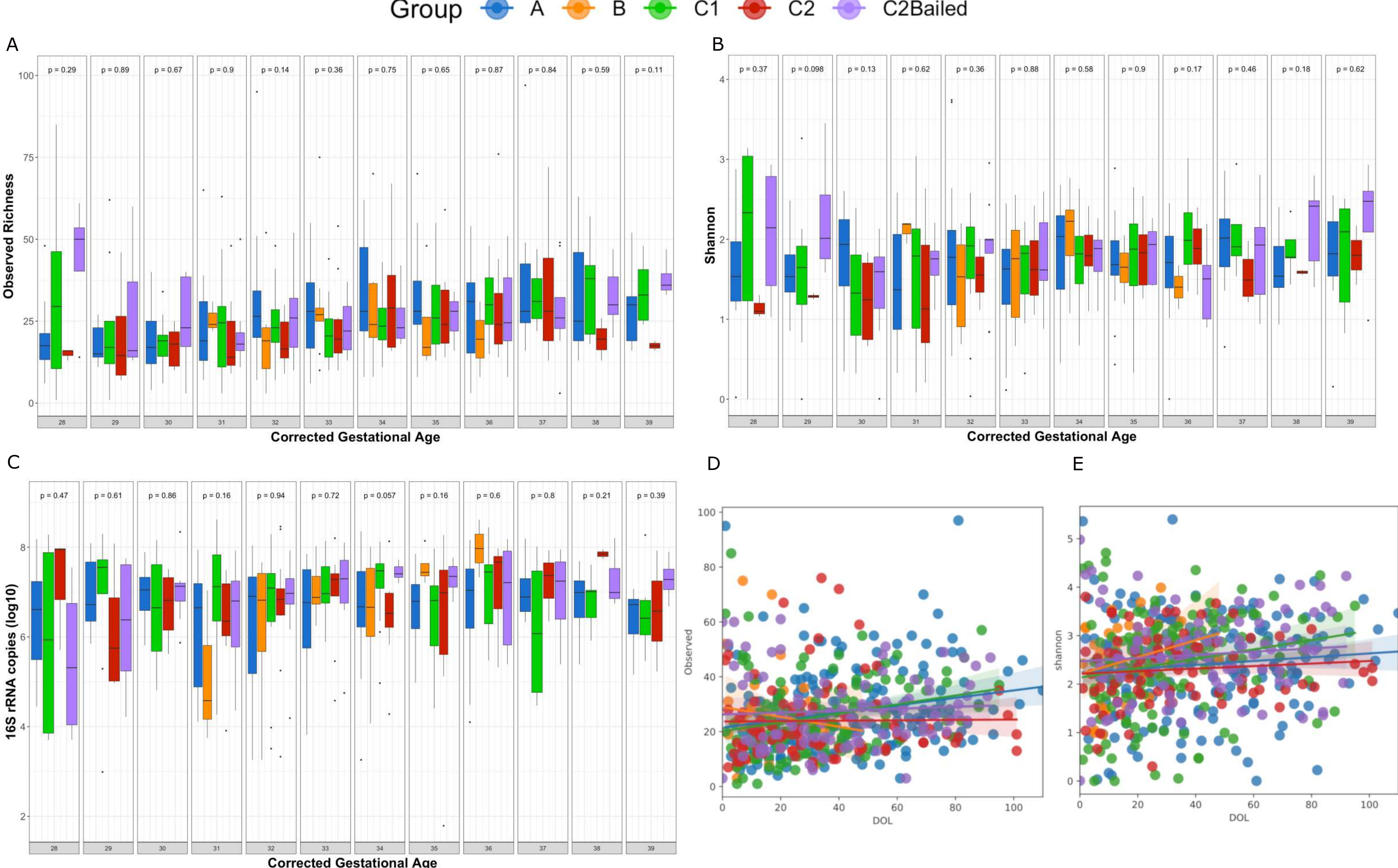
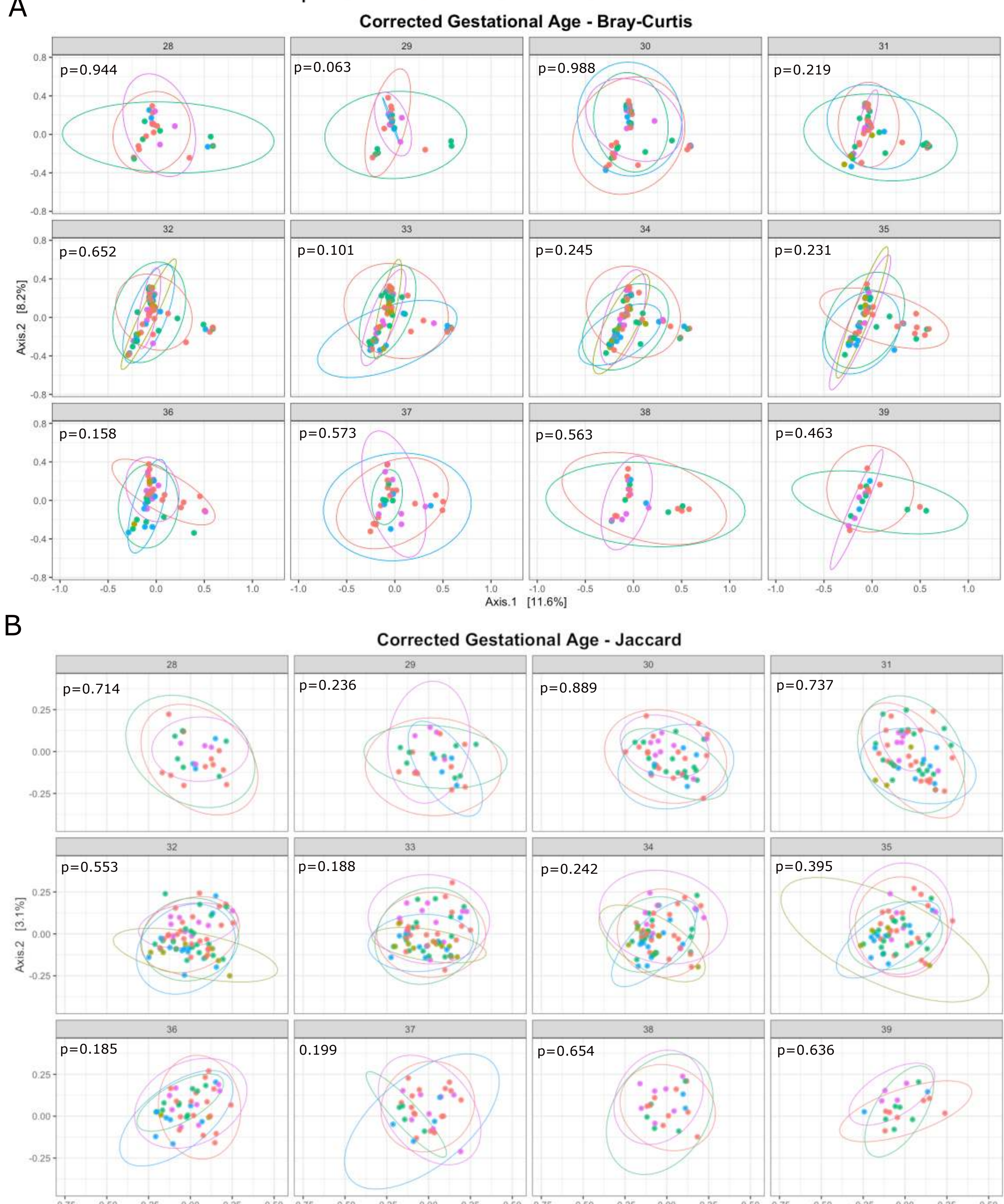
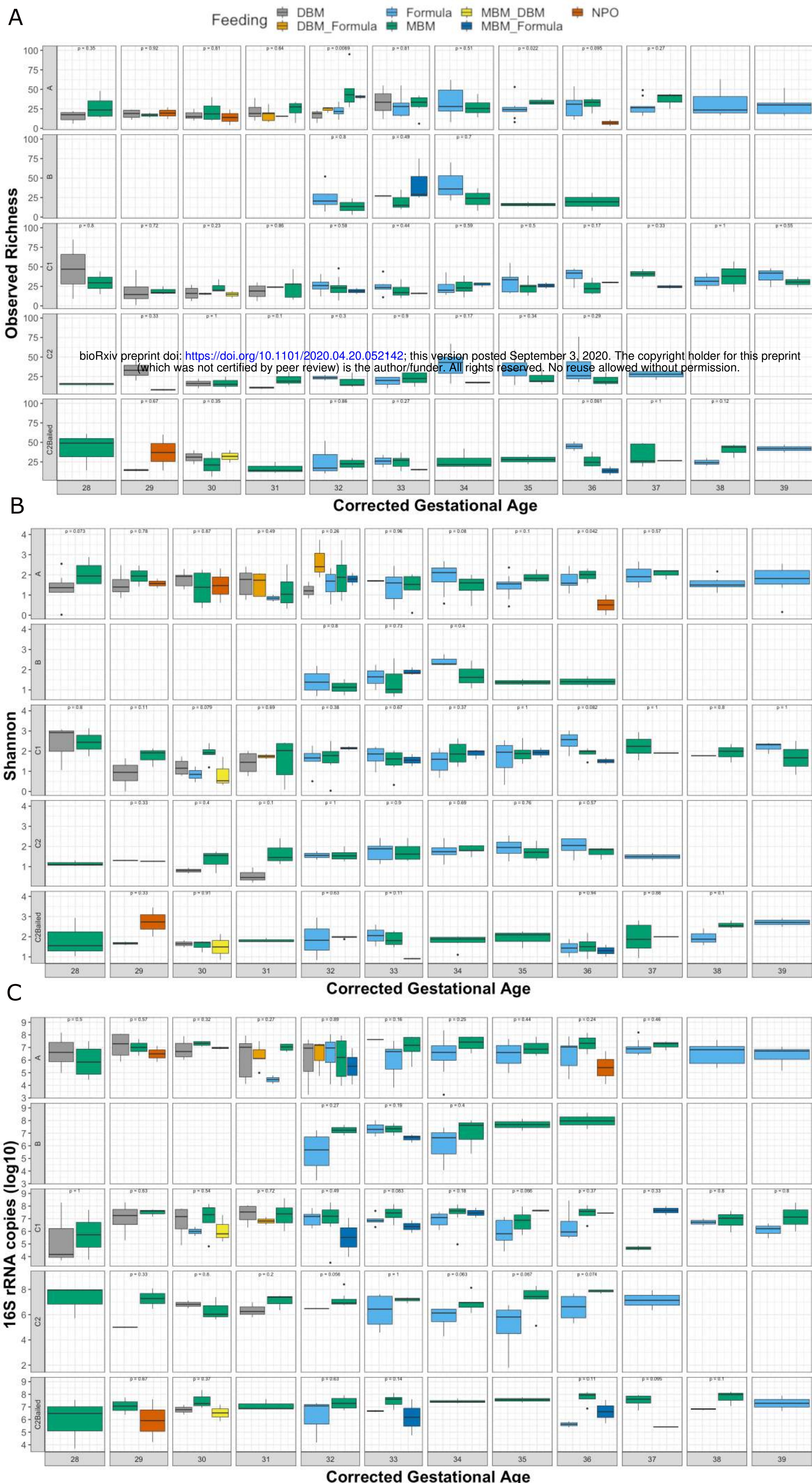
779 summary of the number of infants and samples by group for immune marker analysis is given in

780 Table 1.


Group
A
B
C1
C2
C2Bailed
A
B
C
D
E
Observed Richness
Shannon
16S rRNA copies (log10)
Corrected Gestational Age
Observed
shannon
DOL
p = 0.29
p = 0.89
p = 0.67
p = 0.9
p = 0.14
p = 0.36
p = 0.75
p = 0.65
p = 0.87
p = 0.84
p = 0.59
p = 0.11
p = 0.37
p = 0.098
p = 0.13
p = 0.62
p = 0.36
p = 0.88
p = 0.58
p = 0.9
p = 0.17
p = 0.46
p = 0.18
p = 0.62
p = 0.47
p = 0.61
p = 0.86
p = 0.16
p = 0.94
p = 0.72
p = 0.057
p = 0.16
p = 0.6
p = 0.8
p = 0.21
p = 0.39



Group
A
B
C1
C2
C2Bailed
A
Corrected Gestational Age - Bray-Curtis
28
29
30
31
p=0.944
p=0.063
p=0.988
p=0.219
32
33
34
35
p=0.652
p=0.101
p=0.245
p=0.231
36
37
38
39
p=0.158
p=0.573
p=0.563
p=0.463
Axis.2 [8.2%]
Axis.1 [11.6%]
B
Corrected Gestational Age - Jaccard
28
29
30
31
p=0.714
p=0.236
p=0.889
p=0.737
32
33
34
35
p=0.553
p=0.188
p=0.242
p=0.395
36
37
38
39
p=0.185
0.199
p=0.654
p=0.636
Axis.2 [3.1%]
Axis.1 [3.7%]



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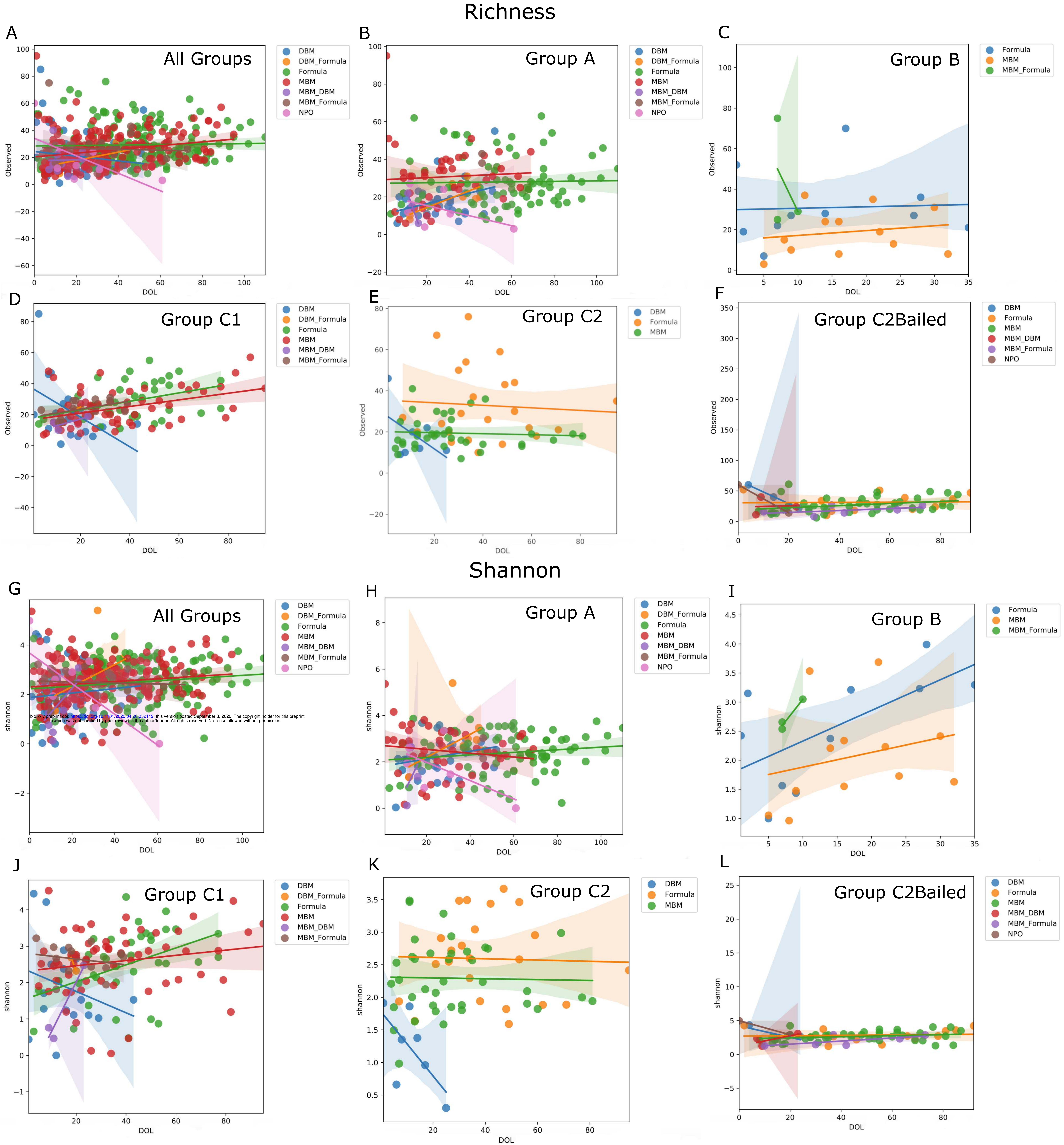

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Infant 1, Group C (randomized to NO Antibiotics, Bailed 0 days post birth), GA 28wks

Adverse Clinical Events

CoNS CoNS

F E A A F E B

Antibiotics

Vancomycin
Oxacillin
Gentamicin
Ampicillin

Bacterial Abundance

Stool Collection

4.60 6.70 6.48 7.61 6.93 7.62 7.92 7.10 7.29 7.35 6.87 7.30 7.07 6.68

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Feeding

HMF

NPO
MBM/DBM
MBM
Formula/MBM
Formula
DBM

Immune Markers Blood

CRP

0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95

Days Post Birth

Bacterial Taxa

Actinomyces
Alloprevotella
Asaccharobacter
Bifidobacterium
Chryseobacterium
Clostridium XIV
Deinococcus
Enterococcus
Finegoldia
Fusobacterium
Klebsiella
Morganella
Pantoea
Prevotella
Pseudomonas
Romboutsia
Selenomonas
Streptococcus
Veillonella
Aerococcus
Anaerococcus
Atopobium
Blautia
Citrobacter
Collinsella
Dialister
Escherichia/Shigella
Franconibacter
Gemella
Lactobacillus
Mycoplasma
Parabacteroides
Propionibacterium
Raoultella
Roseburia
Serratia
Corynebacterium
Enterobacter
Faecalibacterium
Ureaplasma
Alistipes
Aquabacterium
Bacteroides
Clostridium sensu stricto
Fusicatenibacter
Haemophilus
Megasphaera
Negativicoccus
Peptinophilus
Proteus
Robinsoniella
Ruminococcus
Staphylococcus
Terrisporobacter
Unclassified_Enterobacteriaceae
Unclassified_Ruminococcaceae
Unclassified_Peptostreptococcaceae
Unclassified_Coriobacteriaceae
Unclasssifed_Bifidobacteriaceae
Unclassified_Lachnospiraceae
Unclassified_Veillonellaceae

Clinical Events

A - Bacteremia
B - Sepsis
C - Fungemia
D - NEC
E - NEC rule out
F - Sepsis rule out
G - SIP
H - Pneumonia
I - Umbilical abscess or peritonitis
J - Conjunctivitis
K - Bowel stricture or resection
L - OR central line placement
M - Thrombophlebitis
N - Sever IVH
O - Death

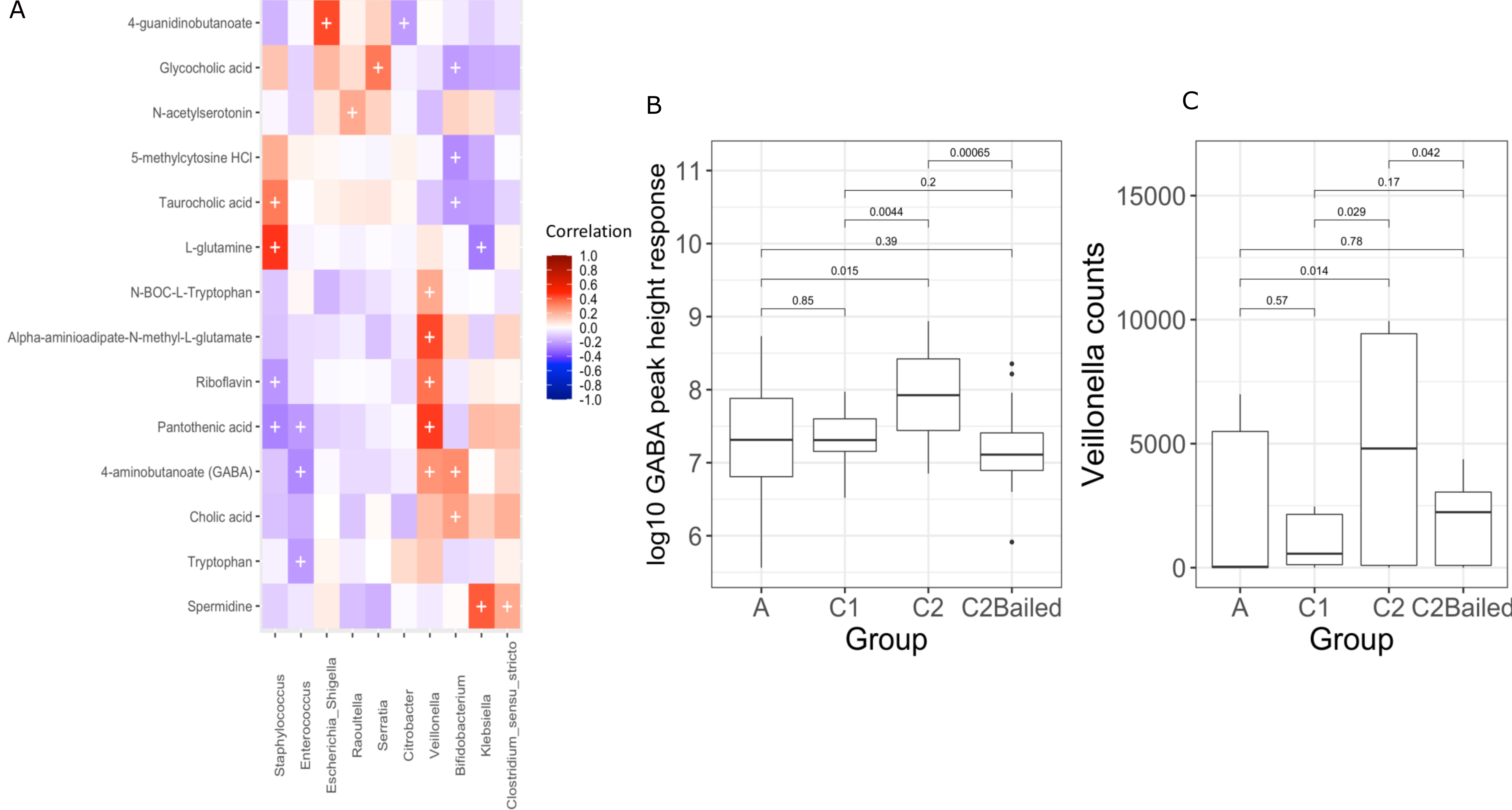
Candida - Candida infection *blood

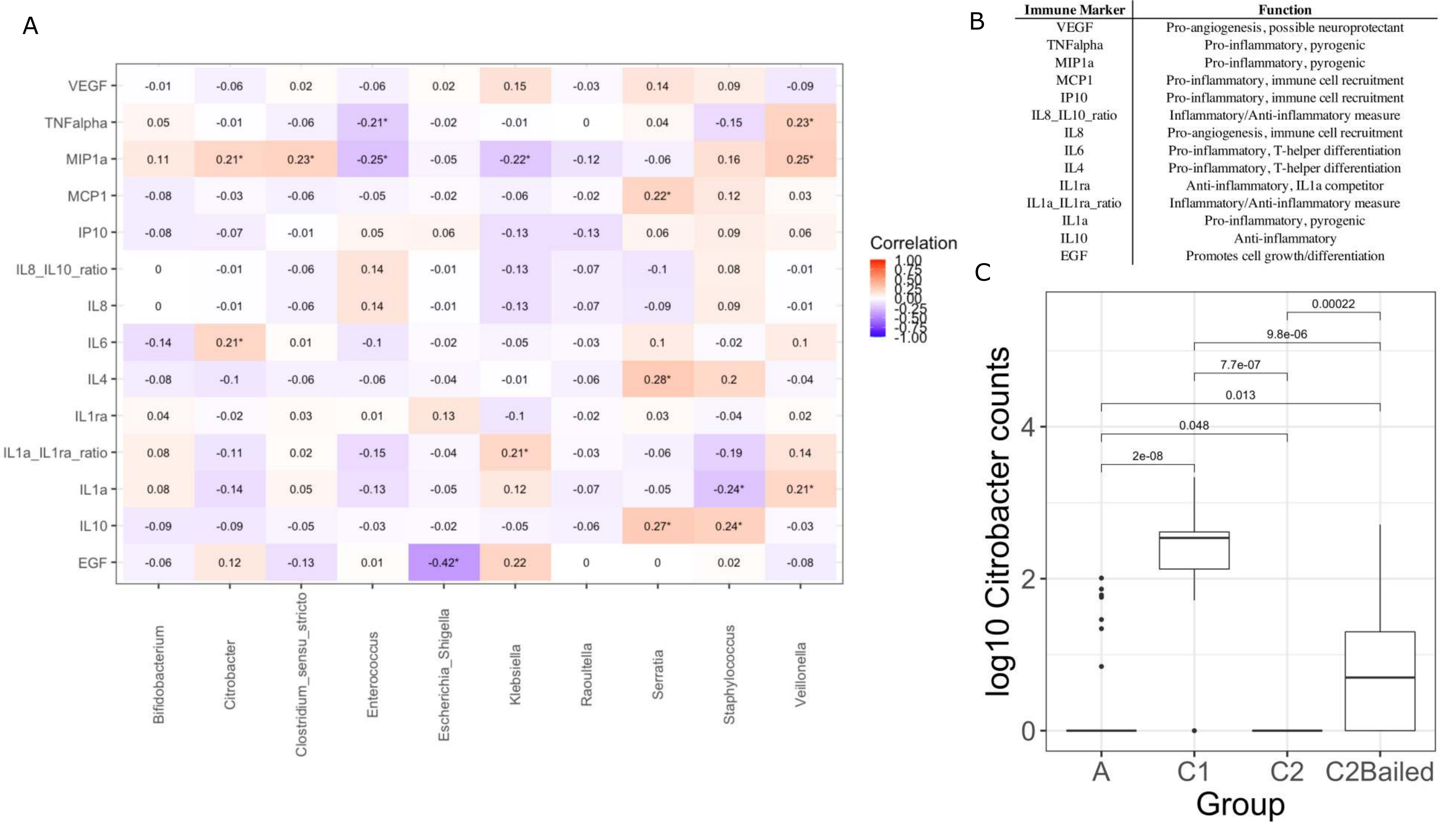
CoNS - Coagulase neg. Staph. *blood

MRSA - MRSA infection *w=wound, s=swab, b=blood

Entero - Enterobacteria infection *w=wound, s=swab, b=blood

HMF - Human milk fortifier


A
4-guanidinobutanoate
Glycocholic acid
N-acetylserotonin
5-methylcytosine HCl
Taurocholic acid
L-glutamine
N-BOC-L-Tryptophan
Alpha-aminioadipate-N-methyl-L-glutamate
Riboflavin
Pantothenic acid
4-aminobutanoate (GABA)
Cholic acid
Tryptophan
Spermidine
Staphylococcus
Enterococcus
Escherichia_Shigella
Raoultella
Serratia
Citrobacter
Veillonella
Bifidobacterium
Klebsiella
Clostridium_sensu_stricto
Correlation
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
B
log10 GABA peak height response
0.00065
0.2
0.0044
0.39
0.015
0.85
A
C1
C2
C2Bailed
Group
C
Veillonella counts
0.042
0.17
0.029
0.78
0.014
0.57
15000
10000
5000
0
A
C1
C2
C2Bailed
Group



Immune Marker	Function
VEGF	Pro-angiogenesis, possible neuroprotectant
TNFalpha	Pro-inflammatory, pyrogenic
MIP1a	Pro-inflammatory, pyrogenic
MCP1	Pro-inflammatory, immune cell recruitment
IP10	Pro-inflammatory, immune cell recruitment
IL8_IL10_ratio	Inflammatory/Anti-inflammatory measure
IL8	Pro-angiogenesis, immune cell recruitment
IL6	Pro-inflammatory, T-helper differentiation
IL4	Pro-inflammatory, T-helper differentiation
IL1ra	Anti-inflammatory, IL1a competitor
IL1a_IL1ra_ratio	Inflammatory/Anti-inflammatory measure
IL1a	Pro-inflammatory, pyrogenic
IL10	Anti-inflammatory
EGF	Promotes cell growth/differentiation