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Assessing the relationship between the gut microbiota and growth traits in Chinese indigenous pig breeds

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Abstract

Background Gut microbiota plays crucial roles in host metabolism, diseases and development. It has also been reported to be associated with growth performance in pigs. However, the bacterial species influencing pig growth performance have not been isolated, and the mechanisms remain unclear.

Results In this study, we collected 500 gut microbial samples from two Chinese indigenous pig breeds, including 244 fecal samples from Bamaxiang (BMX) pigs and 256 cecum content samples from Erhualian (EHL) pigs, to investigate the relationship between gut microbiota and pig growth traits. Bacterial compositions were determined by 16 S rRNA gene sequencing, and association analysis was performed using a two-part model. We found that the Firmicutes-to-Bacteroidota ratio in fecal samples from BMX pigs was negatively associated with average daily gain ($P = 0.0085$). Amplicon sequence variants (ASVs) belonging to *Prevotella* and three ASVs annotated to Oscillospiraceae were negatively associated with pig growth traits, while ASVs annotated to Muribaculaceae and Rikenellaceae showed positive correlations with growth traits in BMX fecal samples. In cecum content samples from EHL pigs, ASVs belonging to *Prevotella*, *Lactobacillus delbrueckii*, and Lachnospiraceae were negatively associated with growth performance, whereas one ASV belonging to Rikenellaceae demonstrated a positive association. Predicted functional capacity analysis revealed that metabolic pathways related to the digestive system, glycan biosynthesis and metabolism, signaling molecules and interactions, and xenobiotics biodegradation and metabolism were positively associated with pig growth traits. Conversely, the excretory system pathway showed a negative correlation. These pathways were found to correlate with growth trait-associated bacterial ASVs, suggesting that alterations in gut bacterial composition led to functional capacity shifts in the gut microbiome, subsequently affecting porcine growth.

Conclusions Our results gave significant insights about the effect of gut microbiota on pig growth and provided important evidence to support further isolation of bacterial taxa that influence pig growth for elucidating their mechanisms.

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Keywords Swine, Gut microbiota, Functional capacities, Growth traits

Background

Pigs are one of the main sources of meats for human consumption. Improvement of pig growth and meat production is important to pig industry because it is related to economic benefits. Growth performance of pigs is influenced by many factors, including genetics, health, diets, and gut microbiota. Most of previous studies have focused on the effects of genetics, diets, and diseases on pig growth [1–3]. However, more and more studies have indicated the significant roles of gut microbiota in regulating host growth [4–6].

Gut microbiota is a highly diverse and complex microbial community in the gastrointestinal tract. It contributes to host metabolism by fermenting and degrading diet polysaccharides [7], promoting nutrient absorption [8], and synthesizing vitamins [9], and plays an important role in pathogen resistance, immunity, and development. Numerous studies have shown that the gut microbiota is related to growth in pigs [10–12]. For examples, gut microbial dysbiosis in piglets has been shown to impair growth, whereas fecal microbiota transplantation could reduce the incidence of diarrhea and enhance the average daily weight gain in piglets by altering the gut microbiota [13]. A previous study found that *Prevotella* enterotype-like group was positively correlated with body weight [6]. Ramayo-Caldas *et al.* identified that the enterotype dominated by *Ruminococcus* and *Treponema*, and the enterotype dominated by *Prevotella* and *Mitsuokella*, showed a significant association with average daily gain [5]. Additionally, Han *et al.* found that pigs with high relative abundance of *Anaerotruncus* in the gut had low body weight. However, pigs with high body weight exhibited an enrichment of metabolic pathways involved in xenobiotic degradation [11]. Our previous studies found the correlations between the gut microbiota and growth performance-related traits in pigs, including feed efficiency [14, 15] and fatness traits [16, 17]. These studies have suggested that the gut microbiota plays an important role in regulating growth performance in pigs.

The possible mechanisms of the gut microbiota affecting host growth have been proposed by several studies [18–20]. For example, gut microbiota contributes to host nutrient digestion and absorption [21, 22]. Commercial formula diets provided to pigs have always contained crude fiber. The gut microbiota can degrade and metabolize dietary fiber in feeds to produce short chain fatty acids (SCFAs) which provide energy to the host [5]. A recent study found that ileal infusion of SCFAs could improve growth performance of growing pigs [23]. On the other hand, the gut microbiota could enhance host health and disease resistance that benefit for pig growth [24–26]. *Lactobacillus plantarum* ZLP001 strain, isolated from a healthy piglet, could enhance intestinal barrier and resist enterotoxigenic *Escherichia coli* infection [27, 28].

To the best of our knowledge, most of existing studies have been performed in fecal samples. As we have known, different gut locations showed significantly different gut microbial compositions. Therefore, systematically investigating the effect of gut microbial taxa in different gut locations on host growth performance is important to improve pig health and growth. Furthermore, there is no bacterial species (strains) that have yet been confirmed to causally affect pig growth. Western commercial pigs have shown significantly different growth speed compared to Chinese indigenous pig breeds [29]. Whether this different growth speed should be caused by different gut microbial composition remains unknown.

Therefore, the main objective of this study was to systematically investigate the contribution of feces and cecum microbiota on pig growth performance by using two representative Chinese indigenous pig breeds of Bamaxiang (BMX) and Erhualian (EHL), and identify the bacterial taxa and potential functional capacities of gut microbiome associated with pig growth traits. This study provided novel insights into how gut microbiota affects pig growth performance.

Methods

Animals, phenotype measurement, and sample collection

A total of 500 Chinese indigenous pigs from two breeds raised in the same farm were used in this study, including 244 BMX and 256 EHL pigs. All experimental pigs were healthy and had not received any antibiotics or other drugs within two months before sampling. The feeding, management, and sample collection of experimental pigs have been described in our previous publications [17, 30, 31]. In brief, BMX and EHL pigs were raised in the same farm with natural lighting and ventilation. All experimental pigs were housed in pens (10 pigs per pen) with solid concrete floors, and were provided the same commercial formula diets twice daily (9:00 am and 16:00 pm). The ingredient compositions and nutrient levels of commercial formula diets are shown in Table S1, following the Chinese National Feeding Standard (GB, 2004). Water was provided *ad libitum via* nipple drinkers. After being fasted for 24 h, all experimental pigs (aged 300 ± 3 days) were slaughtered at a commercial slaughterhouse through bleeding following electrical stunning. Fresh fecal samples from BMX pigs were collected in the day before slaughter. Cecum content samples were harvested from EHL pigs within 30 min after slaughter. All samples

were immediately frozen in liquid nitrogen, and then stored at − 80 °C until use.

Body weight (BW) (kg) at 240 and 300 days of age (defined as BW240 and BW300) were phenotyped for all experimental pigs with an electronic floor scale (XK3190-A12 + E, YAOHUA, China) by three experienced panelists. Average daily gain (ADG) of each experimental pig from 240 to 300 days was calculated by (BW300–BW240)/60 [32].

Microbial DNA extraction and 16 S rRNA gene sequencing

Microbial DNA was extracted from fecal and cecum content samples with QIAamp DNA Stool Mini Kit (Qiagen, Germany) according to the manufacturer´s standard protocol [33]. The concentration and integrity of extracted microbial DNA were determined by a Nanodrop-1000 (ThermoFisher Scientific, USA) and 0.8% agarose gel electrophoresis. We used conservative primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3') that were reported previously [34] to amplify the V4 hypervariable region of the 16 S rRNA gene. The PCR products were sequenced using the paired-end strategy on a MiSeq platform (Illumina, USA).

Bioinformatics analysis of 16 S rRNA gene sequencing data

Bioinformatic analysis of 16 S rRNA gene sequencing data was performed using Quantitative Insights into Microbial Ecology 2 (QIIME2, 2021.11) software pipeline [35]. Briefly, raw reads/sequences under FASTQ file format were imported into QIIME2, and demultiplexed with the q2-demux plugin. After quality control, dereplication, and chimeras filtration, paired-end clean reads were assembled into tags. And then, all tags were denoised and clustered into amplicon sequence variants (ASVs) with the q2-dada2 [36]. The classifier was trained with the sequences of 16 S rRNA gene V4 region from the Silva database (version 138) [37], and each ASV was assigned to a taxonomy at the confidence threshold of 0.7. After removing mitochondria and chloroplast sequences, a total of 5,262 ASVs were obtained. Those ASVs that were presented in < 5% of experimental pigs were removed from analysis. We predicted functional capacities of gut microbiota using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States 2 (PICRUSt2) [38]. Microbial phenotypes were predicted using BugBase (http://bugbase.cs.umn.edu) [39].

Microbial composition analysis and visualization were performed using R (version 4.1.0). The α-diversity of gut microbiota including Shannon index and inverse Simpson index was calculated with the vegan package (version 2.5.7) [40]. The relative abundances of Firmicutes and Bacteroidota were calculated by summing the abundances of all ASVs belonging to each of these two phyla. The Firmicutes/Bacteroidota (F/B) ratio were obtained with the relative abundances of Firmicutes and Bacteroidota.

Construction of co-abundance groups (CAGs)

ASVs identified in at least 20% of Bamaxiang pigs (394 ASVs) or Erhualian pigs (338 ASVs) were chosen to construct CAGs. The correlations between pairs of ASVs among 394 ASVs were calculated by the Sparse Correlations for Compositional data algorithm (SparCC) [41] via the SpiecEasi (version 1.1.1) in R package. And then, the correlation values were converted to a distance matrix (1-correlation value). Using the WGCNA [42] in the R package (version 4.1.0), 394 ASVs in fecal samples of BMX pigs and 338 ASVs in cecal content samples of EHL pigs were clustered into 17 and 24 CAGs, respectively, by using the Ward clustering algorithm. The sum value of relative abundances of ASVs belonging to each CAG represented the relative abundance of that CAG. The CAG networks were visualized by Cytoscape (version 3.8.0) [43].

Statistical analyses

After quality control, a total of 244 fecal samples from BMX pigs and 256 cecum contents from EHL pigs were obtained 16 S rRNA gene sequencing data. Among them, 204 BMX pigs (distributed in nine slaughter batches) and 238 EHL pigs (11 slaughter batches) were also well phenotyped on growth traits. Therefore, we selected those pigs having both 16 S rRNA gene sequencing data and phenotypic data for association study.

The effects of sex and slaughter batch on growth traits were corrected using the linear regression model. The residuals of phenotypic values of porcine growth traits were used for further association analysis. The relative abundance of most of gut bacterial ASVs were not normally distributed. Here, we employed a two-part model to identify ASVs associated with growth traits [44]. In brief, the two-part model consisted of both binary model and quantitative model. The binary model accounted for the effect of presence/absence of a microbe on porcine growth traits. The quantitative model was used to analyze the association between the abundance of the detected microbes and porcine growth traits. To further evaluate the effect of both binary and quantitative features, a meta-analysis was performed with an unweighted Z score method. The minimum of P-values (the most stringent P-value) from binary analysis, quantitative analysis, and meta-analysis was chosen as the final P-value of the association analysis. The Z score was calculated with Z distribution. The 1000 × permutation test was performed to control the false discovery rate (FDR). FDR < 0.1 was set as the significance threshold.

The relationships between host growth traits and the α-diversity of gut microbiota were assessed using the Spearman rank correlation analysis. The correlations of bacterial features including F/B ratio, CAGs and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways with growth traits were analyzed by the Spearman correlation analysis. Additionally, the relationships between growth traits-associated microbes and KEGG pathways were also assessed using the Spearman correlation analysis. The threshold of $P < 0.05$ was considered as statistical significance level. The correlations were visualized using the ggplot2, ggpubr, pheatmap and circlize package in R software (version 4.1.0).

To investigate phenotypic variation of porcine growth traits explained by the gut microbial communities, we performed the 100 × cross-validation [44]. In each run, the dataset was randomly split into a 70% discovery dataset and a 30% validation dataset. In the discovery dataset, ASVs that were significantly associated with the phenotypes at each *P*-value threshold were identified by using the two-part model. And then, we estimated the effect sizes of both binary and quantitative features (β_1 and β_2) of each ASV. In the validation dataset, the risk of the gut microbial communities on growth traits (r_m) for each individual was computed using an additive model:

$$r_m = \sum_{j=1}^{n} \left(\beta_1 + b_j + \beta_{2j}\, q_j \right)$$

where b_j is the binary feature of j ASV; and q_j is the quantitative feature of j ASV, β_1 represents the estimated effect of the presence or absence of a microbe, β_2 indicates the estimated effect on bacterial abundance. The squared correlation coefficient (R^2) values were calculated between the growth trait values corrected for sex and batch and r_m. The R^2 represents the phenotypic variance explained by the gut microbial communities. To further guarantee the stability and validity of the estimation, we performed a 100 × cross-validation and calculated the average of variances explained.

Results

Phenotypic values of growth traits in BMX and EHL pigs

A total of 204 BMX and 238 EHL pigs having both 16 S rRNA gene sequencing and phenotyping data were included in the correlation analysis between phenotypic values and relative abundances of gut microbial taxa. Phenotypic values of BW240, BW300, and ADG in two pig populations followed the normal distribution. The summarized descriptions for phenotypic values of each trait are shown in Table 1.

The phylogenetic composition and category characterization of the gut microbiota in feces of BMX pigs and cecum contents of EHL pigs

Fecal samples collected from 244 BMX pigs and cecum content samples from 256 EHL pigs were used in this study. All these 500 samples were performed 16 S rRNA gene sequencing. An average of 27,359 clean tags per sample was obtained. Based on 100% sequence identity, a total of 5,266 ASVs were clustered in all fecal and cecal samples. Those ASVs present in > 5% of samples were retained for further analyses. As a result, 927 unique ASVs passed quality control in all 500 samples. ASVs were taxonomically assigned to the phylum (100%), class (99.89%), order (98.92%), and family (97.09%) level with high percentage of successful annotation rate. However, only 85% of ASVs could be annotated to the genus level. In details, for fecal samples from BMX pigs, we obtained 821 ASVs with an average of 231 ± 31 (mean ± SD) ASVs per sample, ranging from 155 to 311 ASVs. At the phylum level, a total of 18 phyla were identified in the fecal microbiota, which was dominated by Firmicutes (45.07%), followed by Bacteroidota (29.92%) and Spirochaetota (16.44%). *Treponema* (16.13%), *UCG-005* (7.40%), *Rikenellaceae_RC9_gut_group* (6.49%), *Prevotellaceae_NK3B31_group* (4.83%), *Streptococcus* (4.53%), *Lactobacillus* (3.96%), *UCG-010* (3.32%), and *Prevotella* (3.06%) were the top eight bacterial genera in relative abundance in fecal samples (Fig. 1A).

An average of 203 ASVs per sample was obtained for 256 cecal samples from EHL pigs, ranging from 34 to 303. At the phylum level, a total of 19 phyla were identified. Bacteroidota (51.37%), Firmicutes (31.01%), and Spirochaetota (7.11%) were ranked in the top three in relative abundances. *Prevotellaceae_NK3B31_group* (7.28%), *Treponema* (6.99%), *Alloprevotella* (6.79%), *Prevotella* (6.29%), *Rikenellaceae_RC9_gut_group* (5.89%), *Bacteroides* (4.87%), *Prevotellaceae_UCG-003* (4.81%),

Table 1 Growth performance of Bamaxiang and Erhualian pigs.

	Bamaxiang (n = 204)			Erhualian (n = 238)		
Growth traits	**Mean**	**SD**	**Skewness**	**Mean**	**SD**	**Skewness**
BW240[1], kg	47.86	7.47	0.06	62.36	12.63	0.005
BW300[2], kg	61.35	9.1	0.12	83.95	13.7	-0.085
ADG[3], kg/d	0.22	0.06	0.33	0.36	0.08	-0.18

[1]BW240 = Body weight at 240 days of age

[2]BW300 = Body weight at 300 days of age.

[3]ADG = Average daily gain

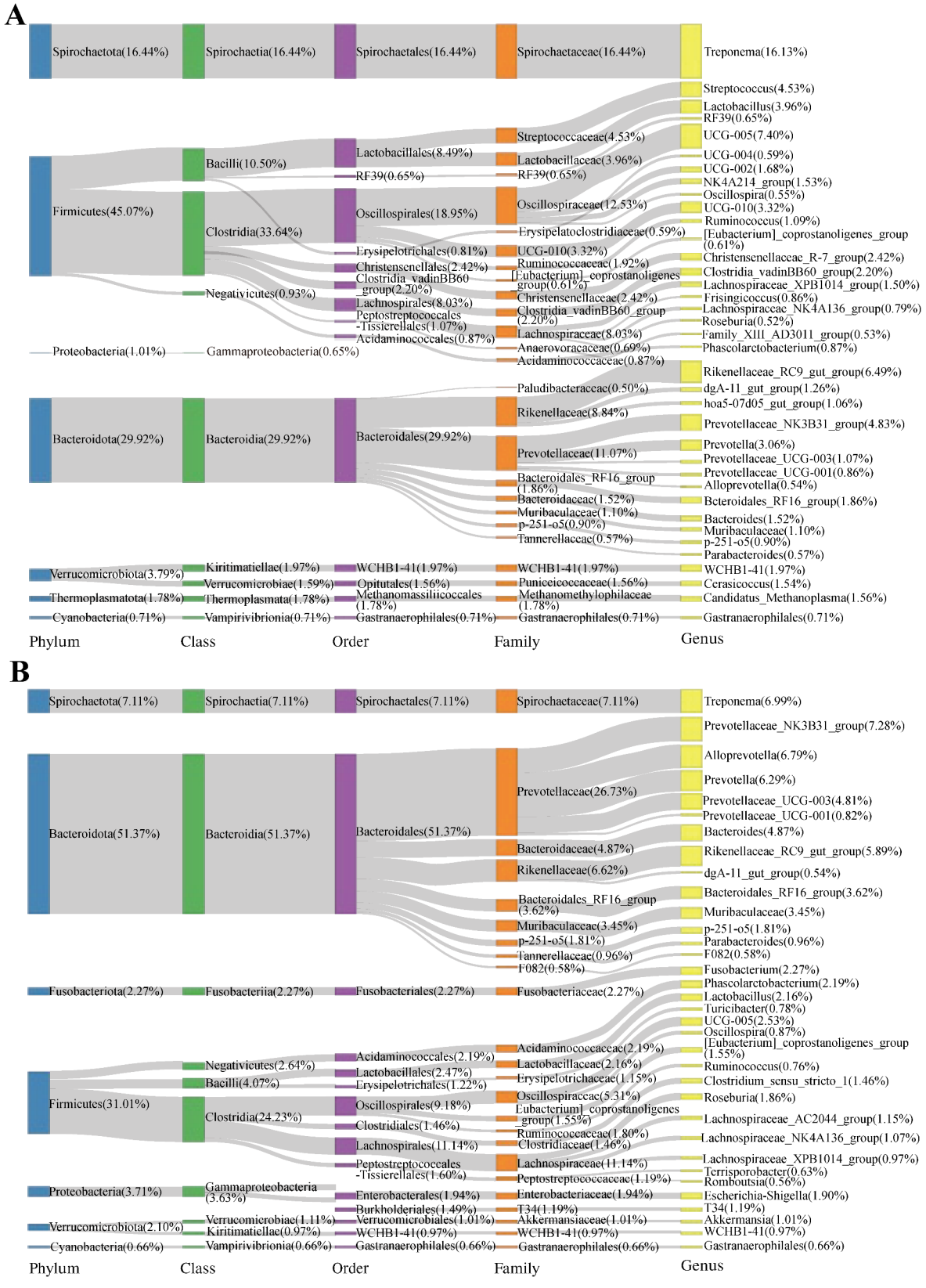

Fig. 1 Sankey diagram showing the composition of bacterial taxa in fecal samples from Bamaxiang (**A**) and cecal samples from Erhualian (**B**). The colors of the rectangles from left to right represent different taxonomy levels. The percentages in bracket indicate the relative abundance of each taxonomy

and *Bacteroidales_RF16_group* (3.62%) were the most abundant genera in cecal samples (Fig. 1B).

Additionally, the category compositions of pig gut microbial taxa according to predicted characteristics were also described. The microbial characteristic traits including aerobe, anaerobe, facultative anaerobe, containing mobile elements, forming biofilms, Gram-negative, Gram-positive, potentially pathogens, and stress-tolerance were determined in fecal samples of BMX pigs. The relative abundances of these characteristic traits were 3.31 ± 2.19% (aerobe), 72.64 ± 4.13% (anaerobe), 66.55 ± 7.64% (facultative anaerobe), 3.37 ± 1.68% (containing mobile elements), 20.61 ± 7.25% (forming biofilms), 48.57 ± 9.03% (Gram-negative), 51.43 ± 9.03% (Gram-positive), 51.77 ± 6.51% (potentially pathogens), and 79.84 ± 5.58% (stress-tolerance) (Fig. 2A). The scatter plot shows the distribution (prevalence) of each ASV in 244 fecal samples (Fig. 2C). Among 821 ASVs, 39 (4.75%) were commonly identified in at least 90% of fecal samples and considered as the core bacteria (Table S2). These ASVs were annotated to Firmicutes, Bacteroidota, Verrucomicrobiota, Spirochaetota, Thermoplasmatota and Euryarchaeota.

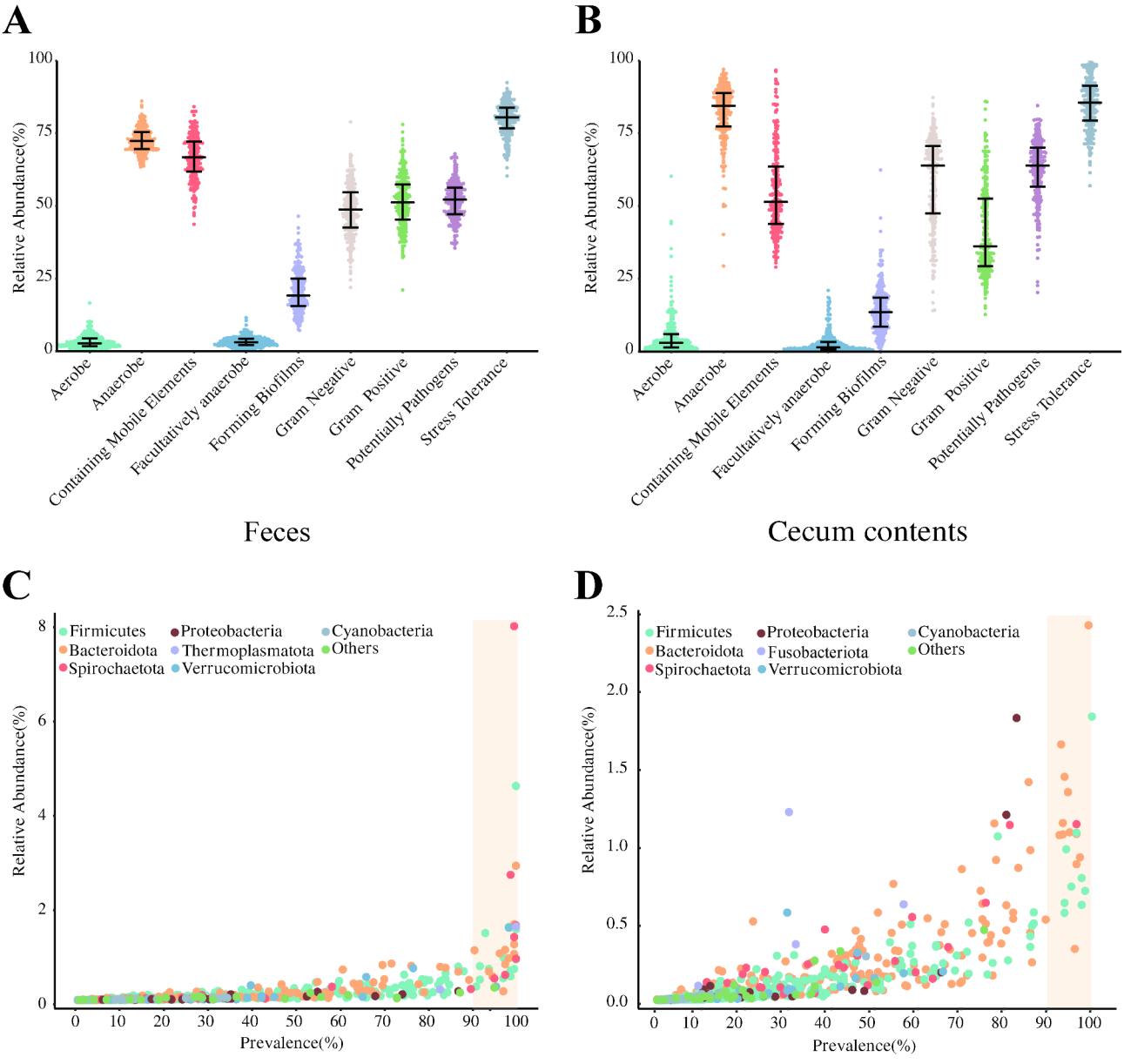

Fig. 2 The characteristic traits of pig gut microbiota in fecal (**A**) and cecal (**B**) samples, and scatter plot of commonly identified core bacteria in fecal (**C**) and cecal (**D**) samples. Prevalence = (number of the samples observed specific ASV/total samples) ∗ 100%. The colors of the circles represent different bacterial phyla

For cecal samples from EHL pigs, the relative abundance of nine characteristic traits including aerobe, anaerobe, facultative anaerobe, containing mobile elements, forming biofilms, Gram-negative, Gram-positive, potentially pathogens, and stress-tolerance bacteria were 5.07 ± 7.13%, 82.21 ± 9.63%, 55.09 ± 14.90%, 2.79 ± 3.66%, 14.25 ± 8.09%, 58.82 ± 15.18%, 41.18 ± 15.18%, 62.35 ± 10.77%, and 85.23 ± 8.65%, respectively (Fig. 2B). The scatter plot shows the prevalence of microbial taxa in 256 cecal samples (Fig. 2D). A total of 22 ASVs annotated to Bacteroidota, Firmicutes, and Spirochaetota were commonly present in pig cecal samples (prevalence ≥ 90%) and defined as core bacteria (Table S3).

Association of the global changes in pig gut Microbiome with pig growth traits

To examine how the variation of growth traits was associated with the global changes in pig gut microbiome, we first performed the correlation analysis between the phenotypic values of growth traits and the α-diversity of the intestinal microbiota. There were no statistically significant correlations between the indices of the α-diversity (Shannon index or inverse Simpson index) and growth traits in both pig populations (Fig. S1). However, we found that the F/B ratio (a synthetic measure of gut microbial dysbiosis) in fecal samples of BMX pigs showed a negative association with the phenotypic values of ADG ($P = 0.0085$) (Fig. 3A).

As the gut microbiota plays roles as functional groups in the microecological system [45], we next established CAGs of the gut microbiota to identify the microbial CAGs associated with growth traits in both populations. ASVs presented in > 20% of tested samples were clustered by SparCC correlation based on their relative abundances. For fecal samples of BMX pigs, 394 ASVs were clustered into 17 CAGs (Fig. 3B). Using the Spearman correlation analysis, we identified CAG9, CAG13 (it was not shown in Fig. 3B because the absolute values of the correlation coefficient between ASV pairs were less than 0.4), and CAG15 showed significantly negative correlations with BW300 and ADG (Fig. 3C, Table S4). In these three CAGs, most ASVs were annotated to Oscillospiraceae and Muribaculaceae (Table S4). The CAG4 including 95 ASVs was positively related to ADG, and ASV44 (*p-251-o5*) was the hub ASV of CAG4 (Fig. 3C, Table S4). For cecal samples from EHL pigs, 338 ASVs were divided into 24 CAGs (Fig. 3D). The CAG22 in which a Ruminococcaceae ASV (ASV565) was the hub in the network, was positively associated with BW240, BW300 and ADG. Four CAGs including CAG18, CAG29, CAG34, and CAG36 displayed positive correlations with BW240, while CAG39 and CAG41 were negatively correlated with BW240 and BW300 (Fig. 3E, Table S5).

Identification of the gut bacterial taxa associated with porcine growth traits in BMX and EHL pigs

The two-part model was used to further identify the gut bacterial taxa associated with pig growth traits. The phenotypic values were firstly adjusted for the effects of sex and slaughter batch. And then, the residuals were used for association analyses with those ASVs having the relative abundance > 0.05% and existing in > 20% of tested samples. At the significance threshold of FDR < 0.1, we found no significant correlations between growth traits and ASVs. However, when the significance threshold was set at $P < 0.01$, in BMX pigs, we identified a total of 14 ASVs that were significantly associated with growth traits including BW240, BW300, and ADG (Fig. 4, Table S6). Among them, six ASVs (ASV105, ASV195, ASV282, ASV305, ASV78, and ASV92) showed significantly negative correlations with BW240. These ASVs were annotated to Peptococcaceae, Rhodospirillales, *Rikenellaceae_RC9_gut_group*, *[Eubacterium]_ruminantium_group* and *Prevotella*. However, we did not observe any ASVs that were positively associated with the phenotypic values of BW240. In addition, we found that the ASV378 (*Muribaculaceae*) were positively correlated with BW300, whereas ASV326 (*Oscillospirales_UCG-010*), ASV78 (*Prevotella*), and ASV92 (*Prevotella*) were negatively correlated with BW300 ($P < 0.01$). Similarly, seven ASVs were associated with ADG, including ASV11 (*Rikenellaceae_RC9_gut_group*), ASV160 (*Bacteroides*), ASV378 (*Muribaculaceae*), ASV172 (*Prevotellaceae_NK3B31_group*), and ASV180 (*Treponema succinifaciens*), which were positively correlated with ADG, and ASV266 (*Oscillospiraceae_UCG-005*) and ASV51 (*Oscillospiraceae_NK4A214_group*) which showed negative correlations with ADG.

In EHL pigs, five ASVs were identified to be positively associated with BW240. Among these five ASVs, ASV255 was annotated to *[Eubacterium]_coprostanoligenes_group*, and the other four ASVs were annotated at the genus level (Fig. 4). Three ASVs showed negative correlations with BW240. These three ASVs were annotated to *Lachnospiraceae_NK4B4_group*, *Prevotella*, and *Lactobacillus delbrueckii*. Three ASVs were associated with BW300 (Fig. 4), including ASV122 (*Lactobacillus delbrueckii*) and ASV426 (*Lachnospiraceae_NK4B4_group*) showing negative correlations, and ASV258 (*Quinella*) having positive associations. Only one ASV was negatively associated with ADG (ASV445 annotated to Lachnospiraceae) ($P < 0.01$) (Fig. 4, Table S7).

Association of predicted metagenomic functional capacities of the gut microbiota with pig growth traits

In order to characterize potential functional capacities of pig gut microbiota, KEGG pathways were predicted based on 16 S rRNA gene sequencing data with the

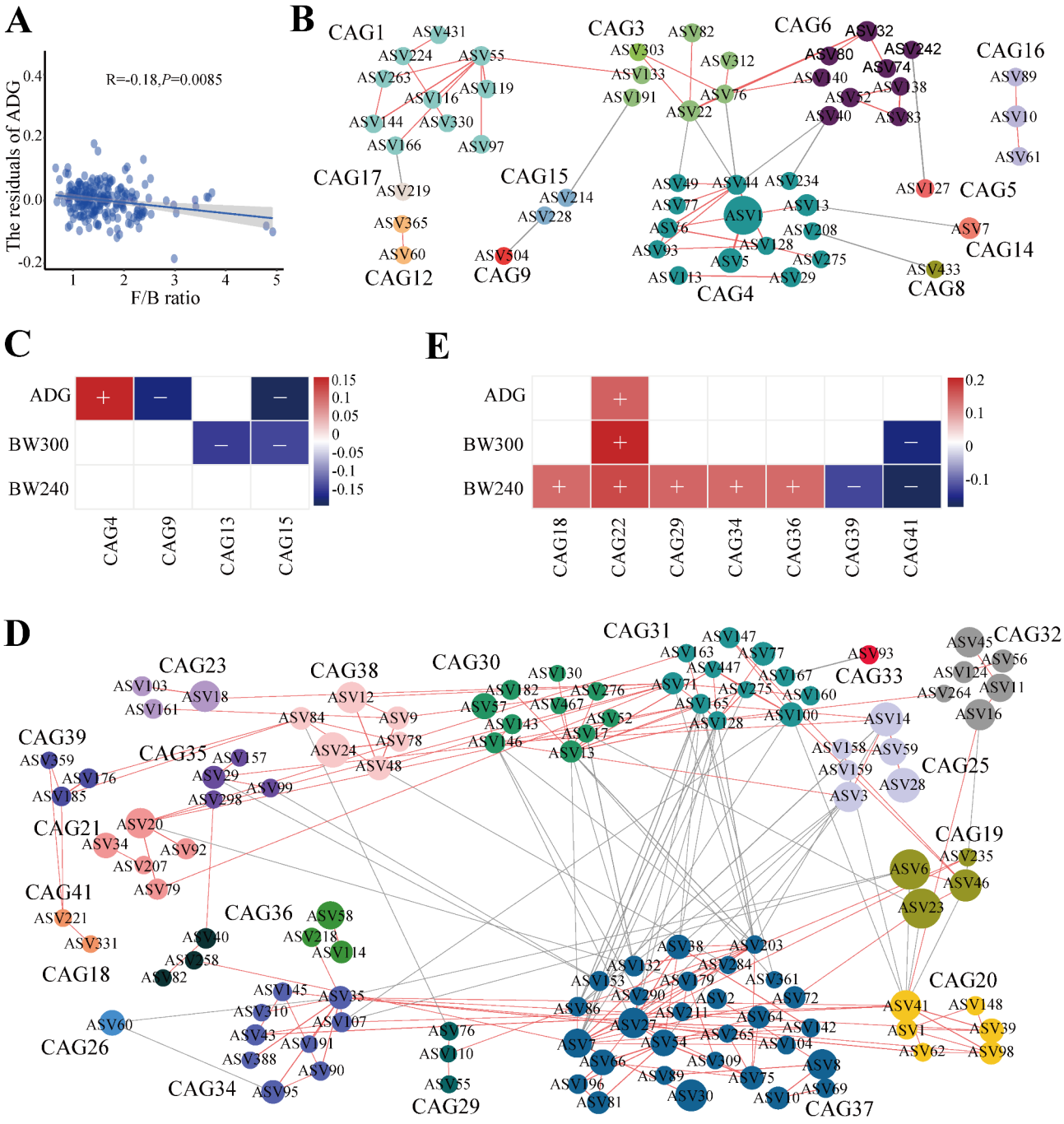

Fig. 3 Co-abundance groups (CAGs) of the gut microbiota based on ASVs in fecal and cecal samples, and the correlations of the F/B ratios and CAGs with growth traits. (**A**) A negative correlation between ADG values and the F/B ratios in fecal samples of Bamaxiang pigs. CAGs constructed for fecal (**B**) and cecal (**D**) samples based on ASVs. The annotations of ASVs are listed in Table S4 and Table S5. The nodes in different size indicate the mean abundances of ASVs in tested samples, and different colors of the nodes represent different CAG types. Lines were not drawn between ASV pairs with correlation coefficient less than 0.4. The thickness of lines represents the strong of the correlations. The red lines indicate positive correlations and the grey lines indicate negative correlations (**B** and **D**). The associations between growth traits and CAGs in fecal (**C**) and cecal (**E**) samples. The red "+" indicates positive correlations and blue "-" shows negative correlations at the significance threshold of $P < 0.05$

PICRUSt2. At the level one, a total of five KEGG pathways including metabolism, genetic information processing, environmental information processing, cellular processes, and organismal systems were identified in both feces and cecum contents. Among them, metabolism and genetic information processing were the main functional pathways with the average of relative abundances of 76.52% and 16.85% in fecal samples, and

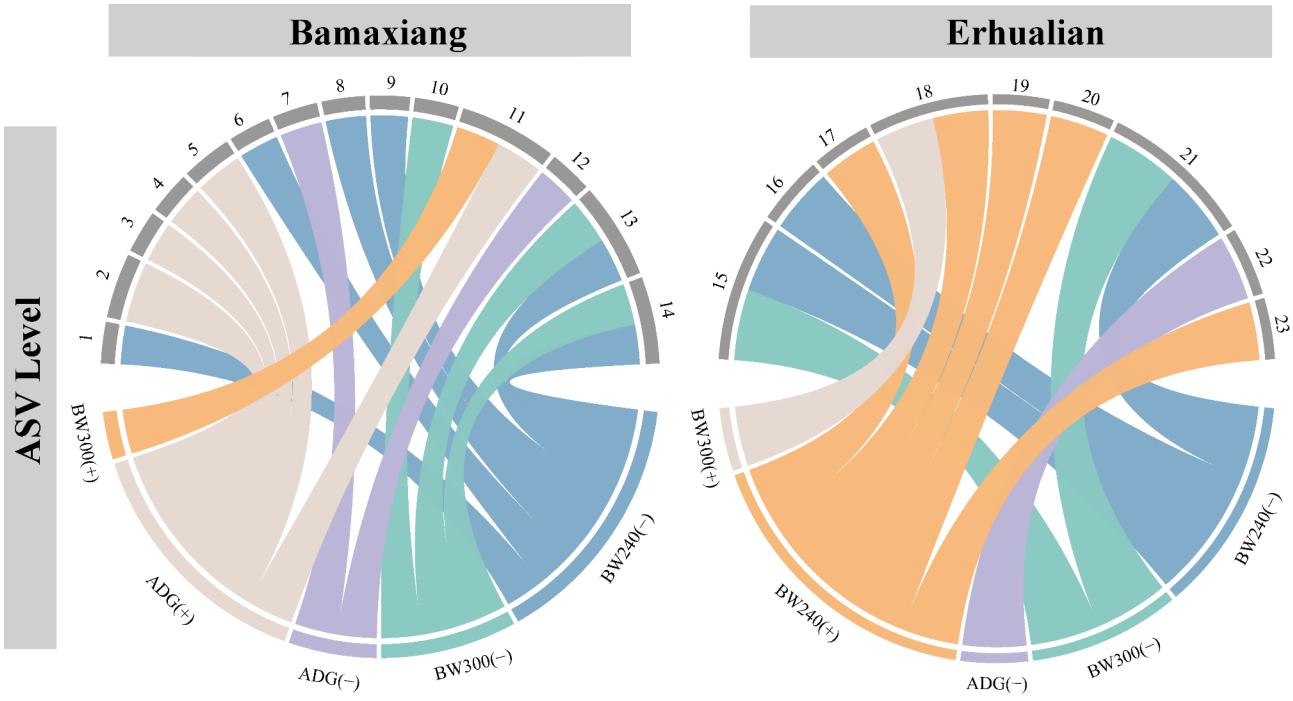

Fig. 4 ASVs significantly associated with growth traits in Bamaxiang and Erhualian pigs. Growth trait-associated ASVs identified by the two-part model. The numbers around the circles represent ASV ID which were annotated below the circles. (+) indicates significantly positive associations, and (-) represents significantly negative associations. Each line indicates a significant association between ASVs and growth traits. The same color lines indicate the associations with the same trait. The significance threshold was set at $P < 0.01$

78.56% and 16.26% in cecal samples, respectively. These five pathways contained 28 KEGG pathways at the level two (Fig. 5A).

To identify KEGG pathways related to porcine growth traits, Spearman correlation analysis was employed between the relative abundances of KEGG pathways and the phenotypic values of growth traits. In BMX pigs, digestive system, and glycan biosynthesis and metabolism had positive correlations with ADG ($r = 0.169$ and 0.163, $P = 0.016$ and 0.019). Only the pathway of excretory system showed negative correlations with both BW240 (r = -0.155, $P = 0.026$).and BW300 (r = -0.154, $P = 0.027$) (Fig. 5B). In EHL pigs, signaling molecules and interaction, and xenobiotics biodegradation and metabolism were positively associated with BW240 ($r = 0.176$, $P = 0.007$) and ADG ($r = 0.203$, $P = 0.002$), respectively (Fig. 5C). However, none of the pathways in cecal samples of EHL pigs was significantly associated with BW300.

Subsequently, Spearman correlation analysis were performed to assess the relationships between growth traits-associated microbes and KEGG pathways. Specifically, digestive system was positively associated with seven growth traits-associated ASVs ($r = 0.144$–0.314, $P < 0.05$), but negatively associated with ASV51 (*Oscillospiraceae_NK4A214_group*) (r = -0.342, $P = 5.4 \times 10^{-7}$). Excretory system was positively associated with three growth traits-associated ASVs ($r = 0.151$–0.564, $P < 0.05$), but negatively associated with ASV180 (*Treponema succinifaciens*) (r = -0.173, $P = 0.013$). Glycan biosynthesis and metabolism were positively associated with six growth traits-associated ASVs ($r = 0.170$–0.313, $P < 0.05$), but negatively associated with ASV51 (*Oscillospiraceae_NK4A214_group*) (r = -0.383, $P = 1.54 \times 10^{-8}$). Signaling molecules and interaction were positively associated with five growth traits-associated ASVs ($r = 0.238$–0.949, $P < 0.05$), but negatively associated with ASV176 (*Prevotella*) (r = -0.290, $P = 5.4 \times 10^{-6}$) (Fig. S2).

Phenotypic variance of Porcine growth traits explained by the gut Microbiome

To evaluate the proportion of phenotypic variance of growth traits that could be explained by the gut

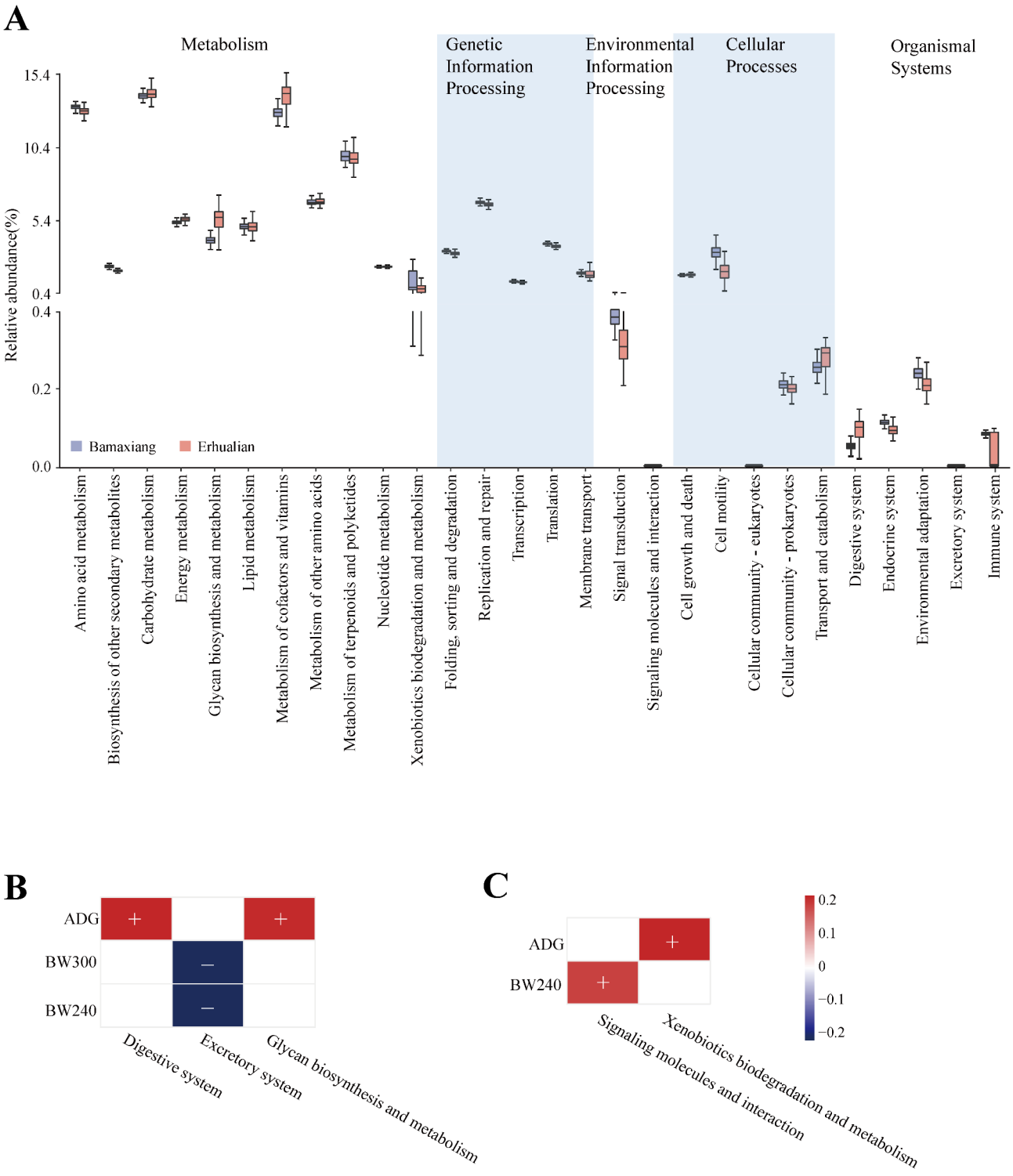

Fig. 5 Potential functional capacities of the gut microbiota predicted based on 16 S rRNA gene sequencing data and its association with pig growth traits. (**A**) KEGG pathways predicted by PICRUSt2 in fecal samples from Bamaxiang (purple) and cecal samples from Erhualian (red). Spearman correlation analysis between growth traits and KEGG pathway items in fecal (**B**) and cecal (**C**) samples. The correlations with a coefficient greater than 0.15 and $P < 0.05$ were plotted. Colored bares (red, positive; blue, negative) marked with plus and minus represent positive and negative correlations

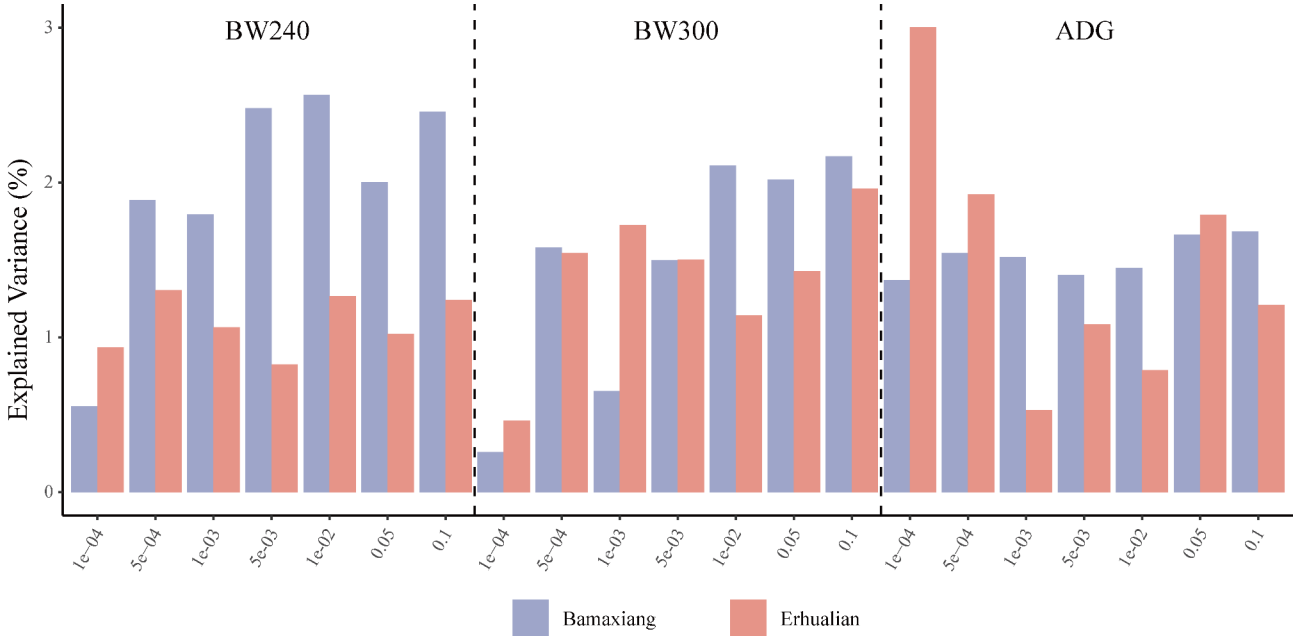

Fig. 6 The contribution of the gut microbiome to the variations of pig growth traits at different significance levels in fecal samples from Bamaxiang (purple) and cecal samples from Erhualian (red)

microbiome, the cross-validation analysis was conducted in 100 times at seven different significance levels (1×10^{-4}, 5×10^{-4}, 1×10^{-3}, 5×10^{-3}, 1×10^{-2}, 0.05, and 0.1). In BMX pigs, we found that growth traits-associated ASVs identified at $P=1\times10^{-4}$ could explain 0.55% phenotypic variation of BW240. With regard to BW300 and ADG, the gut microbiome could explain 0.26% and 1.37% of phenotype variance. At the significance levels of $P=0.1$, the gut microbiome explained 2.46% of the phenotypic variation for BW240, 2.17% for BW300, 1.68% for ADG (Fig. 6). In EHL pigs, the gut microbiome could explain 0.94% phenotypic variations of BW240, 0.46% of BW300, and 3% of ADG at $P=1\times10^{-4}$. The explained variances increased to 1.24% for BW240, 1.96% for BW300, and 1.21% for ADG when the significance threshold was set at $P=0.1$ (Fig. 6).

Discussion

Gut microbiota influences host phenotypes by regulating host metabolism, immunity, and health. In our previous researches, we examined the relationships between gut microbiota and economically important traits in pigs, such as feed efficiency, fatness, and intramuscular fat [14, 17, 30]. Gut microbes might also influence pig growth performance [12]. However, few studies have reported the correlation between gut microbiome and growth traits in Chinese indigenous pig breeds. In this study, we first explored the compositions and potential function capacities of pig fecal and cecum microbiota by 16 S rRNA gene sequencing analysis. And then, we evaluated the contribution of the gut microbiota to pig growth traits.

The utilization of microbial samples from different gut locations in both pig populations, and evaluating the association of gut microbiota in each gut location with growth traits are the optimal experimental design for this study. However, it was sometimes difficult to take into account the sampling from multiple gut locations during slaughtering procedures. All experimental pigs in both pig populations were raised in the same farm and fed with the same formula diets. However, significantly different gut microbial compositions were observed between two experimental pig populations. This was likely due to the reasons of different gut locations (feces vs. cecum contents) and different genetic backgrounds of two pig populations [46]. Some studies have focused on the core gut microbiota in swine. In this study, a total of 39 and 22 of core bacterial ASVs (prevalence ≥ 90%) were identified in fecal samples of BMX pigs and cecum content samples of EHL pigs, respectively, including *Phascolarctobacterium*, *Prevotella*, *Bacteroides*, *Ruminococcus*, *Alloprevotella*, and *Treponema* that were observed in both feces and cecum contents. Remarkably, *Phascolarctobacterium*, *Prevotella*, *Bacteroides*, *Ruminococcus*, and *Treponema* have been reported as core microbial genera in the pig gut in previous studies [47–49], suggesting their essential roles in pig gut microbial ecosystem. There were some different core gut microbial genera between this study and previous reports. This is probably due to the differences in pig breeds, ages, and samples types.

Recent studies have reported the links between the gut microbiota and growth traits (especially BW and ADG) in pigs [4, 5, 11]. Positive correlation between ADG and lean meat percentage (LMP) has been reported in pigs [50]. Previous studies have indicated that an increased ratio of Firmicutes to Bacteroidota promotes fat deposition [51] and increases the capacity to obtain energy from the diets in mice [52]. In this study, the F/B ratio was negatively correlated with ADG in the BMX population, suggesting that the high F/B ratio might decrease lean meat mass that caused the decreased ADG.

ASVs positively associated with body weight and ADG were mostly annotated to *[Eubacterium]_coprostanoligenes_group*, *Bacteroides*, and Muribaculaceae. *Bacteroides* and Muribaculaceae exhibit the capacity to produce butyrate by degrading polysaccharide and complex carbohydrates [53–55]. Butyrate serves as the major energy source for gut bacteria and promotes intestinal development for improving animal growth performance [56]. Some studies reported that higher abundance of *[Eubacterium]_coprostanoligenes_group* was associated with lower plasma cholesterol and diarrhea incidence [57, 58], and contributed to intestinal barrier functions and host health [59]. On the contrary, ASVs annotated to Peptococcaceae, *Prevotella*, and *[Eubacterium]_ruminantium_group* showed negative associations with body weight and ADG. Many studies have shown that the abundance of *Prevotella* in the gut is positively related to porcine fat deposition [17, 30]. Our previous study in commercial Duroc pigs indicated that *Prevotella copri* activated host chronic inflammatory responses and attenuated the genes associated with lipolysis and muscle growth [16]. Additionally, *Prevotella* had lower relative abundance in feces of heavier pigs [11]. All these reports were consistent with our results. Furthermore, *[Eubacterium]_ruminantium_group* and Peptococcaceae are harmful bacteria [60, 61], which are detrimental to pig growth performance. Notably, we found two positive and one negative associations between *Rikenellaceae_RC9_gut_group* members and growth traits in pigs. This should be explained that different species (strains) of *Rikenellaceae_RC9_gut_group* might have different functional capacities and play distinct roles in host growth. *Treponema succinifaciens* was positively associated with ADG. Although the majority species of *Treponema* are pathogenic bacteria [62], *Treponema succinifaciens*, a harmless commensal from the gut of healthy pigs, could produce succinate [63]. Succinate had negative correlations with subcutaneous fat and abdominal fat thickness in broilers [64]. Interestingly, *Lactobacillus delbrueckii*, a beneficial bacterial species, showed a negative correlation with BW. This result was consistent with the previous findings in which the abundance of *Lactobacillus* was negatively associated with ADG in nursery pigs [65]. A recent study found that the administration of the strain of *Lactobacillus delbrueckii subsp. bulgaricus* reduces body weight gain in obese mice [66]. Overall, all results from the association study suggested that bacterial species should affect pig growth performance through regulating host health and energy metabolism.

Functional prediction analysis of gut microbiome revealed positive associations between growth traits (BW and ADG) and several KEGG pathways including digestive system, glycan biosynthesis and metabolism, signaling molecules and interaction, and xenobiotics biodegradation and metabolism. These associations might reflect enhanced nutrient processing capabilities mediated by growth-related bacteria, such as *Bacteroides* and *Prevotellaceae_NK3B31_group* which can degrade dietary polysaccharides [67]. ASV55 (*Oscillospiraceae_UCG-002*) and ASV255 (*[Eubacterium]_coprostanoligenes_group*) were positively associated with both body weight and the KEGG pathway of signaling molecules and interaction. This may be related to the establishment of communication between microbes and hosts. We speculated that the ASV55 and ASV255 might change the level of signaling molecules and interaction, and subsequently increased body weight in pigs. Previous study also revealed that signaling molecules and interaction were more enriched in the cecal microbiota of faster growth pigs [68]. Furthermore, genes related to xenobiotics biodegradation and metabolism were associated with ADG in pigs. This result was also consistent with the findings from other studies which showed that genes involved in xenobiotics biodegradation and metabolism were more abundant in fecal microbiota of heavier pigs [11, 18]. Excretory system of gut microbiome was negatively associated with body weight. As previous reports, the pathogen invasion to host cells have an impact on the host health [69, 70]. Some pathogenic Gram-negative bacteria can excrete protein toxins into the host via the excretory system to disrupt host physiological functions, such as intestinal epithelial barrier [71–73]. An earlier study compared the components of the excretory system of Gram-negative and Gram-positive bacteria, and found that toxicity was associated with bacterial structural components [74]. It should be speculated that protein toxins excreted by some bacteria through excretory system might impair host health, and then affected pig growth. However, further experiments would need to confirm these mechanisms of gut microbiota affecting pig growth.

We showed that the gut microbiome could explain 2.17-3.00% of phenotypic variance of growth traits. The effect size was similar to that previously reported in most quantitative trait loci (QTL) of porcine growth traits [75]. This result suggested that the gut microbiota should be considered in improving growth performances of pigs.

Conclusions

In summary, we characterized the compositions of pig gut microbiota in different breeds and gut locations, and identified a number of ASVs that were significantly associated with porcine growth traits. The growth traits-associated ASVs belonged to the bacteria that were mainly involved in fermenting polysaccharide and complex carbohydrates to produce butyrate. Potential functional capacities (pathways) related to the metabolisms of glycan and xenobiotics was significantly associated with porcine growth traits. We established that the gut microbiome could be an important contributing factor to porcine growth. Based on these results, we provided new insights into the roles of gut microbes in influencing porcine growth traits, and gave the basic knowledge for improving pig growth through regulating the gut microbiota. However, low taxonomic resolution of 16 S rRNA gene sequencing limited the identification of bacterial species associated with pig growth. Potential functional capacities related to pig growth performance were only inferred from 16 S rRNA sequencing data. Furthermore, further experiments need to be performed to isolate the growth-related bacterial species for validating its causality and elucidating the mechanism.

Abbreviations

ADG	Average daily gain
ASV	Amplicon sequence variant
BMX	Bamaxiang
BW	Body weight
CAG	Co-abundance group
EHL	Erhualian
F/B	Firmicutes to Bacteroidota
FDR	False discovery rate
KEGG	Kyoto Encyclopedia of Genes and Genomes
QTL	Quantitative trait loci
SCFAs	Short chain fatty acids
SparCC	Sparse Correlations for Compositional data algorithm

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Author contributions

M.Z.: performed the experiments, analyzed the data, and wrote the manuscript; L.W. assisted in the analysis of 16 S rRNA sequencing data and revised the manuscript, X.S., M.L., and Y.W.: performed the experiments; B.Y. and H.A.: contributed materials; C.C.: conceived and designed the experiments, discussed the results and revised the manuscript; L.H.: conceived and designed the experiments, and revised the manuscript. All authors read and approved the final manuscript.

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Data availability

The 16 S rRNA gene sequencing data generated in this study were deposited in NCBI Sequence Read Archive (SRA) under accession numbers: SRR4422912, SRR4422947, SRR4422914, SRR4422951, SRR4431318, SRR4431319, SRR4431321, SRR4454082, SRR4454119 and SRR4431322.

Declarations

Ethics approval and consent to participate

All procedures involving animals were performed in accordance with the Guidelines for the Care and Use of Experimental Animals of the Ministry of Agriculture and Rural Affairs of China. Informed verbal consent was obtained from the experimental pigs' owner prior to sample collection. The experiments were also approved by the Animal Care and Use Committee of Jiangxi Agricultural University.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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