

1 **Sequence-based analysis of the intestinal microbiota of sows and their offspring fed**
2 **genetically modified Bt maize in a trans-generational study**

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17 Running title: Trans-generational effects of feeding GM maize to pigs

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20 **ABSTRACT**

21 The aim was to investigate trans-generational effects of feeding genetically modified (GM)
22 Bt maize to sows and offspring on maternal and offspring intestinal microbiota. Sows were
23 assigned to either non-GM or GM maize dietary treatments during gestation and lactation. At
24 weaning, offspring were assigned within sow treatment to non-GM or GM maize diets for 115
25 days i.e. 1) non-GM maize-fed sow/non-GM maize-fed offspring (**non-GM/non-GM**); 2) non-
26 GM maize-fed sow/GM maize-fed offspring (**non-GM/GM**); 3) GM maize-fed sow/non-GM
27 maize-fed offspring (**GM/non-GM**); and 4) GM maize-fed sow/GM maize-fed offspring
28 (**GM/GM**). Offspring of GM maize-fed sows had higher counts of fecal total anaerobes and
29 *Enterobacteriaceae* at day 70 and 100 post-weaning, respectively. At day 115 post-weaning,
30 GM/non-GM offspring had lower ileal *Enterobacteriaceae* than non-GM/non-GM or GM/GM
31 offspring and lower ileal total anaerobes than pigs on other treatments. GM maize-fed offspring
32 also had higher ileal total anaerobe counts than non-GM maize-fed offspring and cecal total
33 anaerobes were lower in non-GM/GM and GM/non-GM offspring than in non-GM/non-GM. The
34 only differences observed for major bacterial phyla using 16S rRNA gene sequencing were that
35 fecal *Proteobacteria* were less abundant in GM maize-fed sows prior to farrowing and in offspring
36 at weaning, with fecal *Firmicutes* more abundant in offspring. While other differences occurred,
37 they were not observed consistently in offspring, were mostly encountered for low-abundance, low-
38 frequency bacterial taxa and were not associated with pathology. Therefore, their biological
39 relevance is questionable. This confirms the lack of adverse effects of GM maize [on the intestinal](#)
40 [microbiota of pigs](#), even following trans-generational consumption.

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43 **INTRODUCTION**

44 Genetically modified (GM) maize is one of the most widely grown GM crops worldwide.
45 A large proportion of this is Bt maize, which expresses a truncated form of the Cry1Ab protein
46 from *Bacillus thuringiensis*, which confers resistance to certain maize pests (1, 2). While, to date,
47 adverse effects of Bt maize consumption have not been definitively documented, the safety of
48 GM food and feed is an intensely-debated subject (3).

49 Numerous studies have investigated the effects of Bt maize consumption on production
50 and health characteristics in different animal species (3-10); however, apart from those
51 conducted by our group, few studies have been performed in pigs. Furthermore, while a number
52 of studies have investigated the effects of Bt maize on the intestinal microbiota of ruminants (6,
53 11, 12), our group was the first to examine its impact on the porcine intestinal microbiota (13,
54 14). Moreover, we were the first to employ high-throughput 16S rRNA gene sequencing to
55 determine if consumption of GM food/feed influences intestinal microbial communities. Such
56 studies are warranted, considering *in vitro* observations that the Cry1Ab protein is antimicrobial
57 against intestinal bacteria, such as *Clostridium*, both in its intact and fragmented form, at
58 concentrations of 25 – 63 µg/ml (15). Furthermore, the Cry1Ab protein is not completely
59 degraded during intestinal transit when administered in feed at concentrations of 0.17 – 0.52 µg/g
60 feed and persists in the intestine at concentrations of 0.003 – 0.03 µg/ml (8, 9, 16). In addition, as
61 the intestinal microbiota increases Cry1Ab toxicity in insects (17), it is likely that the microbiota
62 either activates certain gut function that render the Cry1Ab toxic or transform the Cry1Ab into a
63 toxic compound. Furthermore, Finamore et al. revealed changes in local and systemic immune
64 response in mice in response to Bt MON810 maize feeding, which were especially evident in
65 young and old mice i.e. at times of major shifts in the intestinal microbiota (18). As no clear
66 hypothesis is provided to explain the outcomes of the study, it is possible that the differences

67 observed by Finamore et al. may be due to indirect effects of the MON810 maize on the
68 intestinal microbiota.

69 To date, the impact of GM feed consumption on the intestinal microbiota of pregnant
70 females and/or their offspring has not been examined. Due to the physiological changes that
71 occur during pregnancy and the extra demands of the developing fetuses, pregnant females may
72 respond differently to the consumption of Bt maize. Furthermore, any dietary-induced
73 perturbations at the level of the intestinal microbiota could potentially trigger an immune
74 response in the pregnant female that could affect *in-utero* development of the offspring. Any
75 disturbance of the intestinal microbiota of pregnant females may also have consequences for
76 establishment of the piglets' intestinal microbiota and indirectly for maturation of the piglet
77 immune system. This is because the newborn's first microbial contact is with that of the mother
78 at birth (19) and the maternal microbiota is the inoculum for colonization of the neonatal
79 digestive tract, which is important for maturation of the neonatal immune system (20).
80 Furthermore, as newborn pigs are in constant contact with the mother's feces until weaning, it is
81 likely that establishment of their post-natal pre-weaning intestinal microbiota depends largely on
82 that of the sow (20).

83 Therefore, the aim of this study was to investigate the trans-generational effects of feeding
84 GM maize to sows during gestation and lactation and to their offspring from weaning for 115
85 days on sow fecal and offspring fecal, ileal and cecal bacterial communities using culture-
86 dependent and -independent approaches.

87

88 MATERIALS AND METHODS

89 **Pig feeding study.** The pig study complied with European Union Council Directives
90 91/630/EEC (outlines minimum standards for the protection of pigs) and 98/58/EC (concerns the

91 protection of animals kept for farming purposes) and was approved by, and a license obtained
92 from the Irish Department of Health and Children (license number B100/4147). Ethical approval
93 was obtained from the Teagasc and Waterford Institute of Technology ethics committees.

94 The duration of the feeding study was 36 weeks. At insemination (day 0), 24 Large White
95 × Landrace nulliparous sows (~165 kg) were blocked by body weight and date of insemination
96 before being randomly assigned to one of two dietary treatments: 1) near isogenic parent line
97 maize (Pioneer PR34N43; **non-GM**) or 2) Bt maize (Pioneer PR34N44 event MON810; **GM**).
98 Sows were fed diets from insemination, throughout pregnancy and lactation, until weaning at
99 ~day 28 post-parturition. At weaning, offspring (n = 20/sow treatment) were selected and
100 blocked by sow treatment, sex and body weight and randomly assigned within sow treatment to
101 either a non-GM or GM maize-based diet for 115 days, giving rise to four dietary treatments: 1)
102 non-GM maize-fed sow/non-GM maize-fed offspring (**non-GM/non-GM**); 2) non-GM maize-
103 fed sow/GM maize-fed offspring (**non-GM/GM**); 3) GM maize-fed sow/non-GM maize-fed
104 offspring (**GM/non-GM**); and 4) GM maize-fed sow/GM maize-fed offspring (**GM/GM**). Sow
105 and offspring housing and management have previously been described by Walsh *et al.* (21) and
106 Buzoianu *et al.* (22), respectively.

107 **Maize and diets.** Seeds derived from the Bt MON810 and non-GM parent line maize
108 (PR34N44 and PR34N43, respectively; Pioneer Hi-Bred, Sevilla, Spain) were grown
109 simultaneously side by side in Valtierra, Navarra, Spain under similar management conditions by
110 independent tillage farmers in 2007. This was done to avoid, insofar as possible, compositional
111 differences between the non-GM and the GM maize due to differences in environmental
112 exposure, soil composition and management practices. The GM and non-GM maize were
113 purchased by the authors from the tillage farmers for use in this animal study. Samples from the
114 non-GM and GM maize were tested for the presence of the *cry1Ab* gene, pesticide contaminants,

115 mycotoxins and carbohydrate composition as previously described by Walsh *et al.* (10). All diets
116 were manufactured and analyzed for proximate analysis and amino acid concentration as
117 previously described by Walsh *et al.* (10). All diets were formulated to meet or exceed the
118 National Research Council requirements for pigs at each production stage (23). Details of animal
119 feeding have previously been described by Walsh *et al.* (21) and Buzoianu *et al.* (22).

120 **Sample collection and analysis.** Fecal samples were collected by rectal stimulation from
121 12 sows/treatment at insemination (day 0), day 110 of gestation (~one week prior to parturition)
122 and at day 28 of lactation. Samples were collected into sterile plastic containers and stored at 4°C
123 in anaerobic jars containing Anaerocult A™ gas packs (Merck, Darmstadt, Germany) until
124 analysis (within 12 hours). Fecal samples were collected in a similar manner from offspring at
125 weaning and days 30, 70 and 100 post-weaning. At day 115 post-weaning i.e. when pigs had
126 reached the normal slaughter weight (~105 kg), 10 of the offspring per treatment were harvested in a
127 commercial abattoir using electrical stunning followed by exsanguination. Digesta samples were
128 removed aseptically from the terminal tip of the cecum and from the ileum (15 cm proximal to
129 the ileo-cecal junction), placed in sterile plastic containers and stored anaerobically, as described
130 for the fecal samples. The last meal was provided 3 hours before euthanasia.

131 Enumeration of *Lactobacillus* [indicator of beneficial bacteria (24)] and *Enterobacteriaceae*
132 [indicator of pathogenic bacteria (24)] from individual fecal samples and ileal and cecal digesta was
133 performed as described by Gardiner *et al.* (Gardiner et al. 2004). To inhibit growth of yeasts and
134 moulds, nystatin (Sigma Aldrich Ireland Ltd., Wicklow, Ireland) was added to the *Lactobacillus*
135 selective agar (Becton, Dickinson, Cockeysville, MD) at a concentration of 50 units/mL. Total
136 anaerobic bacteria from individual fecal samples were enumerated as previously described by Rea *et*
137 *al.* (25). To maintain anaerobiosis, manipulation of all samples was performed in a Whitley A85

138 anaerobic workstation (DW Scientific, Shipley, United Kingdom), and the plates were also
139 incubated anaerobically within the workstation.

140 Fecal samples from sows at day 110 of gestation, from offspring at weaning and day 100
141 post-weaning and cecal samples from day 115 post-weaning were frozen at -20°C for subsequent
142 16S rRNA gene sequencing.

143 **DNA extraction and PCR.** DNA was extracted from fecal samples of sows at day 110 of
144 gestation, offspring at weaning and day 100 post-weaning and from cecal samples at day 115 post-
145 weaning as described by Buzoianu *et al.* (13). PCR amplification of the 239 bp V4 region of the
146 16S rRNA gene, gel electrophoresis, amplicon quantification and purification were performed as
147 previously outlined by Buzoianu *et al.* (13).

148 **16S rRNA gene sequencing and bioinformatic analysis.** Sequencing was performed on a
149 454 Genome Sequencer FLX platform (Roche Diagnostics Ltd., Burgess Hill, West Sussex, UK),
150 according to manufacturer's protocols. Resulting raw sequences were quality trimmed as
151 previously described (26). Trimmed FASTA sequences were then BLASTed against a locally
152 installed version of SILVA 16S rRNA database, with the top 50 hits against the database
153 selected. The taxonomic distribution of reads was determined using MEGAN, with modified
154 accession look-up tables for mapping the SILVA assignments to NCBI taxonomy. MEGAN
155 assigns reads to NCBI taxonomies by employing the Lowest Common Ancestor algorithm. Bit
156 scores were used from within MEGAN for filtering the results before tree construction and
157 summarization. A bit-score of 86 was selected as previously used for 16S ribosomal sequence
158 data (27). Phylum, family and counts for each subject were extracted from MEGAN. MOTHUR
159 software was used to compute alpha diversity indices. Sequence reads were clustered into
160 operational taxonomical units (OTUs) using the QIIME suite of software tools. OTUs were
161 aligned and chimeric OTUs were removed using the ChimeraSlayer program. A phylogenetic

162 tree was generated using the FastTreeMP tool. Subsequently, beta diversities of the samples were
163 calculated. Principal coordinate analysis (PCoA) and hierarchical clustering of samples were
164 implanted. PCoA plots were visualised with the KiNG viewer. The number of reads assigned to
165 each taxonomic rank was divided by the number of reads assigned to the highest rank (phylum)
166 to obtain the percentage relative abundance values.

167 **Statistical analysis.** Statistical analysis of data was performed using SAS 9.2
168 (SAS/STAT®9.22 2010). Data were checked for normality using the Shapiro-Wilk test within
169 PROC UNIVARIATE in SAS. In an attempt to ensure normality, bacterial counts were log-
170 transformed to the base 10 and non-normal relative abundance data were transformed using the
171 Box Cox transformation (28). Data that were initially normally distributed or were normalized
172 with the Box Cox transformation were analyzed using PROC MIXED while non-normal data
173 were analyzed using PROC NPAR1WAY. Data analyzed using PROC MIXED are presented as
174 least squares means and 5-95th confidence limits of raw data (for data that were initially normal)
175 or of the back-transformed values (for data normalized using the Box Cox transformation), while
176 bacterial counts are presented as log-values with their corresponding standard error of the mean.
177 Data analyzed using non-parametric tests are presented as medians and 5-95th percentiles (the 5th
178 percentile is larger than 5% of the values and the 95th percentile is larger than 95% of the values).
179 Significance is reported for $P \leq 0.05$. For all variables, the individual pig was the experimental
180 unit.

181 For sows, relative abundance data that were normally-distributed and log-transformed
182 microbial counts were analyzed using PROC MIXED in SAS with treatment as a fixed effect and
183 block as a random effect in the statistical model. For microbial counts, day 0 values were
184 included as covariates in the statistical model and day was included as a repeated variable. The

185 *slice* option was used to test for simple effects at individual time points. Non-normal data were
186 analyzed using the Kolmogorov-Smirnov test within PROC NPAR1WAY.

187 For offspring, data that were normally distributed or that were normalized were analyzed
188 as a 2×2 factorial split-plot design with sow treatment regarded as the main plot and offspring
189 treatment as the sub-plot. Sow and offspring treatment and their interaction were included in the
190 statistical model as fixed effects and block, sow block and the sow block \times sow treatment
191 interaction were included as random effects. Data that were normal or that were normalized for
192 both day 0 and day 100 were analyzed as repeated measures with day included in the statistical
193 model as a repeated variable. The *slice* option was used to test for simple effects at individual
194 time points. *P* values were adjusted for multiple comparisons using the Tukey-Kramer
195 adjustment. For bacterial counts, day 0 values were included as a covariate in the statistical
196 model. For non-normal data, the non-parametric Kolmogorov-Smirnov test was used for sow
197 treatment and offspring treatment effects and the non-parametric Kruskal-Wallis test was used to
198 analyze differences among the four combinations of treatments.

199

200 RESULTS

201 One sow from the non-GM treatment received antibiotic treatment on days 105-107 of
202 gestation. Likewise, four of the offspring (one from the non-GM/GM, two from the non-
203 GM/non-GM and one from the GM/GM treatment) were treated with antibiotics for three days
204 between day 70 and day 100. As a result, data from any of these treated animals were not
205 included for analysis at subsequent sampling points.

206 **Maize and diets.** No major compositional differences were observed between the GM and
207 non-GM maize (10) or between the non-GM and GM diets (Tables S1 and S2). [The Bt maize](#)
208 [was found to have a 2.63% units \(as % of dry matter\) lower enzyme-resistant starch content and](#)

209 a 3.08% units (as % of dry matter) higher overall starch content than the non-GM maize.
210 However, the values remained within the natural variability for maize varieties cited in the
211 literature (13).

212 **Effects of feeding GM maize-based diets to sows during gestation and lactation and to**
213 **offspring for 115 days post-weaning on selected culturable fecal and intestinal microbiota.**

214 Bacterial counts from sow fecal samples are presented in Table S3. There was no effect of
215 feeding GM maize-based diets to sows on counts of culturable fecal *Enterobacteriaceae*,
216 *Lactobacillus* or total anaerobes on day 110 of gestation or day 28 of lactation. While
217 *Lactobacillus* counts increased from day 28 of gestation to day 28 of lactation ($P < 0.05$; data not
218 shown), *Enterobacteriaceae* and total anaerobe counts decreased between these two sampling
219 points ($P < 0.05$; data not shown).

220 Bacterial counts from offspring fecal and intestinal samples are presented in Table 1.
221 There was no sow treatment \times offspring treatment interaction or effect of offspring treatment on
222 fecal *Enterobacteriaceae* at any time during the study ($P > 0.05$). At day 100 post-weaning,
223 offspring from GM maize-fed sows had higher fecal counts of *Enterobacteriaceae* than offspring
224 from non-GM maize-fed sows ($P \leq 0.05$; Table 1). No sow treatment \times offspring treatment
225 interaction was observed for *Lactobacillus* counts in the feces of offspring and neither were any
226 sow or offspring treatment effects seen. A sow treatment effect was observed for fecal total
227 anaerobes at day 70 post-weaning, when offspring of GM maize-fed sows had higher counts than
228 offspring of non-GM maize-fed sows ($P < 0.05$; Table 1). Ileal *Enterobacteriaceae* were lower at
229 day 115 post-weaning in offspring on the GM/non-GM treatment than in offspring on the non-
230 GM/non-GM and GM/GM treatments ($P < 0.05$). No treatment effect was observed for offspring
231 ileal or cecal *Lactobacillus* counts at day 115 post-weaning ($P > 0.05$). However, at day 115
232 post-weaning ileal total anaerobe counts were lower in pigs on the GM/non-GM treatment than

233 in pigs on all other treatments ($P < 0.05$) and this lead to an offspring treatment effect, with GM
234 maize-fed offspring having higher counts than non-GM maize-fed offspring ($P < 0.05$; Table 1).
235 A sow treatment \times offspring treatment interaction was also observed for cecal total anaerobes at
236 day 115 post-weaning, with offspring on the non-GM/GM and GM/non-GM treatments having
237 lower counts than offspring on the non-GM/non-GM treatment ($P < 0.05$).

238 **Effects of feeding GM maize-based diets to sows during gestation and lactation and to**
239 **offspring for 115 days post-weaning on microbial population indices.** A total of 65,890 reads
240 (average of 2,864 (1,068 – 4,176) reads/sample) of the V4 region of the 16S rRNA gene were
241 generated from high-throughput sequencing of fecal samples from sows. In offspring at weaning,
242 188,583 (average of 4,715 reads (3,048 – 6,827) /sample) reads were generated, while at day 100
243 post-weaning, 175,133 reads were generated (average of 4,609 (2,509 – 6881) reads/sample).
244 High-throughput sequencing of cecal samples at day 115 post-weaning yielded 100,601 reads
245 (average of 2,719 (1,400 – 4,682) reads/sample).

246 Population indices were similar among treatments (Table 2). Unweighted beta diversity
247 plots did not show clustering specific to any treatment group (Fig. S1, S2, S3 and S4).

248 **Effect of feeding a GM maize-based diet to sows on the relative abundance of sow**
249 **fecal microbiota at day 110 of gestation.** Results for taxa, abundance of which differed
250 significantly among sow treatments are presented in Table 3 and Fig. 1 (main phyla only shown
251 in the latter). A full outline of all taxa detected in fecal samples of sows can be found in Table
252 S4. At the phylum level, the relative abundance of *Proteobacteria* was lower in the feces of GM
253 maize-fed sows ($P < 0.05$; Fig. 1). *Verrucomicrobia* were detected in only four sows on the non-
254 GM treatment and in none on the GM treatment, resulting in a lower relative abundance of this
255 phylum in GM maize-fed sows ($P < 0.05$; Table 3).

256 A total of 40 bacterial families were identified in the feces of sows at day 110 of gestation
257 (Table S4). The *Rikenellaceae* differed in their relative abundance among treatments, with GM
258 maize-fed sows having lower abundance compared to the non-GM maize-fed sows ($P < 0.05$).
259 The *Prevotellaceae*, *Succinivibrionaceae*, *Rikenellaceae*-related bacteria and *Lactobacillaceae*
260 were also lower in the feces of GM maize-fed sows compared to non-GM maize-fed sows ($P <$
261 0.05).

262 Forty genera were detected in the feces of sows at day 110 of gestation. The relative
263 abundance of the genus *Prevotella* was significantly different between non-GM maize- and GM
264 maize-fed sows, with a lower relative abundance found in the latter ($P < 0.05$; Table 3). The
265 relative abundance of *Lachnospiraceae incertae sedis*, *Anaerobiospirillum* and *Lactobacillus*
266 was also lower in GM maize-fed sows ($P < 0.05$), with the latter due to low frequency of
267 detection in this treatment group.

268 **Effect of feeding a GM maize-based diet to sows on the relative abundance of fecal**
269 **microbiota of offspring at weaning.** Results for taxa, abundance of which differed significantly
270 among treatments are presented in Tables 4, S8, S9 and Fig. 1 (main phyla only shown in the
271 latter). A full outline of all taxa detected in offspring fecal samples at weaning can be found in
272 Table S5. At the phylum level, the relative abundance of *Firmicutes* was higher for offspring of
273 GM maize-fed sows than for offspring of non-GM maize-fed sows ($P \leq 0.05$; Fig. 1). A sow
274 treatment effect was also observed for fecal *Proteobacteria*, with lower relative abundance in
275 offspring from GM maize-fed sows than in offspring of non-GM maize-fed sows ($P < 0.05$; Fig.
276 1).

277 Analysis of fecal bacterial communities in weanling pigs revealed 43 families (Table S5).
278 A higher relative abundance was observed in offspring from GM maize-fed sows compared to
279 offspring from non-GM maize-fed sows for the families *Ruminococcaceae* ($P < 0.05$; Table S8),

280 *Lachnospiraceae* ($P < 0.05$; Table S8) and *Clostridiaceae* ($P < 0.05$; Table S8). Fecal
281 *Clostridiaceae* were also present at a higher relative abundance in the feces of pigs on the
282 GM/non-GM treatment than pigs on the non-GM/GM treatment ($P < 0.05$; Table 4). There was
283 also an overall offspring treatment effect for the *Rikenellaceae* family, with GM maize-fed
284 offspring having higher relative abundance than non-GM maize-fed offspring ($P < 0.05$; Table
285 S9). Offspring of GM maize-fed sows also had higher relative abundance of *Victivallaceae* than
286 offspring of non-GM maize-fed sows ($P < 0.05$; Table S8).

287 A total of 73 genera were detected in the feces of weanling pigs, with offspring of GM
288 maize-fed sows having a higher relative abundance of *Subdoligranulum* than offspring of non-
289 GM maize-fed sows ($P < 0.05$; Table S8). Fecal *Clostridium* was higher in pigs on the GM/non-
290 GM treatment than in pigs on the non-GM/GM treatment ($P < 0.05$; Table 4). Fecal
291 *Butyricimonas* were present at a higher relative abundance in pigs on the GM/GM treatment than
292 in pigs on any of the other treatments ($P < 0.05$; Table 4). Likewise, *Roseburia* was more
293 abundant in feces of pigs on the non-GM/GM treatment than in pigs on all other treatments ($P <$
294 0.05 ; Table 4).

295 **Effect of feeding a GM maize-based diet to sows and their offspring on the relative**
296 **abundance of fecal microbiota in offspring at day 100 post-weaning.** Results for taxa,
297 abundance of that differed significantly among treatments are presented in Tables 4, S8 and S9.
298 A full outline of all taxa detected in offspring fecal samples at day 100 post-weaning can be
299 found in Table S6. At the phylum level, only the *Fibrobacteres* were affected by treatment, being
300 lower in pigs on the non-GM/GM and the GM/non-GM treatments than in pigs on the non-
301 GM/non-GM treatment ($P < 0.05$; Table 4).

302 Thirty-five bacterial families were detected in the feces of pigs at 100 days post-weaning.
303 The relative abundance of fecal *Fibrobacteraceae* was lower in pigs on the non-GM/GM and

304 GM/non-GM treatments than in pigs on the non-GM/non-GM treatment ($P < 0.05$; Table 4).
305 Fecal *Helicobacteraceae* were higher in pigs on the non-GM/GM treatment than pigs on all other
306 treatments ($P < 0.05$; Table 4).

307 Fecal *Ruminococcus* was less abundant in pigs on the GM/GM treatment than in pigs on
308 the non-GM/GM treatment ($P < 0.05$; Table 4), leading to a lower relative abundance of this
309 genus in offspring of GM maize-fed sows than in offspring of non-GM maize-fed sows ($P <$
310 0.05 ; Table S8). *Oscillospira* and *Faecalibacterium* were less abundant in feces of GM maize-
311 fed offspring than in non-GM maize-fed offspring ($P < 0.05$; Table S9). *Fibrobacter* was less
312 abundant in offspring on the non-GM/GM and GM/non-GM treatments than in offspring on the
313 non-GM/non-GM treatment ($P < 0.05$; Table 4). GM maize-fed offspring had lower fecal
314 *Thalassospira* than non-GM maize-fed offspring ($P < 0.05$; Table S9). *Helicobacter* was present
315 at a higher relative abundance in pigs on the non-GM/GM treatment compared to pigs on any of
316 the other treatments ($P < 0.05$; Table 4). *Solobacterium* was less abundant in pigs on the
317 GM/non-GM and GM/GM treatments than in pigs on the non-GM/non-GM treatment ($P < 0.05$;
318 Table 4).

319 **Effect of feeding a GM maize-based diet to sows and their offspring on the relative**
320 **abundance of cecal microbiota of offspring at day 115 post-weaning.** Results for taxa,
321 abundance of that differed significantly among treatments are presented in tables 4, S8 and S9
322 and a full outline of all taxa detected in offspring cecal samples can be found in Table S7. A total
323 of 13 phyla were detected at 115 days post-weaning. However, no phylum was affected by
324 treatment ($P > 0.05$).

325 At the family level, a sow treatment \times offspring treatment interaction was observed for
326 *Clostridiaceae*, with a higher relative abundance observed in the ceca of pigs on the non-
327 GM/GM treatment compared to pigs on the non-GM/non-GM treatment ($P < 0.05$; Table 4). The

328 offspring of GM maize-fed sows had lower cecal *Enterobacteriaceae* than the offspring of non-
329 GM maize-fed sows, irrespective of offspring treatment ($P < 0.05$; Table S8).

330 At the genus level, a sow treatment \times offspring treatment interaction was observed for
331 cecal *Phascolarctobacterium*, with pigs on the non-GM/GM treatment having lower relative
332 abundance than the non-GM/non-GM and the GM/GM treatments ($P < 0.05$; Table 4). The
333 relative abundance of *Anaerotruncus* was also higher in GM maize-fed pigs than in non-GM
334 maize-fed pigs ($P < 0.05$; Table S9).

335

336 DISCUSSION

337 It is well recognized that the intestinal microbiota has a major influence on host health (19,
338 29). As the maternal microbiota provides the inoculum for colonization of the offspring digestive
339 tract (19), any effect that GM maize consumption may have on the intestinal microbiota of
340 pregnant sows could affect the health and lifelong performance of their offspring. A number of
341 studies have investigated the impact of Bt maize consumption on the intestinal microbiota of
342 ruminants (11, 12, 30) and our group has evaluated its influence on the porcine microbiota (13,
343 14). However, results from trans-generational studies are absent from the literature and, to our
344 knowledge, this is the first study to investigate the effects of GM maize on the intestinal
345 microbiota of pregnant sows and their offspring.

346 Furthermore, the differences in *Enterobacteriaceae* observed using culturing were not
347 evident when the unculturable component of this family was accounted for using 16S rRNA gene
348 sequencing analysis. Moreover, these differences, as well as the treatment differences observed
349 for total anaerobes in the ileal and cecal digesta, were not associated with intestinal dysfunction
350 or organ pathology (22) and are therefore, not believed to be of biological relevance. This is in

351 agreement with findings from our previous studies, that showed a lack of adverse effects on
352 culturable intestinal microbiota in pigs fed GM maize for 31 or 110 days (14, 22).

353 **Sows.** If GM maize were to affect the intestinal microbiota, it would be most evident in
354 sows, as they had the highest dietary inclusion of maize. However, GM maize consumption had
355 no significant effects on selected culturable microbiota nor on the dominant bacterial phyla, as
356 assessed by high-throughput sequencing. *Proteobacteria* were less abundant in GM maize-fed
357 sows, most likely as a result of lower relative abundance of members of this phylum (i.e. the
358 family *Succinivibrionaceae* and its member genus *Anaerobiospirillum*). A lower relative
359 abundance of *Proteobacteria* was also observed at weaning for offspring of GM maize-fed sows,
360 and may have been due to the influence of the maternal microbiota; however, it was not
361 associated with treatment differences in any member taxa. These reductions can be considered
362 beneficial, as increased *Proteobacteria* have been linked with intestinal inflammation (31) and
363 this phylum encompasses bacteria known to cause intestinal pathology in humans and animals
364 (32, 33). Interestingly, this reduction in *Proteobacteria* was associated with beneficial immune
365 effects in the same sows (34).

366 The lower relative abundance of *Prevotellaceae* and its member genus *Prevotella* in GM
367 maize-fed sows could be a result of the lower enzyme resistant starch content of the GM maize
368 used in the present study (10), as these bacteria are known to be involved in intestinal fiber
369 fermentation (35). However, this was not reflected in the offspring microbiota at weaning, 100
370 days later or at slaughter-age. The lack of effect at weaning may be explained by the fact that
371 pigs were still suckling up to this point and had therefore not consumed any solid feed.
372 Furthermore, fiber-degrading populations in the GIT do not stabilize before 6 weeks post-
373 weaning (36). The fact that offspring *Prevotellaceae* and *Prevotella* were not impacted by
374 maternal dietary treatment later in life, may be due to the fact that sows are known to have a fully

375 developed hind-gut fiber-fermenting microbiota, while hind-gut fermentation is not at its peak in
376 younger pigs (37). These data also provide evidence that, while maternal microbiota is important
377 for initial colonization of the digestive tract, other factors, such as diet composition and rearing
378 environment have a major influence on lifelong structure of offspring microbiota, being in in
379 agreement with the findings of Schmidt *et al.* (38) and Mulder *et al.* (39).

380 The lower abundance of the family *Lactobacillaceae* in the feces of GM maize-fed sows
381 resulted from a lower abundance of its member genus *Lactobacillus*. Although no difference was
382 observed in fecal *Lactobacillus* counts using traditional culturing methods, this can be attributed
383 to the [inability of culture-based approaches to account for unculturable lactobacilli](#) (40). Lower
384 intestinal lactobacilli would generally be perceived as negative, as this genus is considered to
385 have a beneficial role in the porcine, as well as the human, intestine (41, 42), however no health
386 abnormalities were observed in the sows (21, 34).

387 **Offspring at weaning.** Fecal *Firmicutes* were more abundant in the offspring of GM
388 maize-fed sows at weaning, as a result of differences in member taxa, such as the
389 *Ruminococcaceae*, *Lachnospiraceae* and *Clostridiaceae* families and their member genera,
390 *Subdoligranulum* and *Clostridium*. This increase in *Firmicutes*, although not found in the sow
391 feces, was accompanied by a reduction in *Proteobacteria*, as outlined above. A higher abundance
392 of *Firmicutes* has been associated with a higher energy intake and increased deposition of body
393 mass (43). This is in agreement with our data, as offspring of GM maize-fed sows consumed
394 more feed and grew faster than offspring from non-GM maize-fed sows (22) even though there
395 were no major nutritional differences between the non-GM and GM diets. These animals had
396 improved lifelong performance even though the increase in *Firmicutes* did not persist to day 100
397 post-weaning. This study was not designed to investigate sow reproductive performance and
398 there was insufficient replication to allow statistical analysis of litter size of sows. However, a

399 numerically higher number of pigs were born to GM maize-fed sows (21). This may have lead to
400 marginal *in-utero* growth retardation, that is known to induce leptin resistance and higher
401 appetite in offspring (44, 45). The latter was in fact observed post-weaning in the offspring of
402 GM maize-fed sows (22). Interestingly, the intestinal microbiota of leptin-deficient mice has also
403 been shown to be higher in *Firmicutes* and lower in *Proteobacteria* (43).

404 **Offspring at day 100 post-weaning.** At day 100 post-weaning, the genus *Helicobacter*
405 and its corresponding family, the *Helicobacteraceae* were also altered in the feces of pigs on the
406 non-GM/GM treatment compared to all other treatments, in that they were found at higher
407 relative abundance. However, while these bacteria are normally associated with gastrointestinal
408 pathology, in this instance no health abnormalities or disruptions in intestinal architecture were
409 observed in these pigs (22).

410 While the pattern of relative abundance for *Ruminococcus* resembles the pattern of feed
411 intake for these pigs (22), there are no data in the literature to correlate this fiber-fermenting
412 genus with feed intake. *Faecalibacterium* and *Oscillospira* are detected with low abundance in
413 the human and ruminant digestive tract and seem to be associated with a high fiber diet (46, 47).
414 Therefore, the lower relative abundance of these genera observed in the feces of GM maize-fed
415 pigs at day 100 post-weaning may be a result of the slightly lower enzyme resistant starch (which
416 is considered dietary fiber) in the GM maize used in this study (13).

417 **Offspring at day 115 post-weaning.** The large intestine is the main microbial
418 fermentation site in pigs, with the highest bacterial load (37). However, no major effects of GM
419 maize feeding were observed within the cecal microbiota at slaughter at day 115 post-weaning;
420 for example, no differences were uncovered for the major bacterial phyla or families. Relative
421 abundance of the family *Clostridiaceae*, however, was increased in pigs on the non-GM/GM
422 treatment and *Enterobacteriaceae* were lower in offspring of GM maize-fed sows. However,

423 while members of both of these families are known to cause intestinal pathology in humans and
424 animals (48-50), the differences were numerically small and no changes in intestinal architecture
425 or any adverse health effects were observed in these treatment groups (22).

426 **Overall observations.** Overall, of the total of 436 taxa (phyla, families and genera)
427 detected in this study in sows and their offspring, differences among treatments were observed
428 for only 36 taxa and most of the differences occurred for minor taxa with low frequency of
429 detection whose role in the intestine is not fully established. Some of the differences observed in
430 the offspring microbiota might be attributable to differences in the *in-utero* environment
431 experienced by pigs on each sow treatment; however, a much more likely explanation is that they
432 were due to changes in the maternal microbiota in response to feeding GM maize. This is
433 because the sow plays a major role in microbial colonization of the digestive tract of pre-weaning
434 pigs (20). However, a clear effect of feeding GM maize to sows on the intestinal microbiota of
435 their newly-weaned offspring was not observed and the treatment differences observed at
436 weaning did not persist. Furthermore, no adverse effects were observed on the immune system or
437 organ health in either sows or their offspring (21, 22).

438 The absence of adverse effects of GM maize consumption supports our previous
439 conclusions that cecal bacteria are not majorly affected by either short- or long-term GM maize
440 consumption (13, 14). Similar results have been obtained in cows, where short-term consumption
441 of GM maize did not affect rumen microbiota as assessed by 16S rRNA gene sequencing (11),
442 quantitative PCR (12) or ribosomal intergenic spacer analysis (30). Culturable amyolytic and
443 cellulolytic bacteria were also unaffected by 3 years of feeding GM maize to sheep (6).

444 Apart from investigating the impact of GM maize consumption, the present study also
445 provides, for the first time, an insight into the intestinal microbiota of sows at the end of
446 pregnancy and its impact on offspring microbiota using 16S rRNA gene sequencing. The fecal

447 microbiota of sows and their offspring and the offspring cecal microbiota were dominated for the
448 most part by *Firmicutes* and *Bacteroidetes*, similar to previous findings for the cecal and fecal
449 microbiota of weanling and finisher pigs (13, 14, 51, 52) and is in agreement with data from
450 humans (47, 53). Some similarities are evident between the fecal microbiota of sows and their
451 offspring, providing support for the assumption that maternal flora influences that of progeny
452 (19). For example, *Lachnospiraceae* and *Ruminococcaceae* were the dominant families in sows
453 prior to delivery and in offspring at weaning and thereafter. However, differences were also
454 observed, perhaps the most obvious being the relatively low abundance of the *Spirochaetaceae*
455 family in offspring at weaning and the fact that *Bacteroidetes* were replaced by *Proteobacteria* as
456 the second most dominant phylum in these animals while the *Spirochaetes* were the second most
457 dominant phylum in sows. At the genus level, *Prevotella* are among the most abundant genera
458 within both the sow and offspring microbiota. However, in accordance with its corresponding
459 family, *Spirochaetaceae* and phylum, *Spirochaetes*, *Treponema* are detected at low relative
460 abundance in offspring at weaning in comparison to sows and offspring at later time points.
461 Another difference is that the fecal microbiota of pigs at weaning is low in *Ruminococcus*-related
462 bacteria, while they were a major genus in growing pigs and sows. Overall, these data
463 demonstrate perturbations in the intestinal microbiota at weaning, which is most likely due to
464 maternal separation, as well as social and environmental changes and is in agreement with the
465 findings of others (54, 55). Furthermore, weaning is a time of flux for the intestinal microbiota,
466 and while the maternal microbiota provides the seed inoculum, adult-type bacterial communities
467 take time to establish. However, the data also indicate that most of the main phyla and families
468 were already established and resembled adult patterns as early as day 28 of life, providing further
469 evidence of the influence of early-life environment on gut microbiota composition in adult pigs
470 (39).

471 While long-term GM maize consumption by sows and their offspring impacted the
472 intestinal microbiota, the effects were limited and were not associated with any health
473 abnormalities in either sows or their progeny. Furthermore, differences observed in GM maize-
474 fed sows did not transfer to offspring and effects in offspring were not consistently detected
475 across sampling points. This helps to confirm the lack of adverse effects of GM maize
476 consumption, even following long-term exposure in immuno-deficient animals, i.e. pregnant
477 females and newly-weaned pigs.

478

479 **AUTHORS' CONTRIBUTIONS**

480 Secured the funding for the research: PGL and RPR. Designed the study: PGL and GEG.
481 Conducted the study: SGB, MCW, PGL, GEG and MCR. Conducted the laboratory analysis:
482 SGB, MCW, GEG, and MCR. Analyzed the data: SGB, OO and LQ. Wrote the manuscript:
483 SGB. Revised the manuscript: SGB, GEG, PGL, PDC and MCR. All authors read and approved
484 the final manuscript.

485

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496

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673

674 **TABLE 1** Bacterial counts^a in offspring of sows fed a GM or non-GM maize diet

Sow trt ^b	Non-GM		GM		SEM	P-value			
	Offspring trt ^c	Non-GM	GM	Non-GM		GM	Sow trt	Offspring trt	Sow trt × offspring trt
<i>Fecal Enterobacteriaceae</i>									
day 30 pw ^d		5.61	5.94	5.80	6.24	0.196	0.51	0.29	0.64
day 70 pw		6.72	6.73	6.61	6.78	0.154	0.91	0.73	0.97
day 100 pw		6.78	6.50	7.15	7.18	0.139	0.05	0.61	0.16
<i>Fecal Lactobacillus</i>									
day 30 pw		8.92	9.03	8.94	8.98	0.090	0.94	0.68	0.97
day 70 pw		8.48	8.98	8.71	8.42	0.096	0.41	0.58	0.18
day 100 pw		8.69	8.90	8.76	8.17	0.171	0.34	0.59	0.41
<i>Fecal total anaerobes</i>									
day 30 pw		9.41	9.20	9.60	9.27	0.120	0.41	0.09	0.29
day 70 pw		9.20	9.51	9.70	9.66	0.116	0.03	0.35	0.09
day 100 pw		8.99	9.72	9.53	9.15	0.224	0.96	0.68	0.59
<i>Enterobacteriaceae</i>									
Ileum day 115 pw		7.91 ^A	7.34 ^{AB}	6.31 ^B	8.30 ^A	0.432	0.37	0.06	0.01
Cecum day 115 pw		7.85	7.18	7.05	7.50	0.302	0.41	0.69	0.06
<i>Lactobacillus</i>									
Ileum day 115 pw		7.01	7.25	7.02	7.19	0.383	0.95	0.52	0.91
Cecum day 115 pw		8.34	7.78	7.90	7.92	0.320	0.62	0.38	0.34
<i>Total anaerobes</i>									
Ileum day 115 pw		8.70 ^A	8.63 ^A	7.96 ^B	8.87 ^A	0.192	0.14	0.02	0.01
Cecum day 115 pw		9.54 ^A	8.97 ^B	9.15 ^B	9.19 ^{AB}	0.148	0.55	0.07	0.03

675 ^aData are presented as treatment (trt) least squares means (log₁₀ CFU g⁻¹). Variability present at

676 weaning has been accounted for by including weaning counts as covariates in the statistical

677 model.

678 ^bSows were fed either a non-GM or a GM maize-based diet during gestation and lactation.679 ^cWithin sow treatment, offspring were fed either a non-GM or a GM maize-based diet from

680 weaning for 115 days.

681 ^dpw – post-weaning.682 Within a row, means without common superscripts differ at $P \leq 0.05$. Means separation was

683 performed using Tukey-Kramer adjustment for multiple comparisons.

684 **TABLE 2** Bacterial diversity within samples from sows and offspring fed non-GM or GM

685 maize-based diets^a

Sow trt^b	Non-GM		GM	
Sow fecal microbiota				
Chao 1 richness estimation	1267		1242	
Shannon diversity index	5.92		5.86	
Good's coverage	0.87		0.88	
Offspring trt^c	Non-GM	GM	Non-GM	GM
Offspring fecal microbiota at weaning				
Chao 1 richness estimation	806	812	953	820
Shannon diversity index	5.06	5.02	5.30	5.16
Good's coverage	0.95	0.95	0.94	0.95
Offspring fecal microbiota at day 100 post-weaning				
Chao 1 richness estimation	1106	1098	1125	1156
Shannon diversity index	5.68	5.61	5.54	5.58
Good's coverage	0.93	0.93	0.93	0.93
Offspring cecal microbiota at day 115 post-weaning				
Chao 1 richness estimation	846	938	784	869
Shannon diversity index	5.40	5.53	5.46	5.44
Good's coverage	0.91	0.90	0.90	0.91

686 ^a Estimates of diversity were computed using MOTHUR software and are presented as treatment
687 (trt) means.

688 ^b Sows were fed either a non-GM or a GM maize-based diet from service to weaning.

689 ^c Within sow treatment, offspring were fed either a non-GM or a GM maize-based diet from
690 weaning at ~28 days for 115 days.

691 **TABLE 3** Relative abundance (%) of sow fecal bacteria at day 110 of gestation^a

Taxon	Non-GM^b	GM^b	P - value	n^c
Phylum				
<i>Verrucomicrobia</i> [#]	0 (0 - 0.4)	0 (0)	0.04	4 vs 0
Family				
<i>Rikenellaceae</i> †	5.9 (1.7 - 11.9)	3.6 (1.9 - 6.5)	0.05	11 vs 12
<i>Prevotellaceae</i> †	2.4 (1.3 - 4.4)	1.5 (0.7 - 2.9)	0.01	11 vs 12
<i>Succinivibrionaceae</i> [#]	1.9 (0 - 14.8)	0.5 (0 - 2.3)	0.01	10 vs 10
<i>Rikenellaceae</i> -related bacteria†	1.6 (0 - 4.7)	0.6 (0.2 - 1.2)	0.04	10 vs 12
<i>Lactobacillaceae</i> [#]	0.2 (0 - 1.0)	0 (0 - 0.4)	0.03	9 vs 3
Genus				
<i>Prevotella</i> †	2.4 (1.3 - 4.4)	1.5 (0.7 - 2.9)	0.01	11 vs 12
<i>Lachnospiraceae incertae sedis</i> [#]	1.3 (0.5 - 2.8)	0.8 (0.6 - 1.5)	0.01	11 vs 12
<i>Anaerobiospirillum</i> [#]	1.8 (0 - 14.3)	0.5 (0 - 2.3)	0.01	10 vs 10
<i>Lactobacillus</i> [#]	0.2 (0 - 1.0)	0 (0 - 0.4)	0.03	9 vs 3

692 ^aData are presented as treatment means (with 95% confidence intervals) for data analyzed using
693 parametric tests (†) or medians (with 5-95th percentiles) for data analyzed using non-parametric
694 tests ([#]). The main phyla are shown in Figure 1 and a full outline of the relative abundance of all
695 bacterial taxa detected in sow feces is available in Table S4 in the supplemental material.

696 ^bSows were fed a non-GM or a GM maize-based diet during gestation and lactation.

697 ^cn – number of animals in which the bacterial taxon was present (non-GM vs GM).

698 **TABLE 4** Relative abundance (%) of fecal bacteria in offspring at weaning and 100 days post-weaning and of cecal bacteria at day 15 post-
 699 weaning^a

Sow trt ^b	Non-GM		GM		P - value	n ^e
	Non-GM	GM	Non-GM	GM		
Offspring trt ^c					Sow trt × offspring trt ^d	
Feces						
Weaning (~28 days of age)						
Family						
<i>Clostridiaceae</i> [#]	0.3 (0 - 5.5) ^{AB}	0 (0 - 0.4) ^B	0.9 (0 - 2.4) ^A	0.3 (0 - 1.3) ^{AB}	0.01	7;3;9;9
Genus						
<i>Clostridium</i> [#]	0.1 (0 - 0.6) ^{AB}	0 (0 - 0.2) ^B	0.4 (0 - 2.3) ^A	0.1 (0 - 0.4) ^{AB}	0.03	5;2;8;5
<i>Butyricimonas</i> [#]	0 (0) ^B	0 (0) ^B	0 (0) ^B	0 (0 - 0.2) ^A	0.02	0;0;0;3
<i>Roseburia</i> [#]	0 (0) ^B	0.1 (0 - 1.4) ^A	0 (0 - 0.1) ^B	0 (0 - 0.2) ^B	0.02	0;5;2;1
Day 100 pw^f						
Phylum						
<i>Fibrobacteres</i> [#]	0.4 (0 - 0.8) ^A	0 (0 - 1.1) ^B	0 (0 - 0.4) ^B	0.2 (0 - 1.9) ^{AB}	0.02	8;3;4;7
Family						
<i>Fibrobacteraceae</i> [#]	0.4 (0 - 0.8) ^A	0 (0 - 1.1) ^B	0 (0 - 0.4) ^B	0.2 (0 - 1.9) ^{AB}	0.02	8;3;4;7
<i>Helicobacteraceae</i> [#]	0 (0 - 0.1) ^B	0 (0 - 0.2) ^A	0 (0) ^B	0 (0) ^B	0.01	1;4;0;0
Genus						
<i>Ruminococcus</i> [#]	0.8 (0.3 - 4.1) ^{AB}	1.0 (0.6 - 3.3) ^A	0.7 (0.2 - 1.4) ^{AB}	0.5 (0.3 - 2.2) ^B	0.05	9;9;10;10
<i>Fibrobacter</i> [#]	0.4 (0 - 0.8) ^A	0 (0 - 1.1) ^B	0 (0 - 0.4) ^B	0.2 (0 - 1.9) ^{AB}	0.02	7;3;4;7
<i>Helicobacter</i> [#]	0 (0 - 0.1) ^B	0 (0 - 0.2) ^A	0 (0) ^B	0 (0) ^B	0.01	1;4;0;0
<i>Solobacterium</i> [#]	0 (0 - 0.2) ^A	0 (0 - 0.1) ^{AB}	0 (0) ^B	0 (0) ^B	0.02	4;2;0;0
Cecum						
Family						
<i>Clostridiaceae</i> [†]	1.2 (0.3 - 2.1) ^B	2.3 (1.4 - 3.3) ^A	2.1 (1.2 - 3.0) ^{AB}	1.5 (0.6 - 2.4) ^{AB}	0.04	8;8;10;9
Genus						
<i>Phascolarctobacterium</i> [†]	5.5 (3.9 - 7.1) ^A	3.6 (2.0 - 5.2) ^B	4.0 (2.5 - 5.5) ^{AB}	5.3 (3.9 - 6.8) ^A	0.03	9;8;10;10

700 ^a Data are presented as treatment (trt) means (with 95% confidence intervals) for data analyzed using parametric tests (†) or medians (with 5-

701 95th percentiles) for data analyzed using non-parametric tests ([#]). The main phyla are shown in Figure 1 and a full outline of the relative

702 abundance of all bacterial taxa detected in the feces of offspring at weaning and at day 100 post-weaning is available in Tables S5 and S6 in

703 the supplemental material. A full outline of the relative abundance of all bacterial taxa detected in the cecum of offspring at day 115 post-
704 weaning is available in Table S7 in the supplemental material.

705 ^b Sows were fed either a non-GM or a GM maize-based diet during gestation and lactation.

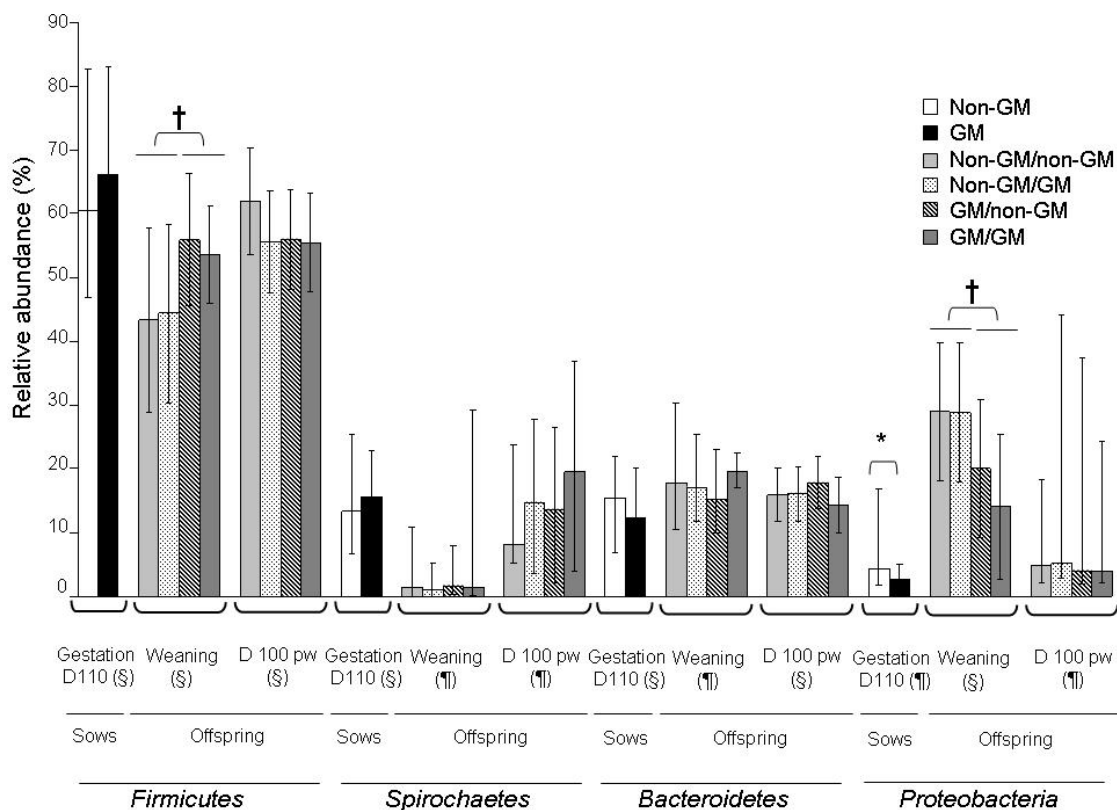
706 ^c Within sow treatment, offspring were fed either a non-GM or a GM maize-based diet for 115 days from weaning.

707 ^d A true sow trt × offspring trt interaction could only be modeled for data that underwent parametric statistical analysis. For non-parametric
708 analysis, the *P* – value from the comparison among the four combinations (non-GM/non-GM, non-GM/GM, GM/non-GM and GM/GM) of
709 treatments is presented.

710 ^e *n* – number of animals in which the bacterial taxon was present (non-GM/non-GM; non-GM/GM; GM/non-GM; GM/GM; n=10/trt).

711 ^f pw – post-weaning.

712 Within a row, means without common superscripts differ at $P \leq 0.05$. Means separation was performed using Tukey-Kramer adjustment for
713 multiple comparisons.



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FIG 1 Main phyla detected in feces of sows and their offspring

Data are presented as treatment means (S) or medians (M) with whiskers corresponding to the 95% confidence interval or 5-95th percentiles, respectively.

Sows were fed either a non-GM or a GM maize-based diet during gestation and lactation. Within sow treatment, offspring were fed either a non-GM or a GM maize-based diet from weaning for 115 days giving rise to four dietary treatments: non-GM maize-fed sow / non-GM maize-fed offspring (non-GM / non-GM); non-GM maize-fed sow / GM maize-fed offspring (non-GM / GM); GM maize-fed sow / non-GM maize-fed offspring (GM / non-GM); and GM maize-fed sow / GM maize-fed offspring (GM / GM).

* - treatment effect at $P < 0.05$; † - maternal treatment effect at $P < 0.05$.

- 13 n = 11 sows on the non-GM treatment, n = 12 sows on the GM treatment, n = 10 offspring/treatment at
14 weaning, n = 9 offspring/treatment on the non-GM /non-GM and non-GM /GM treatments and n = 10
15 offspring/treatment on the GM /non-GM and GM /GM treatments at day 100 postweaning.
16 pw - postweaning
17 A full outline of all taxa detected can be found in tables S4, S5 and S6 in the supplemental material.