AEM Accepts, published online ahead of print on 4 October 2013
Appl. Environ. Microbiol. doi:10.1128/AEM.02937-13

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

Stefan G. Buzoianu[a,b]*, Maria C. Walsh[a]*, Mary C. Rea[c,d], Lisa Quigley[c,e], Orla O'Sullivan[c,d], Paul D. Cotter[c,d], R. Paul Ross[c,d], Gillian E. Gardiner[b#], and Peadar G. Lawlor[a]

Teagasc, Pig Development Department, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland[a]; Department of Chemical and Life Sciences, Waterford Institute of Technology, Waterford, Co. Waterford, Ireland[b]; Teagasc, Food Research Centre, Moorepark, Fermoy, Cork, Ireland[c]; Alimentary Pharmabiotic Centre, Cork, Ireland[d]; Microbiology Department, University College Cork, Cork, Ireland[e].

*These authors are to be considered the joint first authors

[#]Address correspondence to: Gillian E. Gardiner, ggardiner@wit.ie.

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

ABSTRACT

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

INTRODUCTION

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

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

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

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

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

MATERIALS AND METHODS

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

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

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

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

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

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

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

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

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

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

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

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

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

For sows, relative abundance data that were normally-distributed and log-transformed microbial counts were analyzed using PROC MIXED in SAS with treatment as a fixed effect and block as a random effect in the statistical model. For microbial counts, day 0 values were 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 × 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

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

Effects of feeding GM maize-based diets to sows during gestation and lactation and to offspring for 115 days post-weaning on selected culturable fecal and intestinal microbiota. Bacterial counts from sow fecal samples are presented in Table S3. There was no effect of feeding GM maize-based diets to sows on counts of culturable fecal *Enterobacteriaceae*, *Lactobacillus* or total anaerobes on day 110 of gestation or day 28 of lactation. While *Lactobacillus* counts increased from day 28 of gestation to day 28 of lactation ($P < 0.05$; data not shown), *Enterobacteriaceae* and total anaerobe counts decreased between these two sampling points ($P < 0.05$; data not shown).

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

Apart from investigating the impact of GM maize consumption, the present study also provides, for the first time, an insight into the intestinal microbiota of sows at the end of 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).

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

AUTHORS' CONTRIBUTIONS

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

ACKNOWLEDGEMENTS

The research leading to these results received funding from the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement number 211820 and the Teagasc Walsh Fellowship Programme. None of the authors had a financial or personal conflict of interest regarding the present study and the work was conducted independently of any commercial input, financial or otherwise.

The authors would like to acknowledge Fiona Crispie for assistance with 16S rRNA gene sequencing, Lidia Teixeira, Julie Ledoux, Marie Laetitia Gilliot and Julia Chaumier for

494 assistance with sampling and Laurie O'Sullivan, Gemma McCarthy and Maria Luz Prieto for
495 technical assistance with sample processing at harvest.

REFERENCES

1. **Schnepf E, Crickmore N, Van Rie J, Lereclus D, Baum J, Feitelson J, Zeigler DR, and Dean DH.** 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiol. Mol. Biol. Rev. **62(3):**775-806.
2. **James C.** 2010. Global status of commercialized biotech/GM crops: 2010. ISAAA Brief No. 42. ISAAA, Ithaca, NY, USA.
3. **Domingo JL, and Giné Bordonaba J.** 2011. A literature review on the safety assessment of genetically modified plants. Environment International **37(4):**734-742.
4. **Chowdhury EH, Shimada N, Murata H, Mikami O, Sultana P, Miyazaki S, Yoshioka M, Yamanaka N, Hirai N, and Nakajima Y.** 2003. Detection of Cry1Ab protein in gastrointestinal contents but not visceral organs of genetically modified Bt11-fed calves. Vet. Hum. Toxicol. **45(2):72-74.**
5. **Adel-Patient K, Guimaraes VD, Paris A, Drumare M-F, Ah-Leung S, Lamourette P, Nevers M-C, Canlet C, Molina J, Bernard H, Créminon C, and Wal J-M.** 2011. Immunological and metabolomic impacts of administration of Cry1Ab protein and MON 810 maize in mouse. PLoS ONE **6(1)**.
6. **Trabalza-Marinucci M, Brandi G, Rondini C, Avellini L, Giammarini C, Costarelli S, Acuti G, Orlandi C, Filippini G, Chiaradia E, Malatesta M, Crotti S, Antonini C, Amagliani G, Manuali E, Mastrogiacomo AR, Moscati L, Naceur Haouet M, Gaiti A, and Magnani M.** 2008. A three-year longitudinal study on the effects of a diet containing genetically modified Bt176 maize on the health status and performance of sheep. Livest. Sci. **113(2-3):**178-190.

519 7. **Buzoianu SG, Walsh MC, Rea MC, Cassidy JP, Ross RP, Gardiner GE, and Lawlor**
520 **PG.** 2012. Effect of feeding genetically modified Bt MON810 maize to ~40 day old pigs
521 for 110 days on growth and health indicators. Animal **6(10):**1609-1619.
522 8. **Walsh MC, Buzoianu SG, Gardiner GE, Rea MC, Gelencsér E, Jánosi A, Epstein**
523 **MM, Ross RP, and Lawlor PG.** 2011. Fate of transgenic DNA from orally administered
524 Bt MON810 maize and effects on immune response and growth in pigs. PLoS ONE
525 **6(11)**.
526 9. **Walsh MC, Buzoianu SG, Rea MC, O'Donovan O, Gelencsér E, Ujhelyi G, Ross RP,**
527 **Gardiner GE, and Lawlor PG.** 2012. Effects of feeding Bt MON810 maize to pigs for
528 110 days on peripheral immune response and digestive fate of the cry1Ab gene and
529 truncated Bt toxin. PLoS ONE **7(5)**.
530 10. **Walsh MC, Buzoianu SG, Gardiner GE, Cassidy JP, Rea MC, Ross RP, and Lawlor**
531 **PG.** 2012. Effects of short-term feeding of Bt MON810 maize on growth performance,
532 organ morphology and function in pigs. Br. J. Nutr. **107:**364-371.
533 11. **Einspanier R, Lutz B, Rief S, Berezina O, Zverlov V, Schwarz W, and Mayer J.**
534 2004. Tracing residual recombinant feed molecules during digestion and rumen bacterial
535 diversity in cattle fed transgene maize. Eur. Food Res. Technol. **218(3):**269-273.
536 12. **Wiedemann S, Gurtler P, and Albrecht C.** 2007. Effect of feeding cows genetically
537 modified maize on the bacterial community in the bovine rumen. Appl. Environ.
538 Microbiol. **73(24):**8012-8017.
539 13. **Buzoianu SG, Walsh MC, Rea MC, O'Sullivan O, Cotter PD, Ross RP, Gardiner**
540 **GE, and Lawlor PG.** 2012. High-throughput sequence-based analysis of the intestinal
541 microbiota of weanling pigs fed genetically modified MON810 maize expressing *Bacillus*

thuringiensis Cry1Ab (Bt maize) for 31 days. Appl. Environ. Microbiol. **78(12):**4217-4224.

14. **Buzoianu SG, Walsh MC, Rea MC, O'Sullivan O, Crispie F, Cotter PD, Ross RP, Gardiner GE, and Lawlor PG.** 2012. The effect of feeding Bt MON810 maize to pigs for 110 days on intestinal microbiota. PLoS ONE **7(5):**e33668.

15. **Yudina TG, Brioukhanov AL, Zalunin IA, Revina LP, Shestakov AI, Voyushina NE, Chestukhina GG, and Netrusov AI.** 2007. Antimicrobial activity of different proteins and their fragments from *Bacillus thuringiensis* parasporal crystals against clostridia and archaea. Anaerobe **13(1):**6-13.

16. **Chowdhury EH, Kuribara H, Hino A, Sultana P, Mikami O, Shimada N, Guruge KS, Saito M, and Nakajima Y.** 2003. Detection of corn intrinsic and recombinant DNA fragments and Cry1Ab protein in the gastrointestinal contents of pigs fed genetically modified corn Bt11. J. Anim. Sci. **81(10):**2546-2551.

17. **Broderick N, Robinson C, McMahon M, Holt J, Handelsman J, and Raffa K.** 2009. Contributions of gut bacteria to *Bacillus thuringiensis*-induced mortality vary across a range of *Lepidoptera*. BMC Biol. **7(1)**.

18. **Finamore A, Roselli M, Britti S, Monastra G, Ambra R, Turrini A, and Mengheri E.** 2008. Intestinal and peripheral immune response to MON810 maize ingestion in weaning and old mice. J. Agric. Food Chem. **56(23):**11533-11539.

19. **Kelly D, King T, and Aminov R.** 2007. Importance of microbial colonization of the gut in early life to the development of immunity. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **622(1-2):**58-69.

564 20. **Thompson CL, Wang B, and Holmes AJ.** 2008. The immediate environment during
565 postnatal development has long-term impact on gut community structure in pigs. ISME J
566 **2(7):**739-748.
567 21. **Walsh MC, Buzoianu SG, Gardiner GE, Rea MC, O'Donovan O, Ross RP, and**
568 **Lawlor PG.** 2012. Effects of feeding Bt MON810 maize to sows during first gestation
569 and lactation on maternal and offspring health indicators. Br. J. Nutr. **109(5):**873-881.
570 22. **Buzoianu SG, Walsh MC, Rea MC, Cassidy JP, Ryan TP, Ross RP, Gardiner GE,**
571 **and Lawlor PG.** 2013. Trans-generational effects of feeding genetically modified maize
572 to nulliparous sows and offspring on offspring growth and health. J. Anim. Sci. **91:**318-
573 330.
574 23. **NRC.** 1998. Nutrient requirements of swine, 10th ed. National Academy of Sciences,
575 Washington, DC, USA.
576 24. **Castillo M, Martín-Orúe SM, Manzanilla EG, Badiola I, Martín M, and Gasa J.**
577 2006. Quantification of total bacteria, enterobacteria and lactobacilli populations in pig
578 digesta by real-time PCR. Vet. Microbiol. **114(1-2):**165-170.
579 25. **Rea MC, Clayton E, O'Connor PM, Shanahan F, Kiely B, Ross RP, and Hill C.**
580 2007. Antimicrobial activity of lacticin 3147 against clinical *Clostridium difficile* strains.
581 J. Med. Microbiol. **56(7):**940-946.
582 26. **Claesson MJ, O'Sullivan O, Wang Q, Nikkilä J, Marchesi JR, Smidt H, de Vos WM,**
583 **Ross RP, and O'Toole PW.** 2009. Comparative analysis of pyrosequencing and a
584 phylogenetic microarray for exploring microbial community structures in the human
585 distal intestine. PLoS ONE **4(8):**e6669.

27. **Urich T, Lanzen A, Qi J, Huson DH, Schleper C, and Schuster SC.** 2008. Simultaneous assessment of soil microbial community structure and function through analysis of the meta-transcriptome. PLoS ONE **3(6):**e2527.

28. **Osborne JW.** 2010. Improving your data transformations: applying the Box-Cox transformation. Practical Assessment, Research & Evaluation **15**.

29. **Sekirov I, Russell SL, Antunes LC, and Finlay BB.** 2010. Gut microbiota in health and disease. Physiol. Rev. **90(3):**859-904.

30. **Brusetti L, Crotti E, Tamburini A, Cittaro D, Garavaglia V, Rolli E, Sorlini C, Daffonchio D, and Borin S.** 2011. Influence of transgenic Bt176 and non-transgenic corn silage on the structure of rumen bacterial communities. Ann. Microbiol. **61(4):**925-930.

31. **Mukhopadhya I, Hansen R, El-Omar EM, and Hold GL.** 2012. IBD-what role do *Proteobacteria* play? Nat. Rev. Gastroenterol. Hepatol. **9(4):**219-230.

32. **Salyers AA, and Whitt DD.** 2002. Bacterial pathogenesis: a molecular approach. ASM Press, Washington DC, USA.

33. **De Cock HEV, Marks SL, Stacy BA, Zabka TS, Burkitt J, Lu G, Steffen DJ, and Duhamel GE.** 2004. Ileocolitis associated with Anaerobiospirillum in cats. J. Clin. Microbiol. **42(6):**2752-2758.

34. **Buzoianu SG, Walsh MC, Rea MC, O'Donovan O, Gelencsér E, Ujhelyi G, Szabó E, Nagy A, Ross RP, Gardiner GE, and Lawlor PG.** 2012. Effects of feeding Bt maize to sows during gestation and lactation on maternal and offspring immunity and fate of transgenic material. PLoS ONE **7(10):**e47851.

608 35. **Flint HJ, Bayer EA, Rincon MT, Lamed R, and White BA.** 2008. Polysaccharide
609 utilization by gut bacteria: potential for new insights from genomic analysis. Nat. Rev.
610 Microbiol. **6(2):**121-131.
611 36. **Castillo M, Martín-Orúe SM, Anguita M, Pérez JF, and Gasa J.** 2007. Adaptation of
612 gut microbiota to corn physical structure and different types of dietary fibre. Livest. Sci.
613 **109(1-3):**149-152.
614 37. **Lewis AJ, and Southern LL.** 2001. Swine Nutrition, 2nd Edition ed. CRC Press, Boca
615 Raton, FL, USA.
616 38. **Schmidt B, Mulder IE, Musk CC, Aminov RI, Lewis M, Stokes CR, Bailey M,**
617 **Prosser JI, Gill BP, Pluske JR, and Kelly D.** 2011. Establishment of normal gut
618 microbiota is compromised under excessive hygiene conditions. PLoS ONE **6(12)**.
619 39. **Mulder I, Schmidt B, Stokes C, Lewis M, Bailey M, Aminov R, Prosser J, Gill B,**
620 **Pluske J, Mayer C-D, Musk C, and Kelly D.** 2009. Environmentally-acquired bacteria
621 influence microbial diversity and natural innate immune responses at gut surfaces. BMC
622 Biol. **7(79)**.
623 40. **Clarridge JE, III.** 2004. Impact of 16S rRNA gene sequence analysis for identification
624 of bacteria on clinical microbiology and infectious diseases. Clin. Microbiol. Rev.
625 **17(4):**840-862.
626 41. **Reuter G.** 2001. The *Lactobacillus* and *Bifidobacterium* microflora of the human
627 intestine: composition and succession. Curr Issues Intest Microbiol **2(2):**43-53.
628 42. **Walter J.** 2008. Ecological role of lactobacilli in the gastrointestinal tract: implications
629 for fundamental and biomedical research. Appl. Environ. Microbiol. **74(16):**4985-4996.
630 43. **Murphy EF, Cotter PD, Healy S, Marques TM, O'Sullivan O, Fouhy F, Clarke SF,**
631 **O'Toole PW, Quigley EM, Stanton C, Ross PR, O'Doherty RM, and Shanahan F.**

2010. Composition and energy harvesting capacity of the gut microbiota: relationship to diet, obesity and time in mouse models. Gut **59(12):**1635-1642.

44. **Attig L, Djiane J, Gertler A, Rampin O, Larcher T, Boukthir S, Anton PM, Madec JY, Gourdou I, and Abdennebi-Najar L.** 2008. Study of hypothalamic leptin receptor expression in low-birth-weight piglets and effects of leptin supplementation on neonatal growth and development. Am. J. Physiol. Endocrinol. Metab. **295(5):**E1117-E1125.

45. **Ross MG, and Beall MH.** 2008. Adult sequelae of intrauterine growth restriction. Semin. Perinatol. **32(3):**213-218.

46. **Mackie RI, Aminov RI, Hu W, Klieve AV, Ouwerkerk D, Sundset MA, and Kamagata Y.** 2003. Ecology of uncultivated *Oscillospira* species in the rumen of cattle, sheep, and reindeer as assessed by microscopy and molecular approaches. Appl. Environ. Microbiol. **69(11):**6808-6815.

47. **Flint HJ, Duncan SH, Scott KP, and Louis P.** 2007. Interactions and competition within the microbial community of the human colon: links between diet and health. Environ. Microbiol. **9(5):**1101-1111.

48. **Leman AD, Straw BE, Mengeling WL, D'Allaire S, and Taylor DJ.** 1992. Diseases of Swine. Wolfe Publishing, London, UK.

49. **Schierack P, Walk N, Reiter K, Weyrauch KD, and Wieler LH.** 2007. Composition of intestinal *Enterobacteriaceae* populations of healthy domestic pigs. Microbiology **153(11):**3830-3837.

50. **Walk ST, and Young VB.** 2008. Emerging insights into antibiotic-associated diarrhea and *Clostridium difficile* infection through the lens of microbial ecology. Interdiscip. Perspect. Infect. Dis. **2008**.

51. **Poroyko V, White JR, Wang M, Donovan S, Alverdy J, Liu DC, and Morowitz MJ.** 2010. Gut microbial gene expression in mother-fed and formula-fed piglets. PLoS ONE **5(8):**e12459.

52. **Kim HB, Borewicz K, White BA, Singer RS, Sreevatsan S, Tu ZJ, and Isaacson RE.** 2011. Longitudinal investigation of the age-related bacterial diversity in the feces of commercial pigs. Vet. Microbiol. **153(1-2):**124-133.

53. **Larsen N, Vogensen FK, van den Berg FWJ, Nielsen DS, Andreasen AS, Pedersen BK, Al-Soud WA, Sørensen SJ, Hansen LH, and Jakobsen M.** 2010. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. PLoS ONE **5(2)**.

54. **Konstantinov SR, Awati AA, Williams BA, Miller BG, Jones P, Stokes CR, Akkermans ADL, Smidt H, and De Vos WM.** 2006. Post-natal development of the porcine microbiota composition and activities. Environ. Microbiol. **8(7):**1191-1199.

55. **Konstantinov SR, Christine FF, Wei Yun Z, Barbara AW, Jeannette KÃ, Wolfgang-Bernhard S, Willem MdV, Antoon DLA, and Hauke S.** 2004. Microbial diversity studies of the porcine gastrointestinal ecosystem during weaning transition. Anim. Res. **53(4):**317-324.

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

Sow trt[b]	Non-GM		GM			*P*-value		
Offspring trt[c]	Non-GM	GM	Non-GM	GM	SEM	Sow trt	Offspring trt	Sow trt × offspring trt
Fecal *Enterobacteriaceae*								
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
Fecal *Lactobacillus*								
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
Fecal total anaerobes								
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
Enterobacteriaceae								
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
Lactobacillus								
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
Total anaerobes								
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 [a] Data are presented as treatment (trt) least squares means ($\log_{10}$ CFU g^{-1}). Variability present at

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

677 model.

678 [b] Sows were fed either a non-GM or a GM maize-based diet during gestation and lactation.

679 [c] Within sow treatment, offspring were fed either a non-GM or a GM maize-based diet from

680 weaning for 115 days.

681 [d] pw – 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				
Verrucomicrobia[#]	0 (0 - 0.4)	0 (0)	0.04	4 vs 0
Family				
Rikenellaceae†	5.9 (1.7 - 11.9)	3.6 (1.9 - 6.5)	0.05	11 vs 12
Prevotellaceae†	2.4 (1.3 - 4.4)	1.5 (0.7 - 2.9)	0.01	11 vs 12
Succinivibrionaceae[#]	1.9 (0 - 14.8)	0.5 (0 - 2.3)	0.01	10 vs 10
Rikenellaceae-related bacteria†	1.6 (0 - 4.7)	0.6 (0.2 - 1.2)	0.04	10 vs 12
Lactobacillaceae[#]	0.2 (0 - 1.0)	0 (0 - 0.4)	0.03	9 vs 3
Genus				
Prevotella†	2.4 (1.3 - 4.4)	1.5 (0.7 - 2.9)	0.01	11 vs 12
Lachnospiraceae incertae sedis[#]	1.3 (0.5 - 2.8)	0.8 (0.6 - 1.5)	0.01	11 vs 12
Anaerobiospirillum[#]	1.8 (0 - 14.3)	0.5 (0 - 2.3)	0.01	10 vs 10
Lactobacillus[#]	0.2 (0 - 1.0)	0 (0 - 0.4)	0.03	9 vs 3

692 [a] Data 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 [b] Sows were fed a non-GM or a GM maize-based diet during gestation and lactation.

697 [c] *n* – number of animals in which the bacterial taxon was present (non-GM vs GM).

TABLE 4 Relative abundance (%) of fecal bacteria in offspring at weaning and 100 days post-weaning and of cecal bacteria at day 15 post-weaning[a]

Sow trt[b]	**Non-GM**		**GM**		***P* - value**	
Offspring trt[c]	**Non-GM**	**GM**	**Non-GM**	**GM**	**Sow trt × offspring trt**[d]	***n***[e]
Feces						
Weaning (~28 days of age)						
Family						
Clostridiaceae[#]	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						
Clostridium[#]	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
Butyricimonas[#]	0 (0)^{B}	0 (0)^{B}	0 (0)^{B}	0 (0 - 0.2)^{A}	0.02	0;0;0;3
Roseburia[#]	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						
Fibrobacteres[#]	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						
Fibrobacteraceae[#]	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
Helicobacteraceae[#]	0 (0 - 0.1)^{B}	0 (0 - 0.2)^{A}	0 (0)^{B}	0 (0)^{B}	0.01	1;4;0;0
Genus						
Ruminococcus[#]	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
Fibrobacter[#]	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
Helicobacter[#]	0 (0 - 0.1)^{B}	0 (0 - 0.2)^{A}	0 (0)^{B}	0 (0)^{B}	0.01	1;4;0;0
Solobacterium[#]	0 (0 - 0.2)^{A}	0 (0 - 0.1)^{AB}	0 (0)^{B}	0 (0)^{B}	0.02	4;2;0;0
Cecum						
Family						
Clostridiaceae†	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						
Phascolarctobacterium†	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

[a] Data are presented as treatment (trt) means (with 95% confidence intervals) for data analyzed using parametric tests (†) or medians (with 5-95th percentiles) for data analyzed using non-parametric tests ([#]). The main phyla are shown in Figure 1 and a full outline of the relative 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

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

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

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

[d] A true sow trt × offspring trt interaction could only be modeled for data that underwent parametric statistical analysis. For non-parametric 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 treatments is presented.

[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).

[f] pw – post-weaning.

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

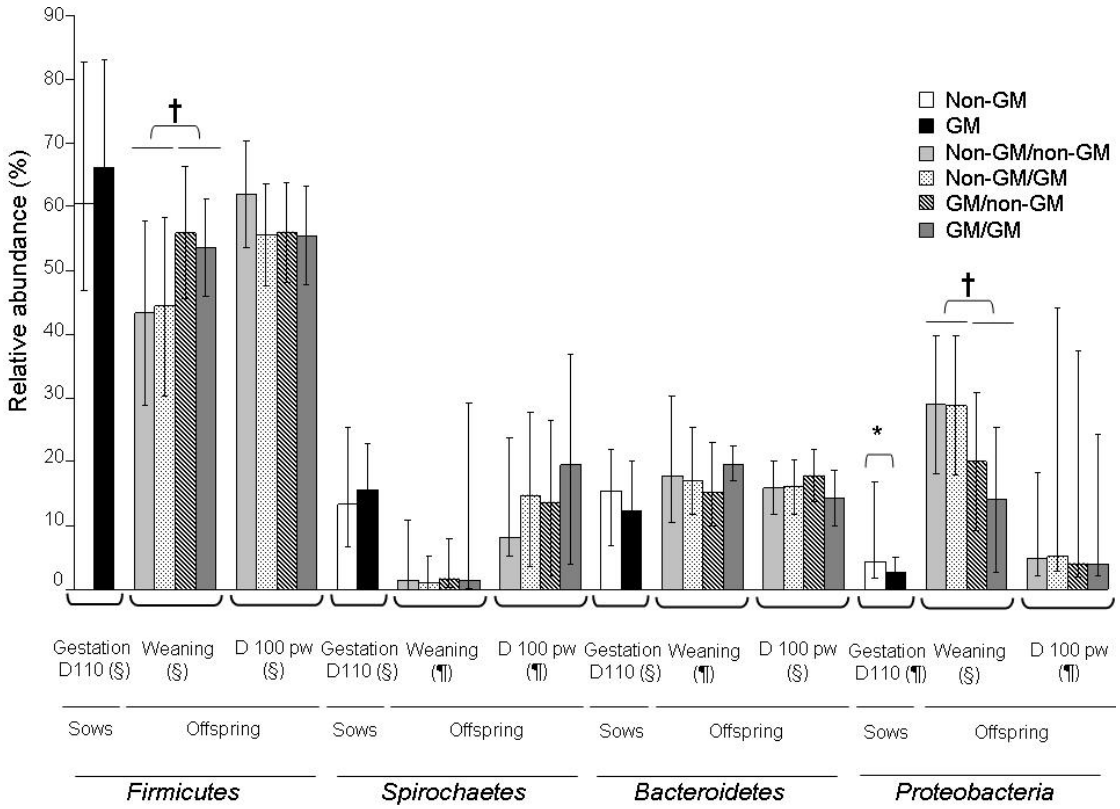

FIG 1 Main phyla detected in feces of sows and their offspring

Data are presented as treatment means (§) or medians (¶) 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 $P < 0.05$.

n = 11 sows on the non-GM treatment, n = 12 sows on the GM treatment, n = 10 offspring/treatment at weaning, n = 9 offspring/treatment on the non-GM /non-GM and non-GM /GM treatments and n = 10 offspring/treatment on the GM /non-GM and GM /GM treatments at day 100 post-weaning.

pw – post-weaning

A full outline of all taxa detected can be found in tables S4, S5 and S6 in the supplemental material.