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The interaction between microbiome and pig efficiency: A review

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Abstract

The existence of genetic control over the abundance of particular taxa and the link of these to energy balance and growth has been documented in model organisms and humans as well as several livestock species. Preliminary evidence of the same mechanisms is currently under investigation in pigs. Future research should expand these results and elicit the extent of genetic control of the gut microbiome population in swine and its relationship with growth efficiency. The quest for a more efficient pig at the interface between the host and its metagenome rests on the central hypothesis that the gut microbiome is an essential component of the variability of growth in all living organisms. Swine do not escape this general rule, and the identification of the significance of the interaction between host and its gut microbiota in the growth process could be a game-changer in the achievement of sustainable and efficient lean meat production. Standard sampling protocols, sequencing techniques, bioinformatic pipelines and methods of analysis will be paramount for the portability of results across experiments and populations. Likewise, characterizing and accounting for temporal and spatial variability will be a necessary step if microbiome is to be utilized routinely as an aid to selection.

KEYWORDS
genetic selection, microbiome, swine

1 | INTRODUCTION

Improved economic conditions in large part of the world in recent decades have led to steady global population growth. This growth has been accompanied by increased demand for access to high-value animal proteins, resulting in an increased pressure for the livestock industry to meet this demand. In the near future identifying methods for producing more food, using fewer inputs and minimizing environmental impact while ensuring the welfare of animals will be the greatest challenge facing the food animal industry. In this, swine could represent an efficient and sustainable approach to help meet the global food demand because of their high nutritional value, diverse manufacturing capabilities and palatability. Livestock profitability is for a large part driven by feed costs and in meat-producing species by the amount and quality meat produced (Hoque, Kadowaki, Shibata, Oikawa, & Suzuki, 2009). Genomic selection for increased feed and growth efficiency has been investigated in swine (Jiao, Maltecca, Gray, & Cassady, 2014; Lu et al., 2017) and is currently implemented in most commercial populations. However, selecting for feed and growth efficiency remains costly and slow.

The gut microbial community is a complex system that co-exists inside each living body. Bacteria in the gut have substantial influence on host nutritional, physiological and immunological processes in various ways. Changes to the gut bacteria composition have been proven to be linked to health problems in human (Backhed et al., 2004; Barman et al., 2008; Collins, Denou, Verdu, & Bercik, 2009; Kassinen et al., 2007; Turnbaugh et al., 2006, 2015), as well as health and production performance of pigs (Celi et al., 2017;

Hermann-Bank et al., 2015; Kim et al., 2012). Nonetheless, the complex interplay between host genetics and the gut microbiome is yet to be investigated on a large scale, including sampling conducted through several stages of the production life in swine (Morota, Ventura, Silva, Koyama, & Fernando, 2019).

The current literature documents a handful of studies in humans that have investigated host genetic contribution to variation in gut microbiome abundance (Davenport et al., 2015; Goodrich, Davenport, Clark, & Ley, 2017; Goodrich et al., 2014; Polderman et al., 2016; Rothschild et al., 2017; Zoetendal & Ak, 2001). Similar efforts are currently underway in cattle (Myer, 2017). In pigs, there are only a few preliminary studies aimed at investigating the host role in the gut bacteria composition (Crespo-Piazuelo et al., 2019). This, although such knowledge could be crucial in meeting the increasing global demand for quality protein, while improving animal health and welfare, in the face of changing environmental conditions.

Investigating the host control of gut microbial composition presents several challenges, particularly in elucidating the genomic architecture of host-microbiome control and identify potential genetic variants influencing the host gut bacterial profile. Addressing these questions requires a large number of individuals having both genotypes data as well as the metagenome sequence of their gut bacteria. The latter might impose a limitation on some genome projects due to the current cost of sequencing gut bacteria.

In Figure 1 is depicted the overall connection of the different system components influencing the total variability of swine growth efficiency. In the next section of the paper, we will try to highlight some of the current efforts and challenges in understanding the connections among all these system elements in swine.

2 | CURRENT KNOWLEDGE OF THE HOST PERFORMANCE AND GUT MICROBIOME INTERPLAY

2.1 | Microbiome association with growth and performance phenotypes in pigs

The link between microbiome and phenotypes has a rich body of literature in humans (Cho & Blaser, 2012; Clemente, Ursell, Parfrey, & Knight, 2012; Sandoval-Motta, Aldana, Martínez-Romero, & Frank, 2015). Gut microbial diversity in pigs has been described and well-characterized from the ecological standpoint (Xiao et al., 2018). Nonetheless in swine when it comes to the gut microbiome and its association with performance, a large portion of the current literature has been characterized by relatively focused studies targeting the manipulation of specific groups of bacteria in the gut at particular times in the animals' lives, often in relation
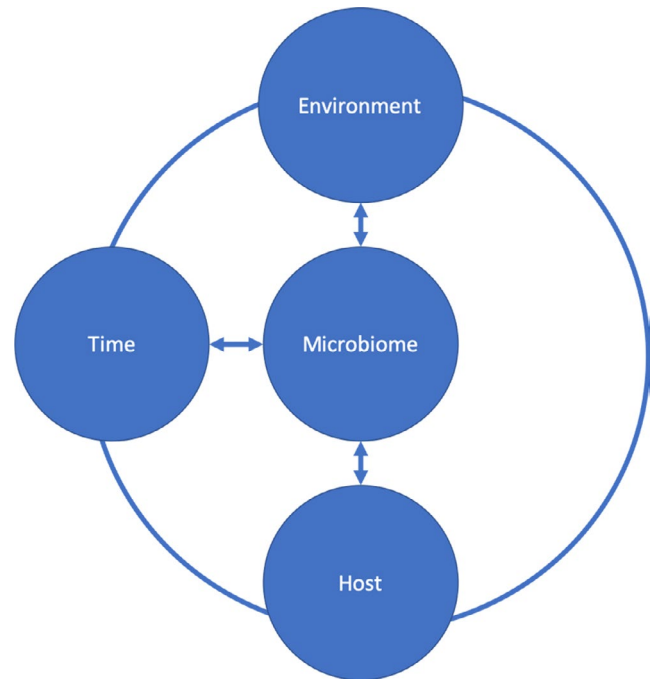
FIGURE 1 Systems components influencing the overall variability of swine growth efficiency

to nutrition studies (Hu et al., 2019; Pedersen, Andersen, Hermann-Bank, Stagsted, & Boye, 2015). Fewer studies have characterized the influence of gut natural variability of microbiome characteristics in relation to growth and carcass traits. Park, Kim, Lee, Rhee, and Kim (2013) described the gut microbiome in pigs as it relates to meat quality and body fat. Yan et al. (2017) reported that microbial transplants from pigs to mice alter metabolic profiles of skeletal muscle. Mach et al. (2015) and Ramayo-Caldas et al. (2019) investigated the early establishment of the microbiome in pigs and identified enterotypes related to growth traits in swine, while Xiao et al. (2016) reported the potential effect of different microbial profiles on lipid metabolism. Similarly, the impact of different microbial communities has been recently linked to the potential for efficient utilization of fibre (Camarinha-Silva et al., 2017; Cheng et al., 2018; McCormack et al., 2019, 2018). Relationships between growth and carcass composition with specific microbial profiles as well as alpha diversity were also reported by Lu et al. (2018).

2.2 | Microbiability heritability and the host genomic architecture of the microbiome composition in swine

The concept of microbiability was proposed first by Difford, Lassen, and Lovendhal (2016) as a way to include the overall microbial composition as part of phenotypic variation while accounting for the relationships between the hosts and their microbial profile. In essence, the idea is that of modelling the effect of an individual microbial profile with standard linear

mixed model machinery by way of using a microbial "relationship" matrix obtained from the Operational Taxonomic Unit (OTU) abundance for each individual in a similar fashion to the genomic relationship matrix.

Briefly a microbial relationship matrix, **O** can be obtained as $\mathbf{O} = \frac{1}{q}\mathbf{X}\mathbf{X}^{\mathbf{T}}$, where **X** is a matrix of dimension of $n \times q$, where n is the number of animals and q is the number of OTU. **X** can in turn be obtained from **S**, a matrix of equivalent dimensions $n \times q$. Each element of **S**, S_{jk}, represents the relative abundance of a particular OTU k in individual j. The elements of X can then be calculated as:

$$X_{ij} = \frac{\log(S_{jk}) - \overline{\log S_k}}{\mathrm{sd}(\log S_k)}$$

where S_k is the vector of the kth column of **S**.

The predictions of individual effects will then represent the overall microbial profile impact for each animal, and the ratio of variance explained by the microbial effect over the total variance will represent the microbiability of the trait. Estimates of microbiability in swine are still scarce in the literature. Camarinha-Silva et al. (2017) identified a sizable microbiability component in pigs, of approximately 0.20 averaged across growth and production traits. Similar results were found by Lu et al. (2018), in a larger swine crossbred population. It is essential to realize that while a microbial relationship matrix accounts for individuals' similarities in microbial structure, microbiability does not have a genetic interpretation per se as it models an environmental component of the overall phenotypic variation for a trait. To investigate the extent of host control over the microbial composition, this last needs to be considered as the dependent variable. Narrow sense heritabilities for different taxa in pigs has been reported by several authors (Camarinha-Silva et al., 2017; Yang et al., 2016) with estimates ranging from low to moderate (~0.05 to 0.4), strongly suggesting a partial genetic control of the microbial gut population in swine. Most of these studies have relied on estimates of microbial composition at a single time point. Furthermore, estimates have been mostly obtained for single taxonomical features (ranging from family to genera). Alternatively, Lu et al. (2018), attempted to model the overall microbial composition of individuals over time as measured by alpha diversity. This measure attempts to describe two key aspects of microbial communities in a determined sample by summarizing both richness (the overall number of features represented) as well as evenness (the overall proportional representation of these features. In their work, they obtained moderate heritability estimates for alpha diversity ranging from ~0.10 to 0.4. Attempts to explicitly model interactions between the genetic effect of the hosts and their microbial profile are still lacking. Lu et al. (2018) obtained direct estimates of genotype by microbiome for growth and carcass traits, but for the most part, these were negligible. For meat quality traits Khanal, Maltecca, Schwab, Gray, and Tiezzi (2019 *submitted*) instead found significant G × M for fatness marbling colour and firmness. Currently, in swine, only few attempts have been performed to identify genomic regions controlling microbial composition through GWAS on relatively small populations. Cheng et al. (2018), identified two potential QTL regions controlling the abundance of particular OTUs on chromosomes 9 and 10. Crespo-Piazuelo et al. (2019), identified 52 single-nucleotide polymorphisms distributed across 17 genomic regions associated with the abundance of six genera: *Akkermansia, CF231, Phascolarctobacterium, Prevotella, SMB53, and Streptococcus*. Future efforts with larger populations will provide additional evidence of the direct control of the host over either single features of aggregated measures of microbial composition.

2.3 | The ability of microbiome to predict future performance in pigs

Microbial variability in swine sits at the crossroad of the metagenome genomic variability, that of the host, and the constraints posed by other environmental factors (management, diet, but also farm conditions, climate, etc.). Attempts to mechanistically elicit the relationships between these various components will require a considerable time. While the ultimate goal from a breeder's perspective is to exploit the host variability in developing and maintaining a favourable microbial composition for fast lean growth, predictions of phenotypic performance will become critical as system management tools with the spread of precision agriculture technology. Microbiome could provide an important source of information both as a biomarker of the physiological status of an animal, as well as of the environmental condition in which the same animal is performing. To date, the literature on the phenotypic predictive power of the overall microbial profile in pigs is virtually non-existent. Camarinha-Silva et al. (2017) obtained predictive accuracy for growth and carcass traits of approximately 0.40 (averaged across traits) when employing a microbial relationship matrix. Maltecca et al. (2018, 2019) provided preliminary evidence of the power of an individual overall microbial profile to predict growth and carcass composition with the use of machinery employed routinely in genomic prediction as well as non-parametric methods achieving good prediction accuracies for fat (~0.50) and average daily gain (~0.40) but less favourable for other carcass characteristic. A larger amount of data should allow in the near future for better predictive power as well as the application of more sophisticated models such as machine learning and deep learning algorithms that could adequately account for the complex dynamics of microbial interactions, which are currently ignored for the most part.

2.4 | Establishing causality between microbial profiles, host genomic make-up and phenotypic performance

In the previous sections, we have looked at the host and microbiome variability somewhat piecewise. While this is, for the most part, a necessary evil of data analysis, it is crucial to recognize that a successful approach in integrating microbiome information in the attainment of growth efficiency in swine would necessarily require a system approach. Thus, in order to fully exploit the range of variability generated by the host-guest-environment system (which we prefer to the ecological definition holobiont (Estelle, 2019; Theis et al., 2016), since this last tend to deemphasize the human intervention part), it will be paramount to establish causal relationships among all the components of the system.

We can attribute to regression coefficients, that is, the change in the *y* variable given a change in the *x* variable, a causal meaning (Gianola & Sorensen, 2004; Valente et al., 2013; Valente, Rosa, Gianola, Wu, & Weigel, 2007). When investigating the impact of (gut) microbiome on a particular phenotype, in a causal framework, we would be interested in inferring whether a given change in microbiome composition causally *affects* the phenotype (e.g., an increase in the relative abundance of an OTU will result in a decrease in backfat depth). In a Microbiome-Wide Association Study (MWAS) context, we could obtain spurious OTU effects if a model that ignores host genotype is used. Particularly, there would be cases where a given host genomic variant impacts the OTU abundance, which in turns affects the phenotype. Conversely, in a GWAS, markers significance obtained ignoring potential microbial effects could be spurious for the same biological reason (Valente et al., 2013; Leal-Gutiérrez et al., 2018).

Structural equation models have been proposed to estimate effects under complex causal structures (de los Campos, Gianola, Boettcher, & Moroni, 2006; de Maturana et al., 2010; Tiezzi, Valente, Cassandro, & Maltecca, 2009; Varona, Sorensen, & Thompson, 2016). A schematic representation of the possible relationships in a causal network between G (the host genotype), M (the microbiome composition), E (the environment) and P (the phenotype) is reported in the directed acyclic graphs of Figure 2.

The direct effect of G on P (α) determines the proportion of phenotypic variability attributable to the host (heritability). The effect of G on *M* (β) determines what can then be interpreted as the heritable portion of M. The joint effect of G on M and P represent the genetic correlation between the microbiome composition and the phenotype. The effect of M on P (γ) determines the microbiability. Finally, the effect of E on P (ε_p) and the effect of E on *M* (ε_m) can be considered exogenous effects as for example management and diet, respectively. If we ignore interactions among these terms, we can see that the total contribution of G on P can be direct (α)
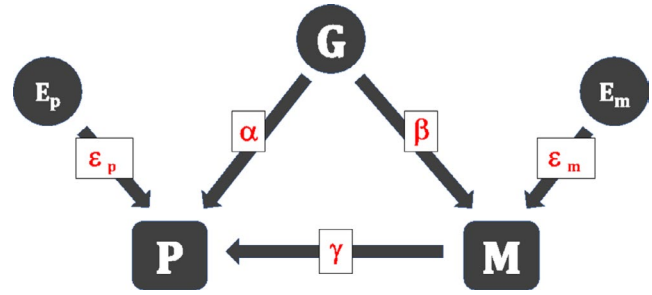
FIGURE 2 Acyclic graphs picturing the possible interplay between the host genotype (G), the gut microbiome (M), the environmental components (E_x) and the phenotype (P) in an animal breeding context

or mediated by M ($\beta*\gamma$). In the case where $\beta*\gamma$ is larger than α, any inference made in the use of host genomic markers should be made with caution: the impact of G on P could vanish if β is altered by external intervention for example by insurgence of disease and/or the use pre/pro/antibiotics. This could have strong repercussions in selection, where the breeding strategy is solely based on the estimate of α.

In addition, there could be other scenarios that an animal breeder should evaluate. In the case were $\alpha > 0$, $\beta = 0$ and $\gamma > 0$, there is no impact of host genome on microbiome composition and G and M play independent roles. When the general model used in animal breeding ($P = \alpha G + \varepsilon_p$) is enhanced with microbiome information ($P = \alpha G + \gamma M + \varepsilon_p$), there could be a reduction of the ε_p component, as a portion of this will likely be absorbed by γM. The reduction of ε_p could in turn have a positive impact on breeding value estimation and accuracy of prediction, by reducing residual error and increasing the power to model systematic variability. In the case, instead, where the inclusion of γM decreases the impact of αG, the breeders should pay particular attention to the β effect, since the collinearity between G and M could be due to an impact of G on *M* (provided that M cannot affect the expression of G, which will be discussed later).

In the case where $\alpha > 0$, $\beta > 0$ and $\gamma = 0$, a genetic correlation between P and M could be estimated. This is the case where the host genome can affect both the phenotype (directly) and the microbiome composition. A mis-specified model (e.g. $P = \gamma M + \varepsilon_p$) could estimate the γ effect, but this model would not allow any inference on the biological nature of such impact of M on P. In this case, an intervention on the microbiome composition (i.e., change in diet, use of pre/pro/antibiotics) would not lead the expected change in P, since such γ effect is spurious but is the host genome to drive P and M, simultaneously.

The cases reported above are over-simplified, and reality will undoubtedly span a variety of different scenarios. Well-balanced experiments will therefore be necessary for the estimation of meaningful causal effects. Carefully designed experiments and appropriate modelling though are only the

first ingredients necessary in establishing causality. For example, in the schematic example provided above, if we assumed that M cannot impact (the effect of) G, while we know that intestinal microbiome can alter gene expression directly or by epigenetic modifications (Aleksandrova, Romero-Mosquera, & Hernandez, 2017; Bultman, 2017), we would be again lacking a key component to allow interpretability of the system. Gene expression and epigenetic modifications would in this case be missing layers in the causal network proposed and the way in which M can alter the impact of G could be misconstrued. In addition, there could be covariance between ε_p and ε_m, which could also make the estimation of the other parameters more difficult. Finally, there could be interactions among all components in the system (as in the case of G × M). We could imagine cases where G can control the contribution of M to P, with different genotypes having different responses to the changes in microbiome composition. At the same time, M could play a different role conditionally on G, that is, the presence (or abundance) of a relative species could have a different impact depending on the host genotype. The use of full, balanced experimental designs will be pivotal in estimating the effect of such interactions.

2.5 | Integrating microbiome information in the selection process

Causal inference would provide a roadmap for the interpretation of microbiome contributions to livestock phenotypes. As a result, economic selection indices could be adjusted in light of such contribution. Given the breeding goal P, in the case where M can account for some systematic variance (currently pushed to the residual) breeders could leverage such reduction of environmental variance for improving the accuracy of the host breeding value, provided that the contribution of M can be controlled. For example, pre/probiotics could shift the microbiome composition towards an optimum, forcing the variance due of M to approach 0. Since such variance is normally reported in the denominator of the ratio to calculate heritability (for explicit modelling in γ or implicit modelling in ε_p), such heritability will increase and would consequently lead to a larger genetic progress.

In the case, instead, of the absence of the γ effect and strong genetic correlation between P and M, the (gut) microbiome composition could be included among the traits in the selection index, as predictor of the breeding goal at the genetic level. This could increase the payoff for traits relatively expensive to measure such as feed efficiency. Lastly, in the case of strong (genetic) association between the breeding goal and some microbial features, and large heritability of these features' abundance, indirect selection could also be effectively performed. Breeders could then produce breeding value predictions for the microbial features of interest, which will be then weighted into a selection index.

3 | TECHNOLOGICAL CONSIDERATIONS

3.1 | Faecal versus intestinal samples

In the investigation of the potential use of microbial information for management and selection purposes, most of the research looking at pig gut microbiome has relied on faecal samples for logistical reasons as well as for ease of collection. While this is approach is unescapable in commercial settings (intestinal samples can currently only be collected by sacrificing the individual, although promising non-invasive alternatives based on ingestive osmotic pills have been proposed (Nejad et al., 2018)), it is important to be aware that the microbial profile of the intestinal tract of swine is variegated and rich, with different niches and taxonomical abundance in different parts of the digestive tract. To date, only a handful of studies have investigated this variability and particularly the relationship among the microbial community of different intestinal tracts and performance traits. Crespo-Piazuelo et al. (2018), characterized the microbial composition of five distinct part of the intestinal tract in Iberian pigs, highlighting how colon and small intestine presented two distinct microbial profiles and how colon samples (the ones closer to the faecal proxy) were more similar among individuals compared to other regions. Similar results were also reported by other authors (Kelly et al., 2017; Yang et al., 2015; Zhao et al., 2001). Future research aimed at understanding microbial variability as a tool for swine selection should pay particular attention at understanding the correlation between microbial profiles at different parts of the digestive tract with specific emphasis on designs that would allow disentagling of breed and familiar effects.

3.2 | 16S ribosomal RNA versus whole-genome sequencing versus genotyping by sequencing

There are two common next-generation sequencing approaches in obtaining microbial profiles in living organisms and specifically in swine. The first is the targeted sequencing of the 16 ribosomal region RNA gene. In this approach, DNA is extracted, and a target variable region of the small ribosomal subunit RNA gene is amplified. After this first step, sequences are clustered through bioinformatics into OTU or amplicon sequence variants (ASV). The technology relies on the fact that the 16s rRNA gene is conserved among bacteria as well as containing a hypervariable region that can be used to reliably taxonomically resolve each OTU (Franzén et al., 2015). This is currently the leading technology in studies of moderate to large sample size in swine, due to its versatility as well as its affordability.

Nonetheless, the achievable resolution is lower than what obtainable with whole-genome sequencing (WGS), which instead does not target a specific gene but the whole microbial genome, thus in principle allowing to capture a more substantial portion of the available microbial community at a more granular level. In this case, the bioinformatics is more involved since the taxonomical assignment is performed through alignment to a reference genome. This in the past was more problematic given the lack of characterized bacterial genomes, but current efforts in providing a better characterization of pigs microbial genomes (Xiao et al., 2018) are making this technology increasingly appealing. The largest limitation remains, in this case, its cost, which makes its application prohibitive for routine collection and selection purposes. The constant decrease in sequencing costs and the employment of long-read technology could nonetheless make this approach more affordable (Dilthey, Jain, Koren, & Phillippy, 2019). A third approach involving a reduced representation through genotyping by sequencing was proposed recently by Hess et al. (2018) in ruminants and could be potentially ported to the swine community providing intermediate resolution at a fraction of the costs of WGS.

3.3 | OTU versus ASV

One of the first steps in any microbiome census is describing the "features" of the community that are to be enumerated. For many years, the molecular OTU concept, borrowed from traditional numerical taxonomy, has informed researchers about how such features are defined in surveys relying on markers such as the bacterial 16S rRNA gene. These OTUs, each consist of a cluster of nucleotide sequences that share some degree of similarity. The exact strategy by which OTUs are defined varies from study to study in terms of the identity cut-offs, clustering algorithms, reference databases, and filtering strategies employed. However, there is accumulating evidence that regardless of the strategy by which OTUs are defined, this paradigm has significant shortcomings that limit the utility of OTU-based analyses (Callahan, McMurdie, & Holmes, 2017). These include: (a) poor resolution (OTUs often group together sequences from disparate organisms), (b) an inability to easily compare results across studies, (c) reliance in some cases on frequently changing reference databases, and (d) the need to define an arbitrary dissimilarity threshold.

Recent advances now allow error to be controlled sufficiently such that a new feature definition, called the ASV, can supplant traditional OTUs as the minimal unit of microbiome analyses. ASVs are directly resolved from Illumina-scale amplicon data using algorithms that attempt to directly infer biological sequences as they exist prior to the introduction of amplification and sequencing errors (Callahan et al., 2016;

Needham, Sachdeva, & Fuhrman, 2014). This inference is accomplished in a de novo manner, so that unlike OTUs, which are generated by clustering sequences on the basis of similarity thresholds for ASV, there is no need to set a similarity cut-off. The result is a set of features (ASVs) that are distinguishable from one another in a census by as little as a single nucleotide. This superior feature resolution allows for better discrimination of closely related taxa, improved differentiation of organisms that may have distinct biological properties, and the identification of more informative biomarkers in marker gene analyses. Several studies also indicate that ASV methods achieve sensitivity and specificity as good or better than OTU methods, while at the same time provide better discriminating ecological patterns (Needham et al., 2014). Importantly, because ASVs are defined as exact sequence variants rather than clusters of different sequences, they are also far more amenable to consistent labelling and comparison across studies.

This consistent labelling of features will allow in the future to perform more powerful meta-analyses, test the reproducibility of the results and make predictions about biomarkers identified here in a way that would not be possible with traditional OTUs.

4 | EXPERIMENTAL DESIGN CONSIDERATIONS

4.1 | Temporal and spatial variation

Changes in individual microbiome composition over time have been reported by several authors (Lu et al., 2018; Mach et al., 2015). Most of the published work in swine has focused on individual and group variation but for the most part, has ignored the influence of time and physical location along with interactions among individuals in shaping the microbial communities. While microbial communities have now been ecologically characterized over a wide range of geographical and environmental conditions (Xiao et al., 2018) a systematic attempt to model this influence in commercial settings and more importantly to understand its evolution and implications for selection and management purposes is currently missing. As an example, in Figure 3, is reported the effect of time and space in the similarity of microbiome composition across an experiment. In the figure are depicted three confusion matrices obtained with a random forest model representing each a specific census time point for the microbiome in an experimental design of crossbred swine reported elsewhere (Lu et al., 2018). Each box of the three matrices represents a replicate, the combination of a room-barn (Rep 1–6). The larger red squares represent different (adjacent) barns. Rooms within barn were filled sequentially during the experiment (so Rep1 would be first room first barn Rep2 s room first barn etc.). Each
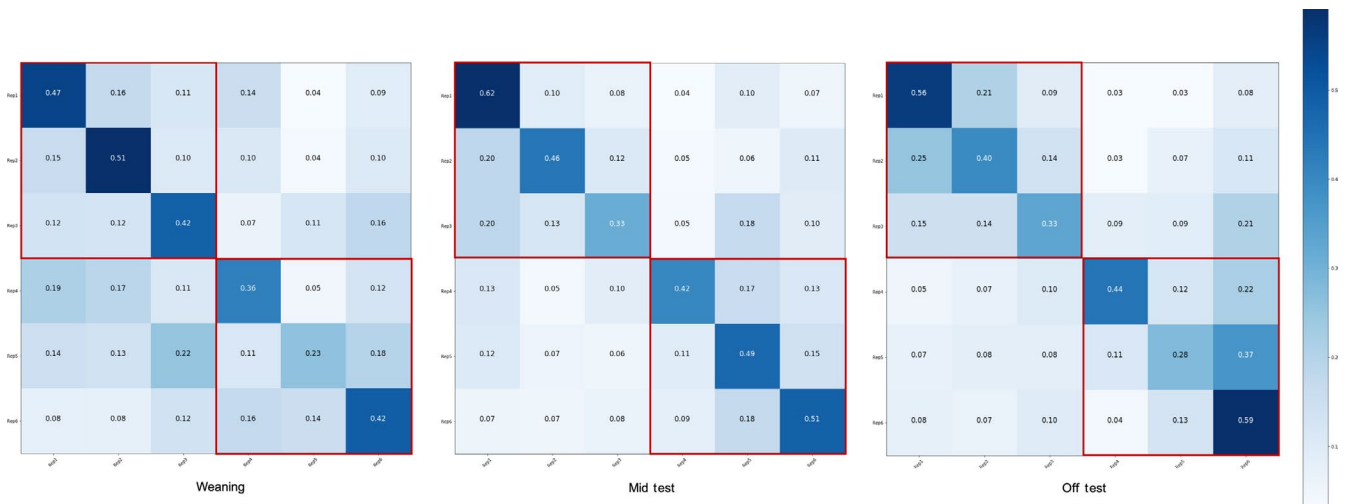
FIGURE 3 Changes in microbial similarity over space and time

confusion matrix reports the ability to correctly classify a sample as belonging to a particular replicate; a perfect classifier would have 1 on the diagonal and 0 elsewhere. In the figure, it is possible to see that as time passes rooms within a barn become more similar (higher misclassification rate) and also that the two barns used for the experiment tend to become two distinct environments and individuals within these units will resemble more microbially than others. The experiment, in this case, was not set up to disentangle time and spatial effects, so that these can only be interpreted as suggestive arguments, but it seems logical to expect that microbial populations will be affected by both space and time. As standard machinery exists for the modelling of this kind of effects, future research experiment should pay particular attention in designing trials that would allow accounting for it effectively.

5 | FUTURE OUTLOOK

The ratio of genomic variability existing in the metagenome compared with the host is high (approximately 9:1), making it simpler and faster to manipulate. Evidence exists that the metagenome can be influenced and shaped by external interventions (diet, management, supplementation), making de-facto the "selection" of the gut ecosystem possible. Conversely though, if the host genetic component of microbiome composition is sizable, neglecting it might significantly hamper the efficacy of gut ecosystem manipulation in achieving efficient lean growth.

The overall effect of the host-guest genomic make-up could instead be exploited in conjunction with diet and management to achieve efficient individuals at a faster pace in different environments.

The ability to maintain a healthy gut microbiota composition would be crucial in understanding an individual's energy homeostasis as well as the ability of an individual to efficiently grow over a broad spectrum of diet conditions. This is particularly important since it has been shown that feed efficiency in swine has a sizeable $G \times E$ component and individuals efficient on high-energy diets often lose their advantage on low energy rations (Knap & Wang, 2012). Currently, there is insufficient knowledge on the extent of genomic control of microbiota composition in swine. The quantification of the importance of microbiome composition for efficient growth could dramatically change the industry's strategies for manipulation of microbiome through both breeding and diet. For the industry, this could be accomplished by adding microbiome composition as part of the breeding objective or by directly manipulating the microbiome to be deployed in populations under selection through diet or other artificial means. The potential benefit of exploiting both microbiome and host genetic variation in the quest to improve efficiency by lowering feed costs while simultaneously reducing the environmental impact and improving the wellbeing of individual pigs could be game-changing. The information presented in previous sections suggest that there is considerable variability in the amount of predictive power for growth and carcass traits that microbial populations provide at different sampling times. The following are what we believe some important points for future efforts aimed at including microbial variability in the attainment of efficiency in pigs.

If metagenomic information is to be employed in the attainment of efficient growth under commercial conditions, a low number of reliably predictive sampling time points need to be identified to justify the investment in this technology and maximize its economic return. To date, no studies of sizable magnitude have characterized the evolution of microbial communities in growing pigs. This should be an important area for future research.

Currently, most of the tools employed in characterizing microbial populations are inherited from the microbial

ecology scientific community. In this respect, they provide a wealth of information but often lack ease of interpretation and portability. Future research should strive to characterize and summarize the critical parameters of the longitudinal microbiome development in swine. This, in turn, will allow us to easily summarize, compare and contrast microbial composition across a variety of factors (e.g., sex, breed, age, family), in order to efficiently rank individuals for growth and to reduce the amount of information needing to be stored and included in predictive models.

Currently, one of the largest limitations of metagenomic studies is the inability to transfer results from different experiments due to the limitation of microbial sequence clustering as well as a large proportion of completely disconnected designs. Future research should seek to harmonize protocols for data and tissue collection sampling times as well as collect the sample of the largest genomic pool as well as the widest environmental conditions.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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