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Age, introduction of solid feed and weaning are more important determinants of gut bacterial succession in piglets than breed and nursing mother as revealed by a reciprocal cross-fostering model

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Summary

A reciprocal cross-fostering model with an obese typical Chinese piglet breed and a lean Western breed was used to identify genetic and maternal effects on the acquisition and development gut bacteria from birth until after weaning. Pyrosequencing of 16S rRNA genes results revealed an age- and diet-dependent bacterial succession process in piglets. During the first 3 days after birth, the bacterial community was relatively simple and dominated by Firmicutes with 79% and 65% relative abundance for Meishan and Yorkshire piglets, respectively. During the suckling period until day 14, the piglet breed and the nursing mother lead to increasing differentiation of the fecal bacterial community, with specific bacteria taxa associated with breed, and others with the nursing sow most likely due to its milk composition. Although the effect of nursing mother and the breed were evident through the suckling period, the introduction of solid feed and subsequent weaning were the major events occurring that dominated succession of the gut microbiota in the early life of piglets. This piglet cross-fostering model is a useful tool for studying the effects of diet, host genetics and the environment on the development and acquisition of the gut microbiota and over longer studies the subsequent impact on growth, health and performance of pigs.

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Introduction

The intestinal microbiota of newborn animals plays a fundamentally important role in the development of intestinal function and the innate immune system (Collado *et al.*, 2012; Hansen *et al.*, 2012; Matamoros *et al.*, 2013). The gut ecosystem of neonates undergoes a dramatic transition from an essentially sterile state to extremely dense colonization, ending with the establishment of an adult-like microbial community (Fanaro *et al.*, 2003; Palmer *et al.*, 2007). In contrast to the established and stable microbiota of adult animals, the gut microbiota of neonates varies more among individuals and is less stable. The fragile ecological system of the neonatal gut is not only a disease risk to the newborn animal but also may have short- and long-term influence on health later in life (Conroy *et al.*, 2009; Hansen *et al.*, 2012; Saavedra and Dattilo, 2012; Arnal *et al.*, 2014). Thus, characterizing the succession of gut microbiota and the factors that affect this process is important.

The establishment of the intestinal microbiota is not only a succession in the ecological sense but also a complex process shaped by internal and external factors. Host genetics has been characterized as a major internal factor that shapes the intestinal microbiota of adults. It was reported that the microbial community was more similar between twins than genetically unrelated individuals (Zoetendal *et al.*, 2001; Turnbaugh *et al.*, 2009; Tims *et al.*, 2013). Furthermore, monozygotic twins have more similar intestinal microbiota than dizygotic twins (Goodrich *et al.*, 2014). With children (0.33–10 years), limited research using denaturing gradient gel electrophoresis technology has shown that the gut microbiota in monozygotic twins was most similar, followed by dizygotic twins, and an unrelated control group (Stewart *et al.*, 2005). However, little is known about the impact of host genetics on the early colonization and development of gut microbiota in neonates.

External factors, including delivery mode (Biasucci *et al*., 2010; Dominguez-Bello *et al*., 2010; Wang *et al*., 2013), early dietary style (breast-fed or formula-fed) (Harmsen *et al*., 2000; Rinne *et al*., 2005; Li *et al*., 2012), and rearing environment (Inman *et al*., 2010), are important factors that affect the early colonization of neonatal intestinal microbiota. Immediately after birth, the neonate is exposed to a microbial world. During the suckling period, the nursing milk is probably the most influential external factor in the development of the infant's microbiota. Differences have been observed in gut microbiota between breast-fed and formula-fed infants (Harmsen *et al*., 2000; Rinne *et al*., 2005), and between sow-fed and formula-fed piglets (Poroyko *et al*., 2010; Li *et al*., 2012). Milk components such as oligosaccharides could affect the gut microbiota (Zivkovic *et al*., 2011; Donovan *et al*., 2012). Recently, in a diabetes-type mouse model, cross-fostering of non-obese diabetic and non-obese diabetic-resistant mice within 48 h after birth permanently shifted the microbiota, and the nursing mother was the critical factor in determining bacterial colonization, rather than the birth mother (Daft *et al*., 2015). However, the impact of a switch of the source of breast milk, that is, from the birth mother to another nursing mother, on the establishment of the gut microbiota in healthy neonates is unknown. Since studies focusing on obesity have demonstrated that obese mammals, including humans, have a different gut microbiota than non-obese subjects (Turnbaugh *et al*., 2009; Goodrich *et al*., 2014), it is important to know whether the breast milk of obese mothers, their fecal microbiota, or a combination of both affects the early colonization of the gut microbiota of neonates, which could have consequences for the offspring's later life.

After weaning, solid feed is one of the most important external factors affecting the gut microbiota of infants. The introduction of solid feed is a key event for the newborn, not only for the development of the body but also intestinal microbial composition (Koenig *et al*., 2011). Much information on the dietary impact on gut microbiota fluctuation in adults is available (Cotillard *et al*., 2013; Wu *et al*., 2013). However, remarkably little information is available regarding the relative impact of the early introduction of solid feed on the development of the gut microbiota.

To address this knowledge gap, we developed a cross-fostering model using newborn piglets of two distinct genetic breeds: Meishan, an obese typical Chinese domestic breed, and Yorkshire, a lean Western breed. We hypothesized that the genetic mother and the nursing mother would contribute to the establishment of the gut microbiota and that these impacts would last after the piglets were weaned and the same solid feed was introduced. With this model, we aimed to elucidate the effects of pig breed (as a proxy for host genetics) and nursing mother on the acquisition and development of fecal bacteria in piglets from birth to 7 weeks of life (i.e. 3 weeks after weaning) while maintaining the piglets in the same environment. This novel approach provides new opportunities in discriminating the impact of host genetics on the gut bacterial community from that of nursing and solid feed, both of which have been shown to affect the development of the microbiota in neonates.

Results

In the present study, the cross-fostering model involved splitting litters of piglets at birth born to Meishan pig and Yorkshire pig and leaving half the litter with the birth sow and cross-fostering an equal number of piglets from the other sow, and vice versa, generating four groups ($n = 10$) (Fig. 1). Power calculations indicated that 10 replicates was sufficient in this experiment. All piglets used in cross-fostering were healthy during the experiment period. Compared with the Yorkshire piglets (Yy and My), the Meishan piglets (Mm and Ym) had lower birth weight and lower body weight gain during the entire 49-day test period but had thicker relative back fat (thickness/body weight). However, when the two nursing sows within each pair were compared, the piglets nursed by Yorkshire sows had thicker back fat than the piglets nursed by Meishan sows on day 28 (Supporting Information Table S1).

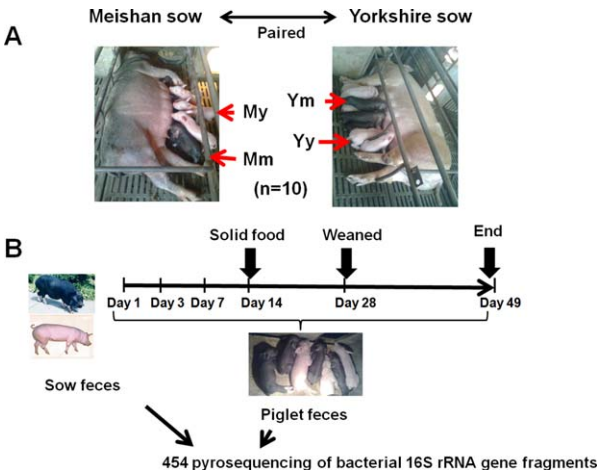

Fig. 1. Experimental design and workflow of sample collection. A. Diagram of the experimental design for the reciprocal piglet cross-fostering model. Half of the piglets from Meishan sow were fostered to a Yorkshire sow with same delivery date (designated Ym piglets), while the other half of the piglets remained with their own mother (Mm). Conversely, half of the piglets born to the Yorkshire sow were fostered to the corresponding Meishan sow (My), with the other half staying with this Yorkshire sow (Yy). B. Overall workflow of sample collection for the fecal bacteria characterization. Fecal sample from sows before delivery and piglets on day 1, 3, 7, 14, 28 and 49 after birth were collected for further analysis.

Bacterial community diversity during succession

Across all 235 samples (20 sow samples and 215 piglet samples), 1 872 432 high-quality sequences were obtained with pyrosequencing analysis and classified as bacterial 16S rRNA genes. A total of 24 bacterial phyla, 52 classes, 92 orders, 155 families and 328 genera or Unclassified groups were identified by matching sequences of known bacteria. Bacterial phylogenetic diversity (richness and evenness) increased gradually with the age of the piglets (Supporting Information Fig. S1). Over the entire experiment period from birth to after weaning on day 49, neither the pig breed nor the nursing mother affected the diversity (Shannon and Simpson indices) of the fecal bacterial community in the piglets.

The overall fecal bacterial community structure in the sow and piglet samples

The overall bacterial community structure showed a clear age-dependent succession, independent of the group (Fig. 2). At the phylum level (Fig. 2A), Firmicutes was predominant in all samples, represented by 65.6% (12.6%–99.6%) of the total 16S rRNA gene sequences. Proteobacteria was relatively dominant on day 1, which was the second dominant phylum for piglets born to Yorkshire sow (Yy1

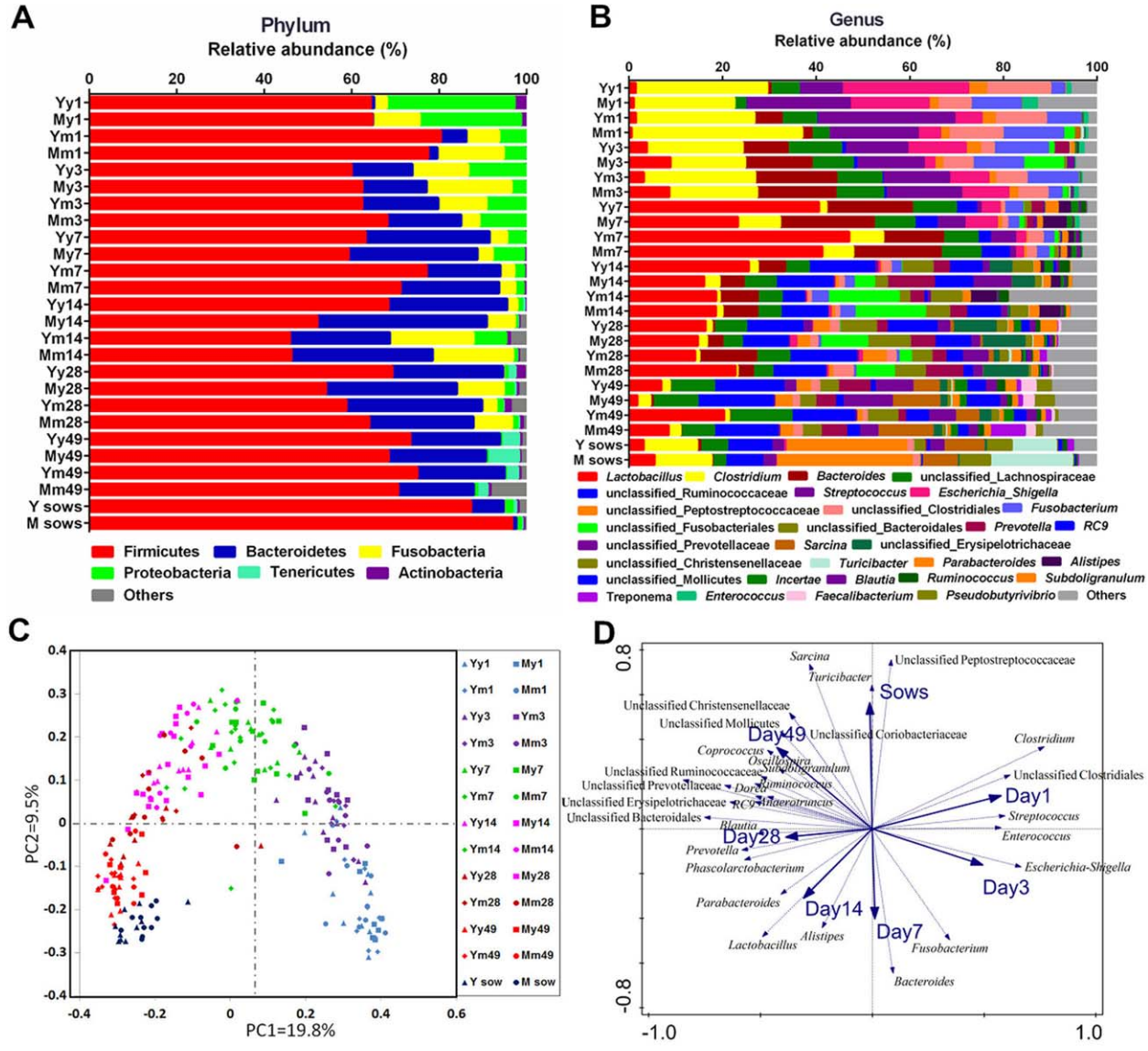

Fig. 2. Fecal bacterial composition of sows and piglets in the cross-fostering experiment. The contribution of sequences (%) of samples evaluated at the phylum (A) and genus (B) levels. Principal coordinates analysis of unweighted UniFrac values (C) and RDA of 30 most predominant bacterial species in relation to different time points (D).

and My1), and decreased significantly ($q < 0.05$) from day 1 to day 14. Bacteroidetes became the second most abundant phylum in the piglet samples from 3 days after birth but was lower in the piglets on day 1 and in the sow samples. Fusobacteria was dominant during the suckling period but almost disappeared at 49 days (Fig. 2A). At the family level, Clostridiaceae, Streptococcaceae, Enterobacteriaceae and Fusobacteriaceae were predominant among the piglet samples on day 1 and 3. Subsequently, members of the Lactobacillaceae became predominant on day 7, 14 and 28, but had lower relative abundance again in the piglet samples taken on day 49 and in the sow samples. Ruminococcaceae, Lachnospiraceae and Prevotellaceae were the most abundant groups on day 28 and 49, whereas Peptostreptococcaceae, Clostridiaceae, Erysipelotrichaceae, Ruminococcaceae and Spirochaetaceae were the most abundant families in the sow samples. At the genus level (Fig. 2B), *Escherichia-Shigella*, *Streptococcus*, *Enterococcus* and *Clostridium* were the most abundant groups on day 1 and 3, whereas the abundance of *Ruminococcus*, *Blautia*, *Prevotella* and *Subdoligranulum* was higher in the piglet samples from day 14 to 49. *Turicibacter*, Unclassified Peptostreptococcaceae, and *Cellulosilyticum* were higher ($P < 0.05$) in the sow samples than the piglet samples. For visualisation, the top 50 bacterial taxa are presented in a heat map (Supporting Information Fig. S2). Furthermore, the PCoA (Fig. 2C) and constrained redundancy analysis (RDA) (Fig. 2D) analysis confirmed that the development of the bacterial community in piglets progressed predominantly with age, with the bacterial community becoming more diverse on day 49.

Similarity of bacterial community structure between groups

To determine the phylogenetic variation within age (Fig. 3A) and groups (Fig. 3B) of piglets, unweighted UniFrac distances were measured. The UniFrac distance of the piglets from all four groups was low on day 1 and day 3, indicating a similar bacterial community in the piglets in the four groups. The UniFrac distance for the four groups increased from day 7 to day 28 (the weaning day), with the highest value on day 28 over the test period, suggesting increasing differentiation of the fecal bacterial community until weaning. After the piglets were weaned and offered the same feed, the unweighted UniFrac distance for all groups was decreased on day 49, with values similar to those on day 7, suggesting age-induced convergence of bacterial composition. The UniFrac distance within breed or nursing groups in given time point could identify the driving factors for differentiation of the fecal bacterial community. The UniFrac distance between individual Meishan piglets (Mm and Ym) on day 14 was higher than that of the Yorkshire piglets (My and Yy), indicating a higher variation among Meishan piglets than among Yorkshire piglets during the differentiation period. However, the UniFrac distance within groups of piglets nursed by Meishan sows (Mm and My) was lower than that of piglets nursed by Yorkshire sows on day 14 (0.60 ± 0.01 vs. 0.65 ± 0.01) and 28 (0.62 ± 0.01 vs. 0.65 ± 0.01), but higher among groups on day 49 (0.60 ± 0.01 vs. 0.56 ± 0.01). These results suggest that the Meishan sows as the nursing mothers had a greater impact on the bacterial community during suckling period, but had less impact after the piglets were weaned.

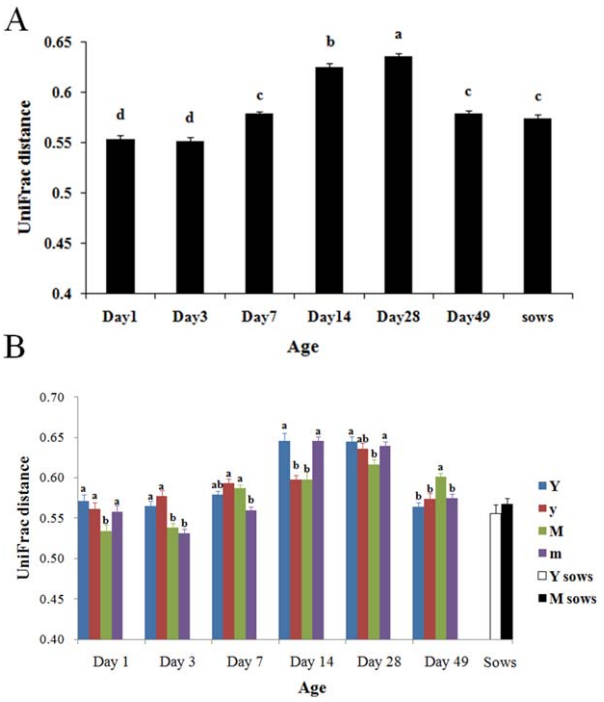

Fig. 3. UniFrac distance among all samples or different piglet group and nursing mother group during the whole experiment period.
A. The UniFrac distance among all samples during the whole experiment period.
B. The UniFrac distance among different piglet groups and nursing mother groups during the whole experiment period. The UniFrac distance among all samples and among different piglet group and nursing mother group on each day. M, the piglets nursed by Meishan sows (Mm and My groups); Y, the piglets nursed by Yorkshire sows (Yy and Ym groups); m, all Meishan piglets (Mm and Ym groups); y, all Yorkshire piglets (Yy and My groups). Means without common letters differ, $P < 0.05$.

Impact of breed, nursing, feed and aging on the bacterial composition

Forward-selection RDA analysis of the fecal bacterial community structure at the genus level across samples from all ages was used to evaluate the relative contribution of the different experimental factors to explain the variation observed in the bacterial composition. Over the entire

experimental period (Fig. 4A), the age of the piglets affected the bacterial composition significantly ($P = 0.001$), and the breed and nursing sows did not significantly contribute to explaining the residual variation (Fig. 4A). At specific ages (Supporting Information Fig. S3), however, breed had a significant impact on the bacterial profile of piglets on day 14 ($P = 0.017$) and day 49 ($P = 0.026$). However, there was no significant effect of the nursing sows on the bacterial profile of piglets at different ages.

Bacterial taxa most strongly affected by the breed or nursing sows

As observed with RDA, the overall dynamics of the bacterial composition during the entire experimental period was not significantly impacted by nursing and breed. To determine whether specific bacterial genera were linked to these factors at a specific age, we assessed for which bacterial taxa the relative abundances were impacted by the pig breed or nursing factor (Fig. 5). Seven taxa were significantly affected by the pig breed on day 14, followed by four taxa on day 28, three taxa on day 49, and one taxon on day 1, 3 and 7. On day 1, the relative abundance of the genera *Escherichia* and *Shigella* were significantly higher in the Yorkshire piglets than in the Meishan piglets (Fig. 5). On day 14, the Meishan piglets had a higher relative abundance of Unclassified Fusobacteriales (15.13% ± 7.21% vs. 2.27% ± 1.23%), while a lower relative abundance of Unclassified Erysipelotrichaceae (0.47% ± 0.18% vs. 6.21% ± 2.68%) was observed compared with the Yorkshire piglets. On day 28, the abundance of the genus *Bacteroides* in the Meishan piglets was higher than in the Yorkshire piglets (7.8% ± 2.8% vs. 1.9% ± 0.7%). On day 49, the abundance of the genus *Lactobacillus* was higher in the Meishan piglets than in the Yorkshire piglets (14.4% ± 4.3% vs. 4.3% ± 2.1%).

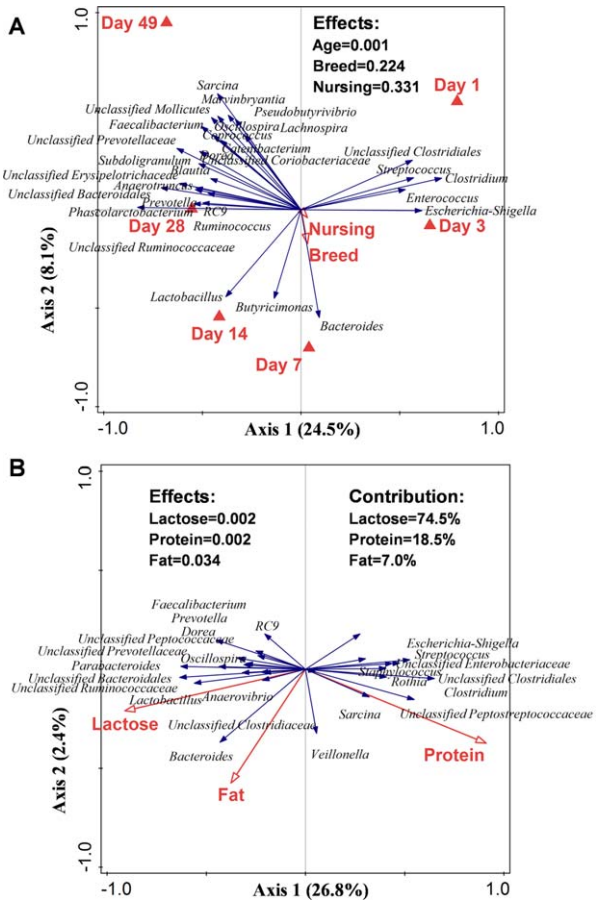

Fig. 4. RDA plots of the fecal bacteria from piglets samples during all age (A) and with milk components (B). The effects of age, breed and nursing on the development of bacterial community during the whole experiment period (A). The effects of milk composition of sows on the fecal bacteria from piglets during the sucking period (B).

Compared with the impact of pig breed, an even smaller number of bacterial taxa were affected by the nursing mother. Five taxa were significantly affected by the nursing mother on day 28, followed by three taxa on day 14, two taxa on day 3, and one taxon on day 49. On day 14, the relative abundance of the taxa *Subdoligranulum* and Unclassified Prevotellaceae was higher for the piglets nursed by Meishan sows than for those nursed by Yorkshire sows (Fig. 5). On day 28, compared with piglets nursed by Yorkshire sows, piglets nursed by Meishan sows had a higher relative abundance of Unclassified Fusobacteriales but a lower relative abundance of taxa *Subdoligranulum* and Unclassified Lachnospiraceae. On day 49, the nursing impact became less evident; only the Unclassified Clostridiaceae was affected by the nursing mother, with the abundance was higher in the piglets nursed by the Meishan sows than those nursed by Yorkshire sows. These results further confirmed the initial differentiation and subsequent post-weaning convergence during the bacterial community development in piglets as observed based on the unweighted UniFrac matrix.

In order to link differences in microbiota development between experimental groups, we also measured the concentrations of lactose, protein and fat in the milk of the sows of the two breeds at different time points (Supporting Information Table S2). The lactose concentration increased with time, and the Meishan sows had a higher level of lactose than the Yorkshire sows on day 7. The milk protein concentration in both breeds decreased with the age of piglets, and no significant difference was observed between the two breeds at any time. The fat concentration was higher on day 3, 7 and 14 than day 1 and 21, and no significant difference was observed between the two breeds.

RDA analysis of the milk composition and relative abundance of bacterial taxa (Fig. 4B) showed that milk lactose ($P = 0.002$), protein ($P = 0.002$) and fat ($P = 0.034$) all had significant impacts on the bacterial profile of piglets. Among these, lactose had the largest contribution to the shaping of

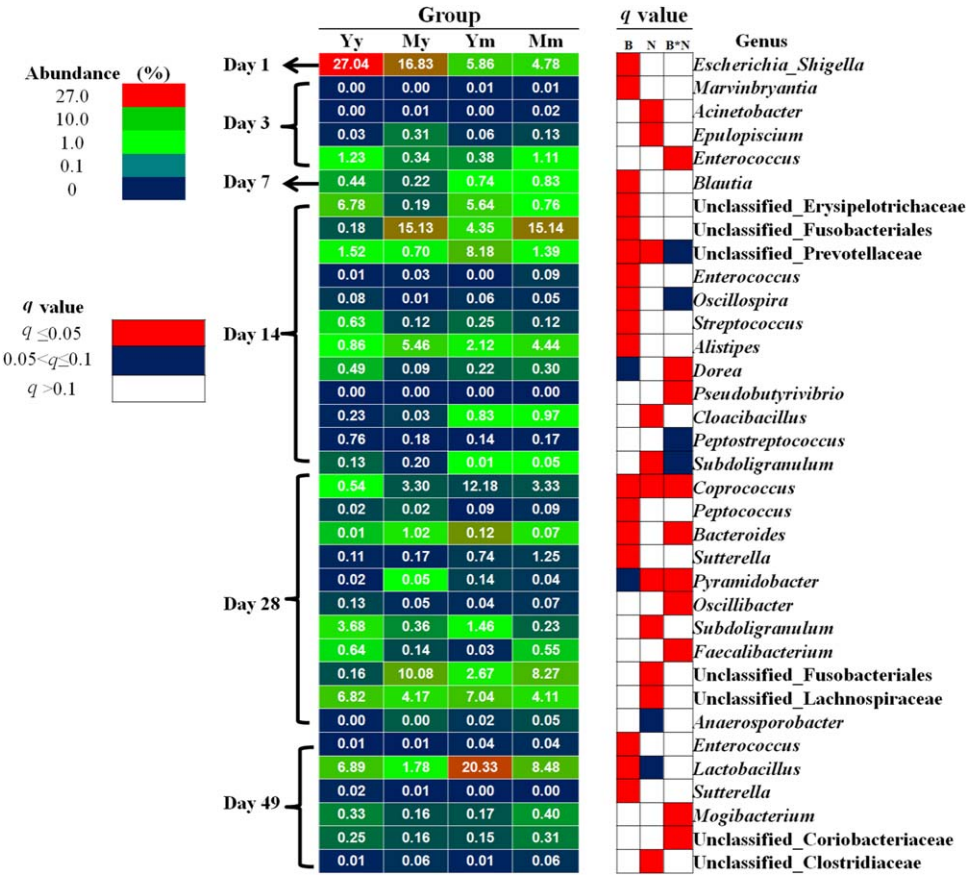

Fig. 5. Bacterial groups affected by breed or nursing mother from piglets samples. The relative abundance (shown as means) of bacterial groups were affected by breed or nursing mother in piglets samples. The major bacterial groups with their relative abundance impacted by the breed or nursing mother in piglets samples. The abundance was calculated as the percentage of all affected genera in all reads of bacteria in certain day.

the bacterial profile of piglets (contribution = 74.5%), followed by protein (contribution = 18.5%) and fat (contribution = 7.0%). Furthermore, the relative abundance of some bacterial taxa was significantly correlated with the milk composition (Fig. 6). The relative abundance of *Lactobacillus*, *Subdoligranulum*, *Coprococcus*, *Oscillospira*, *Faecalibacterium, Bacteroides*, *Prevotella*, *Ruminococcus*, and Unclassified Prevotellaceae was positively associated with the lactose concentration but negatively correlated with the protein concentration. The relative abundance of *Clostridium*, *Streptococcus*, *Escherichia-Shigella*, *Enterococcus*, and *Staphylococcus* was positively correlated with protein concentration but negatively correlated with lactose. *Lactobacillus* and Unclassified Ruminococcaceae were positively linked with milk fat, whereas *Clostridium*, *Staphylococcus*, and Unclassified Clostridiales showed a negative correlation. Among all these groups, *Coprococcus*, *Subdoligranulum*, Unclassified Prevotellaceae, *Lactobacillus*, and *Oscillospira* were associated both with the nursing mother and milk lactose, suggesting that the lactose concentration in the milk may be the key factor in the nursing effect.

Discussion

Host genetics and external factors such as delivery mode (Biasucci *et al.*, 2010; Dominguez-Bello *et al.*, 2010; Wang *et al.*, 2013), nursing milk (Rinne *et al.*, 2005; Li *et al.*, 2012) and solid feed (Koenig *et al.*, 2011) were all shown to contribute to the development of the gut microbiota of infants. In this study, using a cross-fostering piglet model, we for the first time identified the impact of host breed and nursing mother on the early colonization and establishment of the gut bacterial community. We found that the establishment of fecal bacterial communities was a progressive developmental process. In addition to the dominant effect of time after birth, the piglet breed and the nursing mother led to further differentiation of the fecal bacterial community of piglets during the suckling period, whereas the solid feed introduced after weaning decreased the differentiation of the bacterial community that was created during the suckling period.

Successional patterns of bacterial community in newborn piglets

Shortly after birth, all piglets had a similar bacterial community regardless of genotype and fostering sow in the present study. Similarly, when investigating the effects of formula feeding and rearing environment, Inman *et al.* (2010) found that the ileal bacterial community of farm-reared and mother milk-fed piglets was similar to that of isolator-reared and formulate-feed piglets between 2 and 5

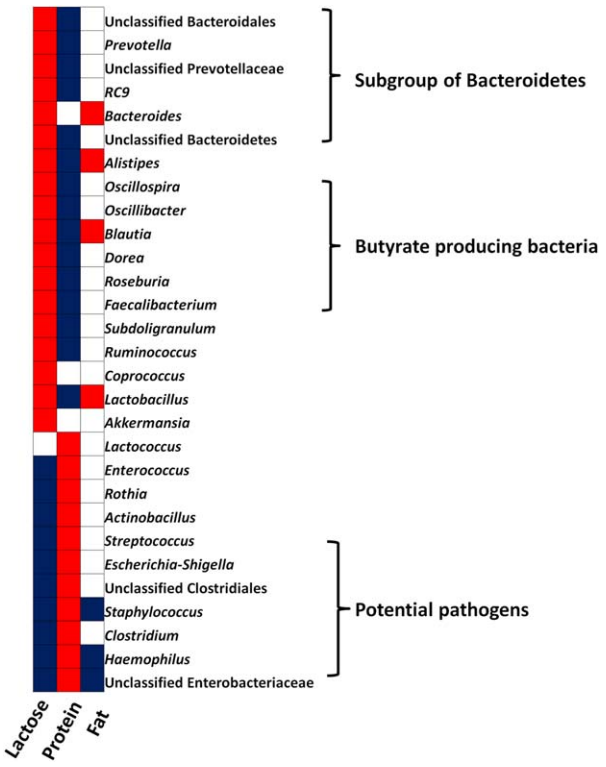

Fig. 6. The correlation between bacterial groups and milk composition during first 2 weeks life. Red colour represents a significant positive correlation ($P < 0.05$), blue colour represents a significant negative correlation ($P < 0.05$), and white colour represents no significant correlation ($P > 0.05$).

days old. These findings suggested that the early bacterial settlers of the gut are basically similar among naturally delivered neonates, and the variation in host genetics, nursing or other environmental factors may not drive the differentiation of the gut bacterial communities of piglets during the first few days. As the piglets grew, the bacterial community became more complex as reflected by the increase in richness and evenness. This is in agreement with other studies in piglets (Inoue *et al.*, 2005) and in human babies (Favier *et al.*, 2003; Palmer *et al.*, 2007).

A novel finding of the present study is the discriminating impact of pig breed and nursing on the differentiation of the bacterial community during the suckling period. The impact of piglet breed was more evident than the nursing factor during the suckling period. Affected by the pig breed and nursing factors, the bacterial community became increasingly differentiated among the different cross-fostering groups. Conversely, the bacterial communities converged among different fostering groups after the piglets were weaned and offered the same solid feed. A previous study reported that the introduction of solid feed led to a large shift in the bacterial phyla composition and metabolites in 2.5-year-old infants (Koenig *et al.*, 2011). This earlier finding is in good agreement with the assumption that the introduction of solid feed induces this post-weaning convergence of the bacterial community in the present study. Separation anxiety at weaning is another potential factor affecting microbiota composition. In humans, separation anxiety raised cortisol levels that can in turn alter the gut microbiota composition through the gut-brain axis (O'Mahony *et al.*, 2011; Kember *et al.*, 2012; Dinan and Cryan, 2013). With piglets, our previous research demonstrated that weaning altered microbiota composition in piglets (Su *et al.*, 2008). However, since all piglets experienced the same separation/weaning experimental protocol, the effect of separation anxiety could not be differentiated from the main effect but could be investigated as an experimental variable in a future study. Nevertheless, our collective findings demonstrated that age, introduction of solid food, and weaning were the main driving forces for the succession and establishment of the bacterial populations in the piglets, and the internal and external factors play an important role in shaping the bacterial community of neonates.

The impact of host genetics

Host genetics can be a major internal factor contributing to the development and establishment of the gut microbiota. In the present study using the cross-fostering model, we for the first time revealed the impact of breed on gut bacterial community development in neonatal piglets and discriminated the breed impact from factors such as diet and environment that could affect bacterial community development. The impact of breed on bacterial community composition was evident during the early suckling period when the piglets received only sows' milk (day 14) but not when the piglets started to receive solid feed while still suckling sows' milk (day 28). Thus, the introduction of solid feed overruled the breed impact. The breed impact on the bacterial composition was evident again on day 49. It is possible that the breed impact persisted throughout the period, but the complexity of the simultaneous intake of individual sow's milk and solid feed for all piglets diluted the breed impact on the bacterial composition.

Our results also showed that the breed factor influenced specific bacterial groups. Meishan piglets harbored relatively high levels of *Lactobacillus* and lower levels of *Escherichia-Shigella*, the latter being generally regarded as potentially harmful bacteria. The Meishan pig is well recognized for its higher ability to resist stress and disease compared with Yorkshire pigs (Reiner *et al.*, 2002). The high abundance of *Lactobacillus* and the low abundance of *Escherichia-Shigella* in Meishan piglets may partly explain the disease resistance of the Meishan pig. Previous studies also showed that some specific bacterial groups were associated with the host genetic variation, such as genetic obese C57BL_6 (*ob_ob*) mice harbouring a high abundance of the phylum Firmicutes (Ley *et al.*, 2005).

However, Everard *et al*. (2011) showed that specific members of the gut bacteria in the Firmicutes and Bacteroidetes phyla could reduce the fat-mass development of genetically obese mice. Meanwhile, a recent study demonstrated that inoculating germ-free mice with *Christensenella minuta*, one of the most heritable members of the human gut microbiome that was furthermore found to be enriched in individuals with a low body mass index, caused loss of weight gain and total adiposity of recipient mice (Goodrich *et al*., 2014). These findings suggested that the host genotype affects the heritability of specific gut bacteria, which may be attractive targets for the promotion of human or animal gut health and welfare.

The impact of nursing mother

In addition to the host's genetic background, the nursing mother probably represents the most influential external factor for the development of neonatal bacteria during suckling. A recent study of a cross-fostering diabetic mouse model demonstrated that the nursing mother, instead of the birth mother, was the critical factor in determining the bacterial colonization and the incidence of diabetes in the offspring (Daft *et al*., 2015). Previous studies have demonstrated that mother's milk and formula milk led to gut bacterial variation in infants (Harmsen *et al*., 2000; Rinne *et al*., 2005) and piglets (Poroyko *et al*., 2010; Li *et al*., 2012). In addition to the milk, the intimate contact with the mother may also affect the gut bacteria of suckling neonates. In our cross-fostering piglet model, we identified the impact of the nursing mother and compared the influence of different mothers' milk. The results indicated that the switch in sows' milk influenced the gut bacterial profile in piglets.

Our findings from RDA analysis of milk composition and bacterial relative abundance suggested that milk lactose, protein and fat all significantly impacted the bacterial profile of piglets, which was in agreement with previous studies (Poroyko *et al*., 2011; Li *et al*., 2012). Among the milk components measured, the lactose concentration made the major contribution (74.5%) to the gut microbiota variation observed, and hence may be the main driving force underlying the nursing effect.

Interestingly, we found that the concentration of milk lactose showed a positive correlation with some generally regarded beneficial bacteria, such as *Lactobacillus*, *Ruminococcus* and *Faecalibacterium*. Human milk oligosaccharides could stimulate the growth and fermentation of members of the genera *Bacteroides*, *Parabacteroides* and *Prevotella* from infant feces in an in vitro study (Shen *et al*., 2011). In contrast, milk protein concentration was positively correlated with some potentially harmful bacteria, such as *Escherichia-Shigella*, *Enterococcus* and *Staphylococcus*. An increase in milk protein content has been shown to increase *Escherichia* spp. in ileal content or adherent to the ileal mucosa of piglets on day 7 (Chatelais *et al*., 2011). In humans, it was reported that infants receiving milk formula with high protein and lower lactose had a lower proportion of bifidobacteria and higher percentage of enterobacteria and clostridia than breast-fed infants (Hascoet *et al*., 2011). Despite these studies, the relationship between milk protein and bacteria is still unclear. These collective results demonstrate that different milk components exert different influences on bacterial groups and that the impact of nursing milk is a combined effect of different milk components. Thus, the dynamic change of milk composition in obese and lean breeds can partly explain the complexity of the nursing impact on the acquisition and development of bacterial community in cross-fostered piglets.

Our findings suggest that the lactose in milk could be beneficial for the gut health of neonates by promoting the growth of potentially beneficial bacteria and inhibiting potential pathobionts. It is difficult to link the high level of milk lactose to the obesity of the Meishan breed and we cannot rule out the influence of differences in milk oligosaccharide patterns, which were not analysed in the present study. Nevertheless, our results suggest that the milk lactose may partly explain the nursing impact of the obese breed Meishan sow on the abundance of *Lactobacillus*, *Ruminococcus* and *Faecalibacterium*. Although the role of the shift in bacterial groups is not clear, our results further imply that feeding higher lactose content of milk could be a possible early nutritional intervention strategy aiming to modulating gut microbiota during the suckling period.

In conclusion, in the present study, the switch of nursing mother milk and host genetics strongly influenced the development of the gut bacterial community in neonates during the sucking period. However, the introduction of solid feed and weaning are likely the major determinants affecting the establishment of the composition and diversity of the gut microbiota in piglets post weaning, while host genetics and nursing could exert influence on some specific groups of microbes. Previous studies demonstrated that the early bacterial profile in the neonatal gut has a long-term influence on later and even adult health (Saavedra and Dattilo, 2012; Arnal *et al*., 2014). Thus, a stable progression and healthy succession of gut microbiota during early life is essential for life-long well-being. Our results imply that the switch of mother' milk or early solid feed intervention could modify the gut bacterial succession of neonates and consequently promote later life health. Thus, our findings may provide important perspectives on early nutritional interventions for modulating the gut microbiota.

Experimental procedures

Ethics

Animals were managed throughout the study in accordance with requirements for the Experimental Animal Care and Use

guidelines of Chinese Science and Technology Committee, 1998.

Experimental animals, cross-fostering operation and sampling

All Meishan and Yorkshire pigs were raised under the same conditions on a commercial farm in Jiangsu Province, China. Before the start of the study, power calculations had identified a required sample size of 10 piglets per treatment group. A randomized complete block design [2 treatments (breed) *2 block (nursing) with 10 replicates per group] was adopted. Candidate sows of each breed with similar expected delivery dates were chosen and intramuscularly injected with cloprostenol (0.2 mg per sow) at 10:00 AM on day 113 of gestation to ensure homophonous delivery. During the cross-fostering operation (Fig. 1A), half of the vaginally-delivered piglets in a litter of one breed were fostered onto the sow of the other breed before the piglets suckled their mothers' colostrum. The other half of each litter remained with the birth mother. Candidate pairs of sows that differed in delivery time by more than 2 h were excluded. In total, 10 sows of each breed with litters of 10 or 12 piglets were used for the cross-fostering operation in delivery-matched pairs as described. The gestational length of all sows was 113–114 d. Cross-fostering for individual pairs was completed within 2 h after birth. Eventually, four groups of piglets (each group $n = 10$) were generated as follows: Meishan piglets fostered by their birth mother (Mm), Yorkshire piglets fostered by Meishan sows (My), Meishan piglets fostered by Yorkshire sows (Ym) and Yorkshire piglets fostered by their birth mother (Yy) (Fig. 1A). From day 14 after birth, all suckling piglets were offered creep feed *ad libitum* and had free access to water (Fig. 1B). All piglets were weaned at 28 days of age. On the weaning day, the sows were removed from the piglets, while the piglets remained in the nursing pens for 3 weeks until end (day 49), to avoid the stress caused by a change in environment. The birth weight and the body weight of the piglets on day 49 were recorded to calculate the body weight gain. One piglet in each group from the same litter was slaughtered on day 28 and 49, and the back fat thickness was measured using calipers.

Fecal sampling days were chosen as follows: Day 1 and 3 represent the first three days for newborn piglets; day 7 constitutes the end of the first week; on day 14, the solid food was introduced; day 28 was the weaning day, day 49 was 3 weeks after weaning, when a relatively stable bacterial community was established and most of the changes would have occurred by then for pigs. Thus, feces from each sow before delivery and two piglets from each litter (one from the origin breed, the other from the fostering breed) at the age of 1 (My = 9, Mm = 9, Yy = 9, Ym = 10), 3 (My = 9, Mm = 9, Yy = 10, Ym = 10), 7 (My = 9, Mm = 9, Yy = 10, Ym = 10), 14 (My = 9, Mm = 9, Yy = 9, Ym = 9), 28 (My = 8, Mm = 9, Yy = 9, Ym = 9) and 49 (My = 9, Mm = 6, Yy = 9, Ym = 7) days were collected (Fig. 1B). A total of 235 fecal samples (20 samples from sows and 215 from piglets) were collected and stored at −25°C for DNA extraction. Milk samples from the sows of both breeds were collected when the piglets were 1, 3, 7, 14 and 21 days old. The milk samples were fixed in 10% formalin overnight at 4°C. The first fecal and milk samples were collected within 12 h after birth, while other samples were collected between 7:00 and 9:00 AM on the sampling day (Fig. 1B).

Milk composition analysis

The concentration of lactose in the sows' milk was analysed with the Teles method (Teles *et al.*, 1978), and the concentration of milk protein was analysed using the Bradford method (Bradford, 1976). Fat analysis was conducted using MilkoScan FT2 (FOSS, Hillerød, Denmark).

DNA extraction

Total fecal DNA was isolated with the bead-beating method as described by (Zoetendal *et al.*, 1998). The concentration of extracted DNA was determined using a Nano-Drop 1000 spectrophotometer (Thermo Scientific Inc., Wilmington, DE, USA).

Polymerase chain reaction amplification, amplicon quantification and pyrosequencing

To analyse the phylogenetic composition of the bacterial community, bacterial 16S ribosomal RNA (rRNA) gene primers (27F 5′-GTG CTC CCC CGC CAA TTC CT -3′ and 533R 5′-TTA CCG CGG CTG CTG GCA C-3') were chosen for the amplification and subsequent pyrosequencing of the polymerase chain reaction (PCR) products (Alm *et al.*, 1996). PCR was carried out in triplicate 50 ml reactions with 10 μl fivefold reaction buffer, 50 ng of DNA, 0.4 mM each primer, 0.5 U Pfu polymerase (TransStart-FastPfu DNA Polymerase, TransGen Biotech, Beijing, China), and 2.5 mM dNTPs. The amplification program consisted of an initial denaturation step at 94°C for 4 min, followed by 25 cycles of 94°C for 30 s (denaturation), 55°C for 30 s (annealing) and 72°C for 30 s (extension) and a final extension at 72°C for 10 min. PCR products were visualized on agarose gels (2% in TBE buffer) containing ethidium bromide and purified with a DNA gel extraction kit (Qiagen, Hilden, Germany).

Before sequencing, the DNA concentration of each PCR product was determined using a Quant-iT PicoGreen double-stranded DNA assay (Invitrogen, Carlsbad, CA, USA) and was quality controlled on an Agilent 2100 bioanalyzer (Agilent Technology, Santa Clara, CA, USA). Amplicon pyrosequencing was performed from the A-end using a 454/Roche A sequencing primer kit on a Roche Genome Sequencer GS-FLX Titanium platform at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). The 16S rRNA gene sequencing data for all the samples analysed in this study was submitted to the Sequence Read Archive (SRA; http://www.ncbi.nlm.nih.gov/Traces/sra/), under accession SRP066893.

Bioinformatic analysis

The sequences were processed using the MOTHUR program (Schloss *et al.*, 2009; Schloss *et al.*, 2011; version 1.34.0; University of Michigan; http://www.mothur.org/wiki/). 16S rRNA gene reads were decoded based on the 5 bp sample-specific barcodes and processed to remove poor-quality sequences. To reduce sequencing errors, the shhh.flows command was

applied, which is the MOTHUR implementation of the AmpliconNoise algorithm (Quince *et al*., 2011). Quality filters were applied to trim and remove sequences with the following characteristics: less than 200 bp in length; average quality score less than 35; homopolymers longer than eight nucleotides; and more than two different bases to the primer. In order to obtain a non-redundant set of sequences, unique sequences were determined and used to align against the SILVA reference alignment database (Pruesse *et al*., 2007); chimeric sequences were removed using chimera.uchime (http://drive5.com/uchime). All analyses were performed with QIIME (Caporaso *et al*., 2010). The heatmap figure was generated using custom Perl scripts, and principal coordinate analysis (PCoA) was conducted based on the unweighted UniFrac distance (Lozupone *et al*., 2011). To show age effect after birth, UniFrac distances among piglets at each time point were calculated using sample from day 1 as the reference sample; the breed effect was calculated using distance between Meishan piglets and Yorkshire piglets at a given age; the nursing effect was calculated using distance between piglets nursed by Meishan sows and Yorkshire sows. RDA analysis was operated at the genus level using Canoco 5.0 software (Lepš and Šmilauer, 2003).

Statistical analysis

Statistical analyses were carried out by conducting tests using the SPSS software package (SPSS version 16, SPSS, Inc., Chicago, IL, USA). The body weight gain, back fat thickness, milk composition, Estimators of diversity, and relative abundance of bacterial taxa at different ages were analysed using the general lineal mode (GLM) procedures as implemented in SPSS according to the following equation: $Y_{ij} = \mu + B_i + N_j + (B*N)_{ij} + e_{ij}$, where Y_{ij} is the observation for the dependent variables, μ is the population mean, B_i is the fixed effect of breed, N_j is the fixed effect of nursing, $(B*N)_{ij}$ is the interaction between breed and nursing and e_{ij} is the residual error assumed to be normally distributed. Unifrac distance was analysed by one-way ANOVA as implemented in SPSS. The normality of the distribution of bacterial taxa was tested using the Shapiro–Wilk test. The correlation between relative abundance of fecal bacteria and milk composition was assessed by Spearman's rank test (non-normal distribution) using GraphPad Prism version 5.00.

Power calculations before the start of the study had identified a required sample size of 10 piglets per treatment group in order to enable detection of an effect size of 0.69 SD for most of the cognitive test scores with 95% power and a type I error of 5%, based on Fish test using one-way ANOVA. Tukey's test was employed to determine significant differences of the body weight gain, back fat thickness, and milk composition among the groups, and *P*-values < 0.05 were regarded as statistically significant. All *P*-values from the analysis of Variance (ANOVA) and multiple comparison analyses of the fecal bacteria community data were adjusted by the false discovery rate (FDR) (Benjamini and Hochberg, 1995). FDR-corrected *P*-values below 0.05 ($q < 0.05$) were considered significant.

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References

Alm, E.W., Oerther, D.B., Larsen, N., Stahl, D.A., and Raskin, L. (1996) The oligonucleotide probe database. *Appl Environ Microbiol* **62:** 3557–3559.

Arnal, M.E., Zhang, J., Messori, S., Bosi, P., Smidt, H., and Lallès, J.P. (2014) Early changes in microbial colonization selectively modulate intestinal enzymes, but not inducible heat shock proteins in young adult swine. *PLoS One* **9:** e87967.

Benjamini, Y., and Hochberg, Y. (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Series B Stat Methodol* **57:** 289–300.

Biasucci, G., Rubini, M., Riboni, S., Morelli, L., Bessi, E., and Retetangos, C. (2010) Mode of delivery affects the bacterial community in the newborn gut. *Early Hum Dev* **86 (Suppl. 1):** 13–15.

Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72:** 248–254.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., *et al*. (2010) QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* **7:** 335–336.

Chatelais, L., Jamin, A., Gras-Le Guen, C., Lalles, J.P., Le Huerou-Luron, I., and Boudry, G. (2011) The level of protein in milk formula modifies ileal sensitivity to LPS later in life in a piglet model. *PLoS One* **6:** e19594.

Collado, M.C., Cernada, M., Bauerl, C., Vento, M., and Perez-Martinez, G. (2012) Microbial ecology and host-microbiota interactions during early life stages. *Gut Microbes* **3:** 352–365.

Conroy, M.E., Shi, H.N., and Walker, W.A. (2009) The long-term health effects of neonatal microbial flora. *Curr Opin Allergy Clin Immunol* **9:** 197–201.

Cotillard, A., Kennedy, S.P., Kong, L.C., Prifti, E., Pons, N., Le Chatelier, E., *et al*. (2013) Dietary intervention impact on gut microbial gene richness. *Nature* **500:** 585–588.

Daft, J.G., Ptacek, T., Kumar, R., Morrow, C., and Lorenz, R.G. (2015) Cross-fostering immediately after birth induces a permanent microbiota shift that is shaped by the nursing mother. *Microbiome* **3:** 17.

Dinan, T.G., and Cryan, J.F. (2013) Melancholic microbes: a link between gut microbiota and depression? *Neurogastroenterol Motil* **25:** 713–719.

Dominguez-Bello, M.G., Costello, E.K., Contreras, M., Magris, M., Hidalgo, G., Fierer, N., and Knight, R. (2010) Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci USA* **107:** 11971–11975.

Donovan, S.M., Wang, M., Li, M., Friedberg, I., Schwartz, S.L., and Chapkin, R.S. (2012) Host-microbe interactions in the neonatal intestine: role of human milk oligosaccharides. *Adv Nutr* **3:** 450S–455S.

Everard, A., Lazarevic, V., Derrien, M., Girard, M., Muccioli, G.G., Neyrinck, A.M., *et al*. (2011) Responses of gut microbiota and glucose and lipid metabolism to prebiotics in genetic obese and diet-induced leptin-resistant mice. *Diabetes* **60:** 2775–2786.

Fanaro, S., Chierici, R., Guerrini, P., and Vigi, V. (2003) Intestinal microflora in early infancy: composition and development. *Acta Paediatr Suppl* **91:** 48–55.

Favier, C.F., de Vos, W.M., and Akkermans, A.D. (2003) Development of bacterial and bifidobacterial communities in feces of newborn babies. *Anaerobe* **9:** 219–229.

Goodrich, J.K., Waters, J.L., Poole, A.C., Sutter, J.L., Koren, O., Blekhman, R., *et al*. (2014) Human genetics shape the gut microbiome. *Cell* **159:** 789–799.

Hansen, C.H., Nielsen, D.S., Kverka, M., Zakostelska, Z., Klimesova, K., Hudcovic, T., *et al*. (2012) Patterns of early gut colonization shape future immune responses of the host. *PLoS One* **7:** e34043.

Harmsen, H.J.M., Wildeboer-Veloo, A.C.M., Raangs, G.C., Wagendorp, A.A., Klijn, N., Bindels, J.G., and Welling, G.W. (2000) Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection methods. *J Pediatr Gastroenterol Nutr* **30:** 61–67.

Hascoet, J.M., Hubert, C., Rochat, F., Legagneur, H., Gaga, S., Emady-Azar, S., and Steenhout, P.G. (2011) Effect of formula composition on the development of infant gut microbiota. *J Pediatr Gastroenterol Nutr* **52:** 756–762.

Inman, C.F., Haverson, K., Konstantinov, S.R., Jones, P.H., Harris, C., Smidt, H., *et al*. (2010) Rearing environment affects development of the immune system in neonates. *Clin Exp Immunol* **160:** 431–439.

Inoue, R., Tsukahara, T., Nakanishi, N., and Ushida, K. (2005) Development of the intestinal microbiota in the piglet. *J Gen Appl Microbiol* **51:** 257–265.

Kember, R.L., Dempster, E.L., Lee, T.H., Schalkwyk, L.C., Mill, J., and Fernandes, C. (2012) Maternal separation is associated with strain-specific responses to stress and epigenetic alterations to Nr3c1, Avp, and Nr4a1 in mouse. *Brain Behav* **2:** 455–467.

Koenig, J.E., Spor, A., Scalfone, N., Fricker, A.D., Stombaugh, J., Knight, R., *et al*. (2011) Succession of microbial consortia in the developing infant gut microbiome. *Proc Natl Acad Sci USA* **108:** 4578–4585.

Lepš, J., and Šmilauer, P. (2003) *Multivariate Analysis of Ecological Data Using CANOCO*. Cambridge, UK: Cambridge University Press.

Ley, R.E., Backhed, F., Turnbaugh, P., Lozupone, C.A., Knight, R.D., and Gordon, J.I. (2005) Obesity alters gut microbial ecology. *Proc Natl Acad Sci USA* **102:** 11070–11075.

Li, M., Bauer, L.L., Chen, X., Wang, M., Kuhlenschmidt, T.B., Kuhlenschmidt, M.S., *et al*. (2012) Microbial composition and in vitro fermentation patterns of human milk oligosaccharides and prebiotics differ between formula-fed and sow-reared piglets. *J Nutr* **142:** 681–689.

Lozupone, C., Lladser, M.E., Knights, D., Stombaugh, J., and Knight, R. (2011) UniFrac: an effective distance metric for microbial community comparison. *ISME J* **5:** 169–172.

Matamoros, S., Gras-Leguen, C., Le Vacon, F., Potel, G., and de La Cochetiere, M.F. (2013) Development of intestinal microbiota in infants and its impact on health. *Trends Microbiol* **21:** 167–173.

O'Mahony, S.M., Hyland, N.P., Dinan, T.G., and Cryan, J.F. (2011) Maternal separation as a model of brain-gut axis dysfunction. *Psychopharmacology* **214:** 71–88.

Palmer, C., Bik, E.M., DiGiulio, D.B., Relman, D.A., and Brown, P.O. (2007) Development of the human infant intestinal microbiota. *PLoS Biol* **5:** e177.

Poroyko, V., White, J.R., Wang, M., Donovan, S., Alverdy, J., Liu, D.C., and Morowitz, M.J. (2010) Gut microbial gene expression in mother-fed and formula-fed piglets. *PLoS One* **5:** e12459.

Poroyko, V., Morowitz, M., Bell, T., Ulanov, A., Wang, M., Donovan, S., *et al*. (2011) Diet creates metabolic niches in the "immature gut" that shape microbial communities. *Nutr Hosp* **26:** 1283–1295.

Pruesse, E., Quast, C., Knittel, K., Fuchs, B.M., Ludwig, W., Peplies, J., and Glöckner, F.O. (2007) SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res* **35:** 7188–7196.

Quince, C., Lanzen, A., Davenport, R.J., and Turnbaugh, P.J. (2011) Removing noise from pyrosequenced amplicons. *BMC Bioinformatics* **12:** 38.

Reiner, G., Melchinger, E., Kramarova, M., Pfaff, E., Buttner, M., Saalmuller, A., and Geldermann, H. (2002) Detection of quantitative trait loci for resistance/susceptibility to pseudorabies virus in swine. *J Gen Virol* **83:** 167–172.

Rinne, M., Kalliomaki, M., Arvilommi, H., Salminen, S., and Isolauri, E. (2005) Effect of probiotics and breastfeeding on the bifidobacterium and lactobacillus/enterococcus microbiota and humoral immune responses. *J Pediatr* **147:** 186–191.

Saavedra, J.M., and Dattilo, A.M. (2012) Early development of intestinal microbiota: implications for future health. *Gastroenterol Clin North Am* **41:** 717–731.

Shen, Q., Tuohy, K.M., Gibson, G.R., and Ward, R.E. (2011) In vitro measurement of the impact of human milk oligosaccharides on the faecal microbiota of weaned formula-fed infants compared to a mixture of prebiotic fructooligosaccharides and galactooligosaccharides. *Lett Appl Microbiol* **52:** 337–343.

Stewart, J.A., Chadwick, V.S., and Murray, A. (2005) Investigations into the influence of host genetics on the predominant eubacteria in the faecal microflora of children. *J Med Microbiol* **54:** 1239–1242.

Su, Y., Yao, W., Perez-Gutierrez, O.N., Smidt, H., and Zhu, W.-Y. (2008) Changes in abundance of Lactobacillus spp. and Streptococcus suis in the stomach, jejunum and ileum of piglets after weaning. *FEMS Microbiol Ecol* **66:** 546–555.

Teles, F., Young, C.K., and Stull, J. (1978) A method for rapid determination of lactose. *J Dairy Sci* **61:** 506–508.

Tims, S., Derom, C., Jonkers, D.M., Vlietinck, R., Saris, W.H., Kleerebezem, M., *et al*. (2013) Microbiota conservation and BMI signatures in adult monozygotic twins. *ISME J* **7:** 707–717.

Turnbaugh, P.J., Hamady, M., Yatsunenko, T., Cantarel, B.L., Duncan, A., Ley, R.E., *et al*. (2009) A core gut microbiome in obese and lean twins. *Nature* **457:** 480–484.

Wang, M., Radlowski, E.C., Monaco, M.H., Fahey, G.C., Jr, Gaskins, H.R., and Donovan, S.M. (2013) Mode of delivery and early nutrition modulate microbial colonization and fermentation products in neonatal piglets. *J Nutr* **143:** 795–803.

Wu, G.D., Bushmanc, F.D., and Lewis, J.D. (2013) Diet, the human gut microbiota, and IBD. *Anaerobe* **24:** 117–120.

Zivkovic, A.M., German, J.B., Lebrilla, C.B., and Mills, D.A. (2011) Human milk glycobiome and its impact on the infant gastrointestinal microbiota. *Proc Natl Acad Sci USA* **108 (Suppl. 1):** 4653–4658.

Zoetendal, E.G., Akkermans, A.D., and De Vos, W.M. (1998) Temperature gradient gel electrophoresis analysis of 16S rRNA from human fecal samples reveals stable and host-specific communities of active bacteria. *Appl Environ Microbiol* **64:** 3854–3859.

Zoetendal, E.G., Akkermans, A.D., Akkermans-Van Vliet, W.M., De Visser, A., and De Vos, W.M. (2001) The host genotype affects the bacterial community in the human gastrointestinal tract. *Microbial Ecol Health Dis* **13:** 129–134.

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Estimators of diversity of the 16S rRNA gene libraries from fecal bacteria of piglets at different ages.
Fig. S2. The Heatmap of the fecal microbiota from sows and piglets samples. The relative abundance of major genera of samples each group in the cross-fostering experiment. The color intensity of the panel is proportional to the abundance of each genus.
Fig. S3. The RDA plots of the fecal bacteria from piglets samples at genus level. The impacts of breed and nursing sows on the bacterial profile on day 1, 3 and 7 (A), day 14 (B), day 28 (C), and day 49 (D).
Table S1. The body weight gain and back fat thickness of piglets at different periods.
Table S2. The concentration of milk composition between two breed sows at different time point.