



Composition and functional diversity of fecal bacterial community of wild boar, commercial pig and domestic native pig as revealed by 16S rRNA gene sequencing

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Abstract

The bacterial community in mammalian gastrointestinal tract is abundant and complex. To date, little is known about the gut microbiota of wild boar. This study aimed to investigate the fecal bacterial diversity of wild boar and compare with commercial pig and domestic native pig. The diet composition showed that the diets of wild boar, commercial pig and domestic native pig were different from each other. More than 1,760,000 quality-filtered sequences were obtained, and the results revealed distinct compositions and diversity of fecal microbiota in three groups. PCoA and NMDS analyses showed that fecal bacterial communities of wild boar, commercial pig and domestic native pig formed distinctly different clusters. Although the three groups shared a large size of OTUs comprising a core microbiota community, a strong distinction existed at family and genus levels. *Ruminococcaceae*, *Prevotellaceae* and *Christensenellaceae* were more abundant in the feces of wild boar than in domestic native pig and commercial pig. At the genus level, the proportion of unidentified *Christensenellaceae* was remarkably higher in wild boar group, while commercial pig and domestic native pig group had a higher abundance of *Streptococcus* and *Lactobacillus*. Tax4Fun predictions of metagenome function showed statistically significant differences in the functions of fecal microbiota in three groups. There were more bacteria genes with amino acid metabolism, cell growth and death, cell motility, energy metabolism, immune system and environmental adaptation observed in wild boar feces, while commercial pig feces contained more bacteria genes with carbohydrate metabolism, drug resistance, aging, infectious diseases, lipid metabolism, endocrine and metabolic diseases. These results indicated that the fecal microbial ecosystem of the wild boar is significantly different from that of domestic native pig and commercial pig, suggesting that diet is an important factor leading to differences in bacterial abundance and diversity in feces.

Keywords Wild boar · Commercial pig · Domestic native pig · High-throughput sequencing · Bacterial diversity · Function prediction

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Introduction

Around 1000 bacterial species have been discovered in mammalian gastrointestinal tract, which influence the physiological, nutritional and immunological processes

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of animals by interaction with host and other microorganisms (Lamendella et al. 2011; Kim and Isaacson 2015). In healthy gut, certain species of microbial community always maintain a large and constant number, which contribute to reuse some nutrients and produce vitamins (Turnbaugh et al. 2006). In addition, the microbial communities could resist the invasion of foreign microorganisms and effectively prevent the colonization of pathogenic microorganisms (Sullivan et al. 2002). It is reported that 10^{10} – 10^{11} microorganisms per gram of lumen content or feces settle in swine gastrointestinal tract (Isaacson and Kim 2012). The microbiota is beneficial to the host in many ways like the production of short-chain fatty acids (SCFA) derived from indigestible dietary fiber (Cummings 1981; Ivarsson et al. 2014). The SCFAs, which are mainly produced by anaerobic bacteria fermentation, regulate the size and function of colonic tregs (Smith et al. 2013). They can provide energy for colonic epithelial cells, reduce the pH value of intestinal tract, inhibit the proliferation of pathogenic bacteria and increase the number of beneficial bacteria (Duncan et al. 2009). The insight into the composition and characteristics of swine intestinal microorganisms can improve understanding these mechanisms. Because nearly 50% of microbiota in gastrointestinal tract cannot be cultured in vitro, the culture-dependent methods cannot provide the characteristics of entire intestinal bacteria. However, next-generation sequencing technologies and bioinformatic analysis provide a more reliable solution to characterize and analyze the microbial community structure and function in animal gut comparing to culture-dependent methods (Kim and Isaacson 2015). This technology allows a direct analysis of the microbiota in feces, which is a representative of the intestinal microorganisms.

The structure of swine intestinal microbiota can be affected by different factors such as breed or diet (Yang et al. 2014; Kim and Isaacson 2015; Zhao et al. 2018). It has been reported that different swine breeds have different compositions of gut microbiota, especially between overseas breeds (lean type) and Chinese breeds (relatively obese type) (Yang et al. 2014). Meanwhile, dietary structure also has a strong influence on swine intestinal microorganisms. It is reported that 5% wheat bran supplementation in diets improved the feed conversion ratio, increased the concentration of butyrate in nursery pigs and altered the relative abundance of microbiota community (Zhao et al. 2018). Antibiotics as a feed additive have been used for many years to improve the growth of livestock animals including pigs in China. It has been suggested that one mechanism by which antibiotics promote the growth of livestock is by altering their gut microbiota. Furthermore, there were different effects on intestinal microbial population when different antibiotics as growth promoters were administered to pigs (Kim and Isaacson 2015).

Due to variations in the natural environment and differences in socioeconomic conditions, more than 100 local swine breeds are reared in China (Jiang et al. 2011). However, most of the native pig breeds are rarely used for industrial production due to their poor growing performance. Therefore, industrial swine breeds in the market, such as Yorkshire, Landrace or Duroc, are mainly imported from other countries. Meanwhile, a large number of wild boars widely distribute in many regions of China, especially in Jiangxi Province. Compared with other pig, wild boar has many advantages, e.g., high disease resistance, high lean meat rate and low-fat content, showing great economic values (Zhang et al. 2015). In addition to the intrinsic physiological differences between wild boar and other pigs, their performance can also be influenced by diets and intestinal microorganisms. Recently, due to the insoluble fibrous components, wheat bran has been evaluated for its potential prebiotic effect in young pigs. The supplementation of wheat bran in piglet diets can be a potential feeding strategy to control post-weaning diarrhea by decreasing digesta retention time and reducing proliferation of pathogens in the intestine (Kim et al. 2012; Heo et al. 2013). The intestinal microorganisms, such as *Lactobacillus*, can induce the expression of cytoprotective heat shock protein (HSP)-27 and HSP-72, which maintain the barrier function and tight junction integrity in IPEC-J2 cells (Liu et al. 2015). Compared with pig, wild boar has more diverse diets, where mast, roots, green plant matter and agricultural crops often constitute the bulk of the food ingested (Schley and Roper 2003). As microbiome communities are important participants during the food digest processes in intestinal tract, it is necessary to figure out the correlation between the microbial community structure and these diverse nutrient sources. For this reason, the insight into animal intestinal microbiota is helpful to reveal the absorption and utilization of nutrients and improve the efficiency of animal breeding. To our knowledge, the intestinal microbiota of wild boar has not been systematically characterized. Therefore, we used high-throughput sequencing of 16S rRNA genes to identify, analyze and compare the composition and structure of fecal bacterial community of wild boar, commercial pig and domestic native pig.

Materials and methods

Animals and sample collection

Twenty-four fecal samples were collected from animals in three groups, including eight wild boars (WB), eight commercial pigs (CP, Duroc × Landrace × Yorkshire) and eight domestic native pigs (NP). The wild boar fecal samples were obtained from wild boar breeding farm, while the native pig fecal samples were collected from different native pig farms

and the commercial pig fecal samples were provided from local commercial breeding farm. All animals were from Yingtan city, Jiangxi Province, China. The diet composition of the animals is shown in Table S1. All animals were female and weighed 50–60 kg. Fecal samples were individually collected after rectal stimulation. They were immediately transferred into a separate sterilized container and transported to the laboratory at 4 °C with long-term storage at –80 °C until DNA extraction. The experiment was conducted according to the animal ethics guidelines of the State Key Laboratory of Food science and Technology and approved by the Animal Ethics Committee of Nanchang University.

Total DNA isolation and PCR amplification

Total DNA was extracted with the QIAamp DNA Stool Mini Kit (Qiagen, Valencia, USA) according to the manufacturer's instructions. The quantity and quality of the DNA were measured with a NanoDrop 2000c spectrophotometer (Thermo Scientific, USA). The concentration of DNA was determined by 1% agarose gel electrophoresis (DNA concentration ≥ 20 ng/ul). The V3–V4 hypervariable region of bacterial 16S rRNA genes was amplified with specific area primers: 338F (5'-ACTCCTACGGGAGGCAGCA-3') and reverse primer 806R (5'-GGACTACHVGGGTWTCTAAT-3') using Phusion® High-Fidelity PCR Master Mix with GC Buffer (Biolabs, New England) and high-fidelity enzyme. The PCR conditions consisted of initial denaturation at 98 °C for 1 min, followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 55 °C for 30 s and elongation at 72 °C for 60 s, and finally, the PCR system was held at 72 °C for 5 min. The PCR products were detected by electrophoresis using a 2% agarose gel. Samples with bright main strip of 400–450 bp were selected for further experiments.

Library preparation and sequencing

The library was constructed using Ion Plus Fragment Library Kit 48 rxns (ThermoFisher). The constructed library was sequenced on Ion S5TMXL platform (ThermoFisher) after being subjected to Qubit quantification and library testing. The sequencing was performed at Novogene Bioinformatics Technology Co., Ltd., Beijing, China.

Bioinformatics analyses

According to the samples' unique barcode, paired-end reads were assigned to different samples. The low-quality part of the reads were cut using Cutadapt(V1.9.1, <http://cutadapt.readthedocs.io/en/stable/>), and then, each sample probes were split from the high-quality reads. Then, the paired-end reads were merged into single, longer sequences using FLASH (version 1.2.7). The raw reads were obtained after

initial trimming by cutting off the barcode and primer sequence, then, quality filtering on the raw tags was performed under specific filtering conditions to obtain high-quality clean tags, and the reads were compared with the species annotation database (SILVA132) (<http://www.arb-silva.de/>) according to QIIME (version 1.7.0) quality controlling process (Caporaso et al. 2010) to obtain the final clean reads. Chimeric sequences were detected and removed using the UCHIME algorithm (Edgar et al. 2011). The clean reads of all samples were clustered and classified into the same OTUs (operational taxonomic units) with an identity of 97% similarity using the UPARSE software (Uparsev7.0.100). According to the algorithm principle, the sequences with the highest occurrence frequency were selected as the representative sequence of OTUs. The method Mothur and SSUrRNA database (Wang et al. 2007b) of SILVA132 (<http://www.arb-silva.de/>) were used for species annotation analysis (with a threshold value of 0.8–1) to obtain taxonomic information and the community composition of each sample at various classification levels including kingdom, phylum, class, order, family, genus, species.

Alpha diversity of observed OTUs, Chao1 index, Shannon index, Goods coverage, phylogenetic diversity and beta diversity of PCoA (principal coordinate analysis), NMDS (non-metric multi-dimensional scaling) analysis and UPGMA (unweighted pair-group method with arithmetic means) sample cluster tree were calculated by QIIME (version 1.9.1) (Caporaso et al. 2010). The linear discriminant analysis (LDA) effect size (LEfSe) algorithm was used to analyze the different taxa found in different groups (Tian et al. 2017). LDA values > 4 were considered to indicate statistical significance. Tax4Fun (Qin et al. 2012) was used to predict the functional gene content of microorganisms.

Results

Analysis of basic sequencing information of all samples

To characterize the fecal bacterial composition of wild boar, commercial pig and domestic native pig, 16S rRNA sequencing was conducted on all samples. The rarefaction curves and rank abundance of all samples are illustrated in Fig. S1. The flat trends span and smoothness of the curves for each sample indicated that the sampling coverage was sufficient. A total of 1,764,381 high-quality sequences were acquired from all twenty-four samples, and all sequences were assigned to 18,727 OTUs with $\geq 97\%$ species similarity. In these samples, WB1 had the maximum number of sequences (84,477), while NP1 had the minimum number of sequences (62,045). Based on the results of OTUs analysis obtained by clustering, Venn diagram was used to analyze

the shared and unique OTUs in three groups. The Venn diagram (Fig. 1a) showed that a total of 1101, 1126 and 1125 OTUs were observed in CP, NP and WB groups, respectively. CP and NP groups shared 969 OTUs. CP and WB groups shared 986 OTUs. NP and WB groups shared 1004 OTUs. About 909 OTUs were shared by three groups, and the unique numbers corresponding to CP, NP and WB were 55, 62 and 44, respectively.

Taxonomic profiles in three groups

Fifteen phyla, 25 classes, 42 orders, 61 families and 130 genera were observed in final results. Figure 2a, b shows the abundance of bacteria taxa in all samples at phylum level. *Firmicutes* were the most abundant phylum, with an average

abundance of 79.45%, 73.26%, 71.14% in CP, NP and WB groups, respectively. The *Bacteroidetes* was the second most dominant taxon followed by *Firmicutes*, accounting for 15.27% (CP), 21.89% (NP) and 23.41% (WB), respectively. Furthermore, the total abundance of *Firmicutes*, *Bacteroidetes* and *Spirochaetes* accounted for 96.71% (CP), 96.20% (NP) and 96.88% (WB), respectively. The rest of the phyla found were *Proteobacteria*, *Euryarchaeota*, *Tenericutes*, *Melainabacteria*, *Actinobacteria*, *Fibrobacteres*, *Cyanobacteria*, *Verrucomicrobia*, *Kiritimatiellaeota*, *Fusobacteria*, *Elusimicrobia*, *Acidobacteria*. The total of unidentified bacteria and ‘others’ accounted for 0.54% (CP), 0.98% (NP) and 0.38% (WB), respectively. The relative abundance of *Firmicutes* and *Bacteroidetes* in CP group was significantly different from those in WB group. The proportion

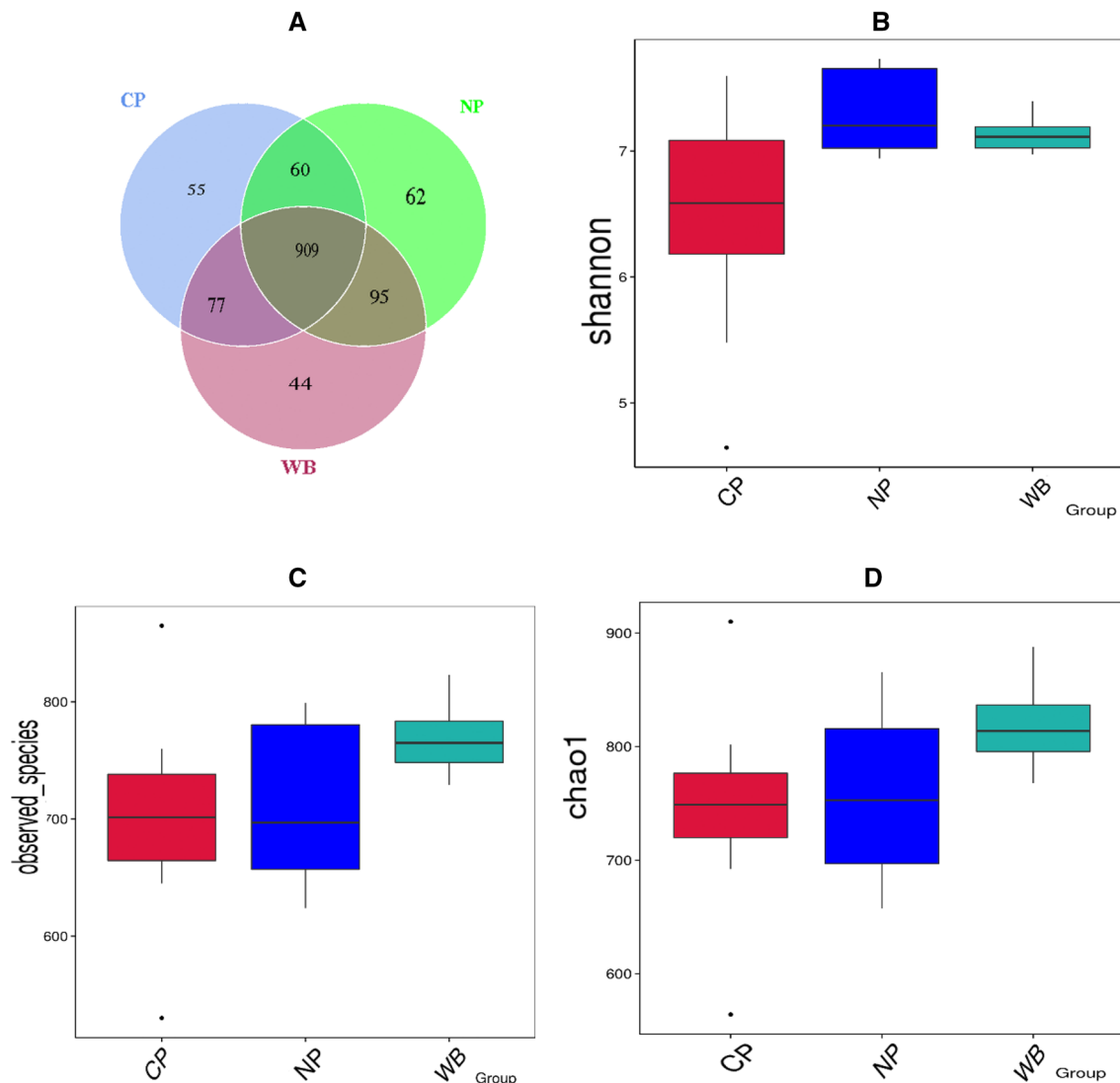


Fig. 1 OTUs analysis and alpha diversity index analysis among CP, WB and NP groups. **a** Venn diagram based on OUT. Box plot of Shannon index (**b**), observed species index (**c**) and Chao1 (1) index (**d**) intergroup differences between CP, WB and NP groups

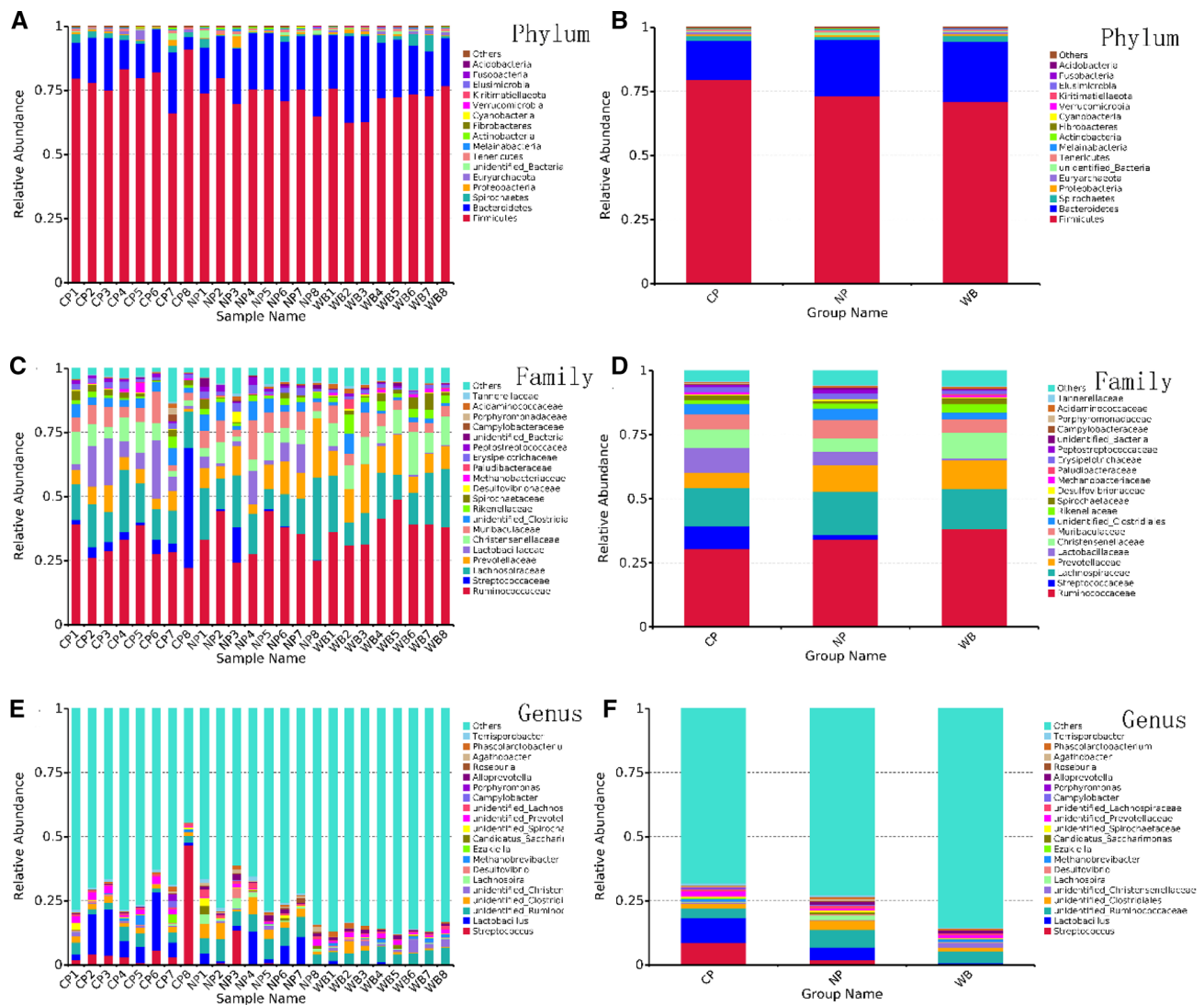


Fig. 2 Relative abundance of bacteria community in CP, NP and WB groups at phylum (a, b), family (c, d) and genus (e, f) levels. CP commercial pig, NP domestic native pig, WB wild boar

of *Euryarchaeota* showed differences among CP, NP and WB group, while NP and WB groups had statistically significantly different relative abundance of *Actinobacteria* (Fig. S2A). Meanwhile, the phylogenetic classification of different samples showed differences in CP, NP and WB groups in terms of 20 main bacterial families (Fig. 2c, d). *Ruminococcaceae* (38.21%), *Lachnospiraceae* (15.76%), *Prevotellaceae* (11.30%) and *Christensenellaceae* (10.1%) were the most abundant families in WB group, while CP group was enriched with *Ruminococcaceae* (30.65%), *Lachnospiraceae* (15.03%), *Streptococcaceae* (8.66%) and *Lactobacillaceae* (9.78%). Meanwhile, *Ruminococcaceae* (34.15%), *Lachnospiraceae* (16.85%), *Prevotellaceae* (10.38%) and *Muribaculaceae* (7.17%) were the most abundant taxa in NP group. The relative richness of *Ruminococcaceae* and *Prevotellaceae* was higher in WB than in CP and

NP, while that of *Streptococcaceae* and *Lactobacillaceae* was higher in CP than in NP and WB.

At the genus level, the relative abundance of *Streptococcus* in CP, NP and WB groups was 8.66%, 1.94% and 0.036%, while *Lactobacillus* accounted for 9.79%, 5.03% and 0.62%, respectively (Figs. 2c, 3a). Compared with WB group, both CP and NP groups had a significantly greater abundance of *Streptococcus* and *Lactobacillus*. Other genera, such as unidentified *Ruminococcaceae* (6.78%), unidentified *Clostridiales* (3.63%) and *Lachnospira* (1.6%), were more abundant in NP group. However, the relative abundance of unidentified *Christensenellaceae* (4.85%) was remarkably higher in WB group. Eight of top ten genus showed significant differences among the three groups by Wilcoxon test ($P < 0.05$) (Fig. S2), including 4 genera in comparison between CP and WB groups, 6 genera between

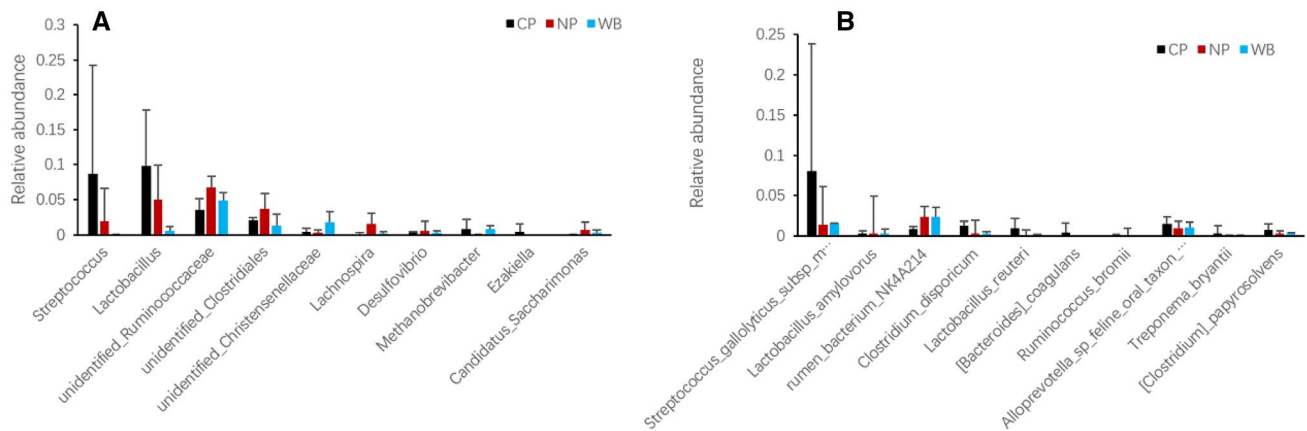


Fig. 3 Comparison of bacterial relative abundance in CP, NP and WB groups at genus level (a) and species level (b). CP commercial pig, NP domestic native pig, WB wild boar

NP and WB groups and 4 genera between CP and NP groups, respectively.

Bacterial diversity of WB, CP and NP

The bacterial alpha diversity indices (observed species, Shannon, Chao1, OTUs, ACE and Simpson) of all samples are shown in Table 1. The alpha diversity indices showed differences among the three groups (Fig. 1). Observed species (768 ± 33) and OTUs (828 ± 32) in WB group were higher than in CP and NP groups, but no significant difference was found among three groups (Fig. 1c). By Wilcoxon test, the Shannon diversity index ($P < 0.05$) differed significantly between CP and NP groups (Fig. 1b), while the Chao1 index had significant difference ($P < 0.05$) between CP and WB groups (Fig. 1d), but both Shannon diversity index and Chao1 index had no significant difference between NP and WB groups. According to PCoA analysis, a significant difference among three groups was observed. Meanwhile, PCoA1 and PCoA2 could explain 21.35% and 15.42% of total variance, respectively. The NMDS analysis (Fig. 4b) also showed a significant difference among three groups. The MRPP analysis (P value < 0.01) results (Table S2) further indicated that the bacterial community composition and structure of three groups were significantly different. However, the samples of CP and WB group had smaller intra-group differences than those of NP group.

Bacterial cluster analysis

UPGMA hierarchical cluster analysis was used to analyze the similarity of bacterial communities in twenty-four fecal samples of three groups (Fig. 5). According to the results, all samples could be divided into two distinct groups. The first group was composed of NP8, WB2 and WB3. The second

group contained the remaining samples. In addition, the second group could be divided into four distinct clusters. In the second group, the first cluster contained six samples (CP1, CP2, CP3, CP4, CP5 and CP6), two samples (CP7 and NP3) constitute the second cluster, while the third cluster was composed of six samples (NP1, NP2, NP4, NP5, NP6, NP7, WB1, WB4, WB5, WB6, WB7 and WB8) (Fig. 5a). As it can be observed in Figs. 4 and 5, animal CP8 seems to be an outlier, which is in accordance with the reduced Shannon diversity (Table 1), indicating a possible subclinical disease. As shown in Fig. 5b, the three groups were divided into two clusters, CP group was a separate cluster, while another cluster contains WB and NP groups. The UPGMA results were consistent with the PCoA analysis and alpha diversity analysis in this study and showed that WB and NP groups were more similar.

Linear discriminate analysis (LDA) effect size (LEfSe) analysis

The LEfSe algorithm (LDA log score threshold ≥ 4) analysis was used for high-dimensional biomarker discovery among CP, NP and WB groups. As shown in Fig. 6a, the results revealed that WB group had significantly higher ($P < 0.05$) relative abundances of five taxa (*Bacteroidetes* phylum, *Bacteroidia* class, *Bacteroidales* order, *Prevotellaceae* and *Christensenellaceae* family), while CP group contained eight taxa (*Firmicutes* phylum, *Lactobacillales* order, *Bacilli* class, *Streptococcaceae* family, *Lactobacillaceae* family, *Lactobacillus* genus, *Streptococcus* genus and *Streptococcus galloyticus subsp macedonicus* specie) with significantly higher relative abundances. At the phylum level, *Bacteroidetes* was the significantly different biomarker in WB group compared to CP and NP groups, while *Firmicutes* was the different biomarker in CP group compared to NP and WB groups.

Table 1 Alpha diversity of twenty-four fecal samples, including eight wild boar fecal samples, eight commercial pig fecal samples and eight domestic native pig fecal samples

Sample	Valid sequences	OTUs	Observed species	Shannon index	Simpson index	Chao1 index	ACE	Goods coverage
CP1	64,038	818	760	7.19	0.982	801.71	799.46	0.998
CP2	68,156	738	671	6.41	0.959	729.04	725.87	0.998
CP3	67,582	746	691	6.56	0.957	733.13	736.79	0.998
CP4	82,074	784	731	6.61	0.953	764.52	779.92	0.998
CP5	74,727	778	712	7.05	0.979	768.23	759.15	0.998
CP6	64,627	574	530	5.48	0.935	564.08	573.08	0.998
CP7	75,657	916	865	7.60	0.987	910.00	913.22	0.998
CP8	74,885	712	645	4.64	0.771	692.26	695.95	0.998
Av (CP)	71,468 ± 6329 ^a	758 ± 97 ^a	701 ± 96 ^a	6.44 ± 0.96 ^a	0.94 ± 0.071 ^a	745.37 ± 98.03 ^a	747.93 ± 96.37 ^a	0.998
NP1	62,045	737	737	7.64	0.99	799.48	798.36	0.998
NP2	68,992	847	799	7.73	0.989	838.09	836.57	0.998
NP3	68,565	815	776	7.15	0.972	808.14	809.50	0.998
NP4	63,602	701	657	6.96	0.975	706.11	698.64	0.998
NP5	80,047	853	794	7.68	0.99	865.54	857.18	0.998
NP6	67,268	708	657	7.25	0.984	692.25	692.19	0.998
NP7	80,041	705	657	6.94	0.976	698.71	703.43	0.998
NP8	80,301	673	624	7.04	0.98	657.60	662.84	0.998
Av (NP)	71,358 ± 7366 ^a	755 ± 72 ^a	713 ± 72 ^a	7.30 ± 0.34 ^a	0.982 ± 0.007 ^a	758.24 ± 78.22 ^a	757.34 ± 75.76 ^a	0.998
WB1	84,477	861	803	7.17	0.971	847.34	859.97	0.998
WB2	77,373	790	731	7.27	0.986	770.51	776.09	0.998
WB3	69,753	817	763	7.12	0.977	804.00	808.80	0.998
WB4	74,931	826	767	7.00	0.972	818.68	818.81	0.998
WB5	80,081	843	777	7.11	0.982	833.10	832.97	0.998
WB6	80,283	881	823	7.39	0.985	887.63	901.29	0.997
WB7	80,044	811	754	7.03	0.97	808.80	814.96	0.998
WB8	74,832	793	729	6.97	0.972	768.01	770.07	0.998
Av (WB)	77,722 ± 4518 ^a	828 ± 32 ^a	768 ± 33 ^a	7.13 ± 0.14 ^a	0.977 ± 0.007 ^a	817.26 ± 39.58 ^a	822.87 ± 42.90 ^a	0.998 ± 0.0003 ^a

CP commercial pig, WB wild boar, NP domestic native pig

Av average value (mean ± SD). ^aSD standard deviation

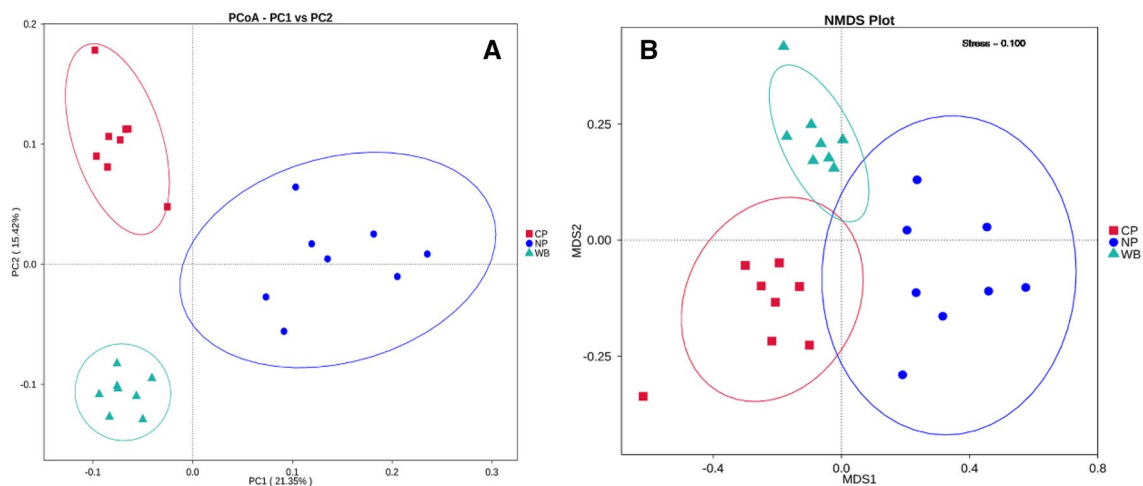


Fig. 4 PCoA and Non-metric multi-dimensional scaling (NMDS) of the data set (24 samples). Red: commercial pig group (CP); blue: domestic native pig group (NP); green: wild boar group (WB) (color figure online)

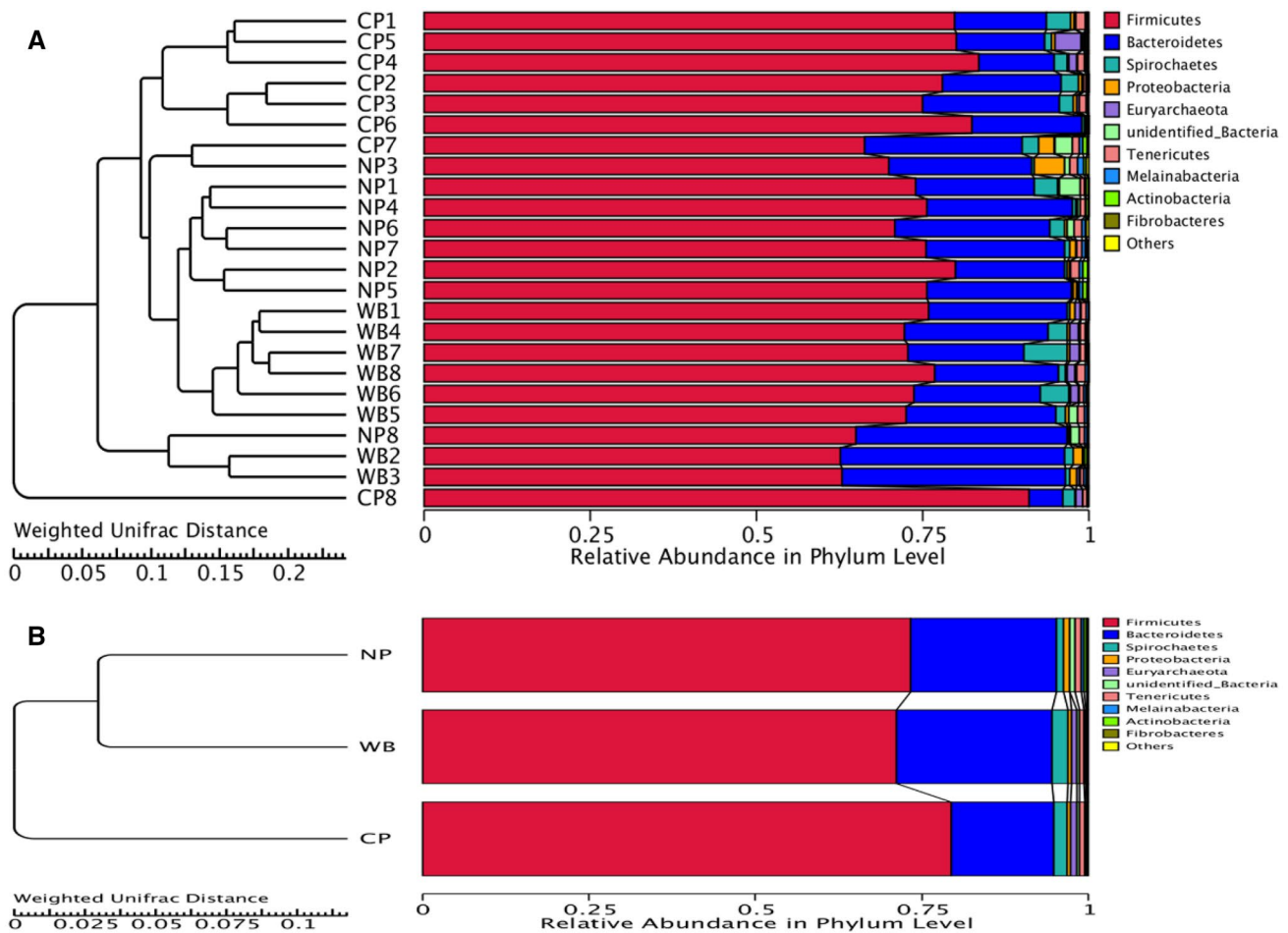


Fig. 5 UPGMA clustering tree based on weighted UniFrac distance. **a** UPGMA clustering tree among 24 samples; **b** UPGMA clustering tree among CP, WB, and NP groups. CP commercial pig, WB wild boar, NP domestic native pig

At the genus level, unidentified *Ruminococcaceae* and unidentified *Clostridiales* were the most differentially abundant taxon in NP group, while *Lactobacillus* and *Streptococcus* had higher relative abundance in CP group. The relative abundance of *Bacteroidia* (class), *Prevotellaceae* (family), *Christensenellaceae* (family) and *Bacteroidales* (order) was significantly higher in WB group, whereas CP group had greater proportions of *Lactobacillales* (order), *Bacilli* (class), *Lactobacillaceae* and *Streptococcaceae* (family) and *Streptococcus gallolyticus subspecies macedonicu* (specie). As shown in Fig. 6b, most bacteria with significant difference in WB group belonged to *Bacteroidetes*, while in NP and CP groups, all bacteria with significant difference belonged to *Firmicutes*. These key phylotypes contributed to the differences of microbiota composition in the three groups.

Predicted functions of microbiota

Tax4Fun analysis was performed to investigate microbiota functions in all samples. The predicted functions are

precalculated for genes in KEGG database (Ogata et al. 1999). Thirty-nine gene families were identified in all samples; many of them were classified into cellular processes, environmental information processing, genetic information processing, human diseases, metabolism and organismal systems (Fig. S4). According to Fig. 7, the differences of bacterial gene functions were found among the three groups. We observed that CP group had higher abundance microbial genes of carbohydrate metabolism, drug resistance, aging, infectious diseases, lipid metabolism, endocrine and metabolic diseases. The fecal microbial genes related to genetic information processing, replication and repair, metabolism of terpenoids and polyketides, folding-sorting and degradation were more abundant in NP group, while WB group had greater proportions microbial genes of amino acid metabolism, cell growth and death, transport and catabolism, cell motility, energy metabolism, immune system, environmental adaptation and endocrine system. Notably, more microbial genes of drug resistance, aging and infectious diseases were found in CP group. However,

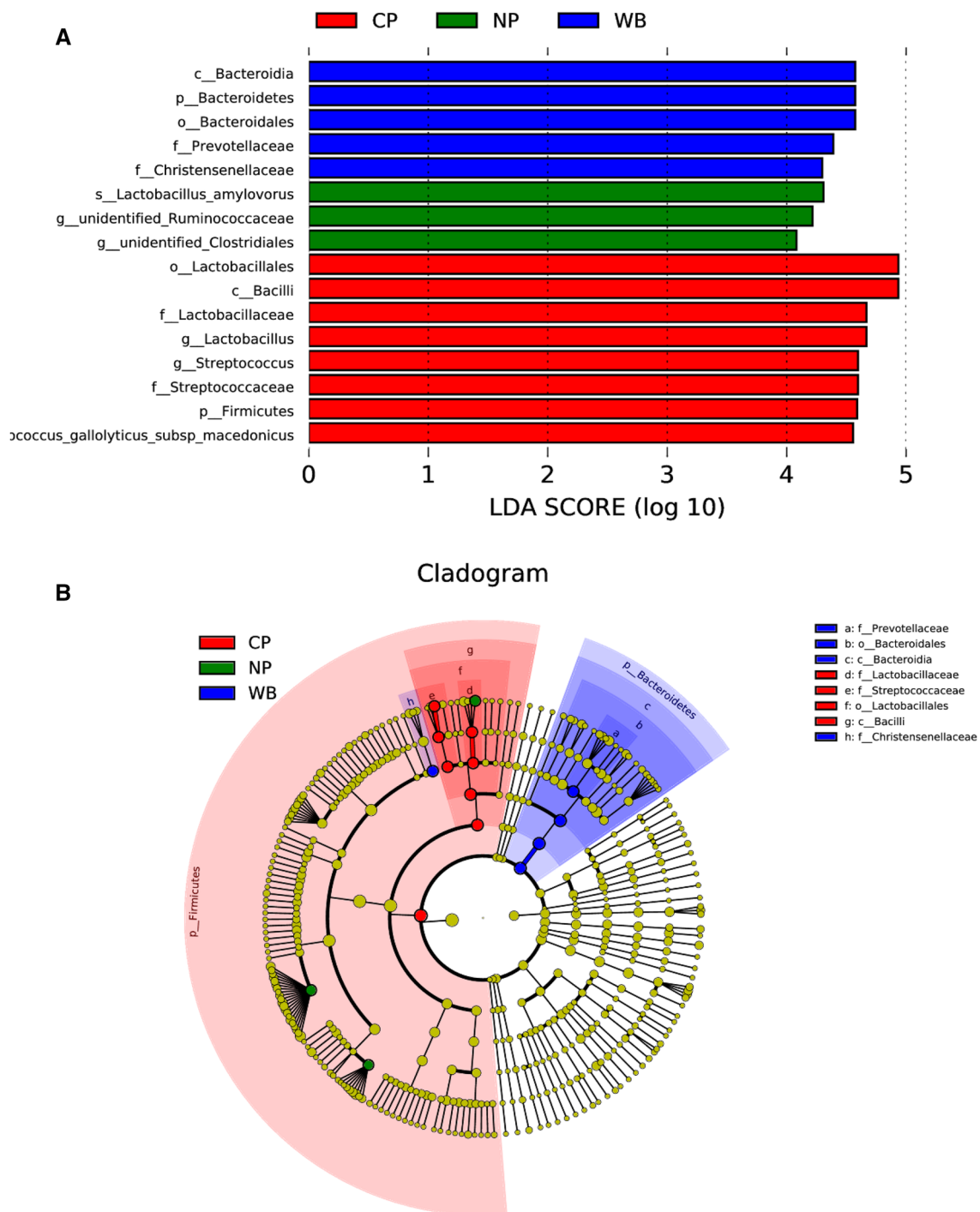


Fig. 6 LefSe analyses of CP, NP and WB groups. Red: commercial pig group (CP); green: domestic native pig (NP). Blue: wild boar group (WB). **a** Histogram of the results of LefSe among CP, NP and

WB groups and their respective effect sizes; *P* values < 0.05 considered significant. **b** Cladogram showed taxonomic representation of differences among CP, NP and WB groups (color figure online)

the relative abundance of microbial genes related to with immune system, environmental adaptation and endocrine system in WB group was greater than in CP and NP groups. Moreover, significant differences (*P* < 0.05) of

fifteen gene functions were observed between CP and WB groups, ten different gene functions were found between CP and NP groups, and only three gene functions were obtained between NP and WB groups (Fig. 8).

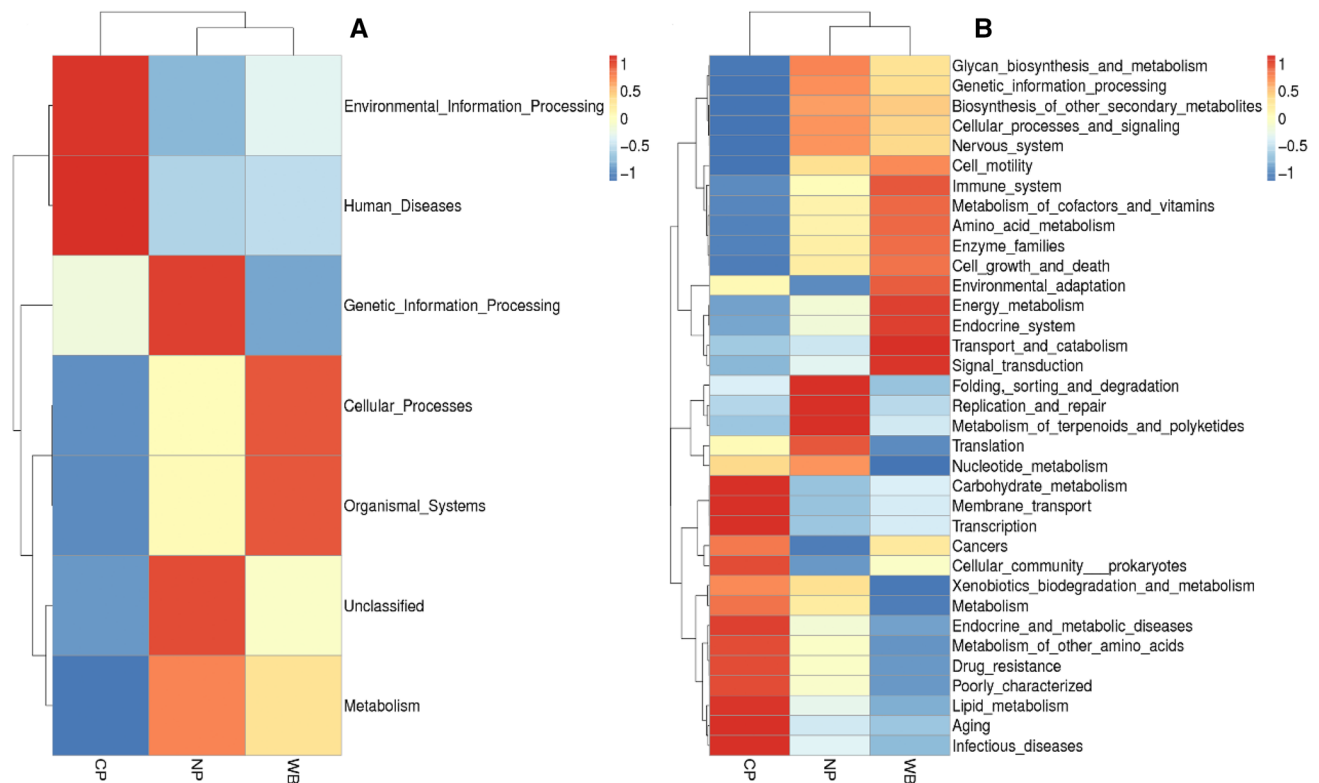


Fig. 7 The heatmap of Tax4Fun-generated functional categories comparison among CP, NP and WB groups at level1 (a) and level2 (b). CP commercial pig, NP domestic native pig, WB wild boar

Discussion

The objective of this study was to investigate the bacterial profiles in feces of wild boar, commercial pig and domestic native pig and then compare their bacterial communities using high-throughput DNA sequencing. As shown in Table 1, the bacterial communities present in the microbiota of group WB had a higher Chao1 index (817) and observed species (768) than that of groups CP and NP indicating higher richness in group WB. This may be due to their different diets. As reported by Lau et al. (2018), a more diverse gut microbiome was observed in the omnivorous cattle, suggesting that microbiota diversity increases as diet variation increases. According to their diet composition, the wild boar's diets are more complex and diverse, mainly including wheat bran, corn and grass due to their wild life habits. The complexity of diet sources might cause high diversity of microbial community in intestinal tract of pigs. The bacterial communities in swine gastrointestinal tract changed with dietary input and other factors such as breed. Högborg reported that both the total gut microflora and the coliform flora in growing pigs were influenced by the dietary non-starch polysaccharides content (Högborg et al. 2004). It also had been reported that the diversity of fecal microflora in the pigs showed an increasing trend with inclusion of whole

crop rice in diet, suggesting that diet had an important influence on intestinal bacterial community. Wang et al. reported that the number of special cellulolytic bacteria in swine rectal samples increased 71% when fed with high-fiber diet for 8 weeks (Wang et al. 2007a). A decrease in bacteria of the *Lactobacillus spp.* group and a loss of microbial diversity occur during weaning, whereas *Clostridium spp.*, *Prevotella spp.* or facultative anaerobes such as *Proteobacteriaceae*, including *E. coli*, were positively impacted (Gresse et al. 2017). The diversity of the microbiota may decrease even more with the extended use of antibiotics, which can increase opportunities for pathogenic microorganisms to colonize and trigger diseases (Fouhse et al. 2016). The composition of the gut microbiota in swine is also likely to be shaped by host genetics or breed. Pajarillo et al. (2014) found differences in the pig fecal microbial community among three 15-week-old purebred pig breeds, including Duroc, Landrace and Yorkshire. The total abundance of *Bacteroides* and *Firmicutes* was more than 90%, but at the genus level, *Catenibacterium*, *Phascolarctobacterium* and *Subdoligranulum* were more abundant in Duroc pigs, whereas *Dialister* was more abundant in Yorkshire pigs. Yang et al. (2014) reported that different swine breeds have different compositions of gut microbiota, especially between overseas breeds (lean type) and Chinese breeds (relatively obese type). In our study,

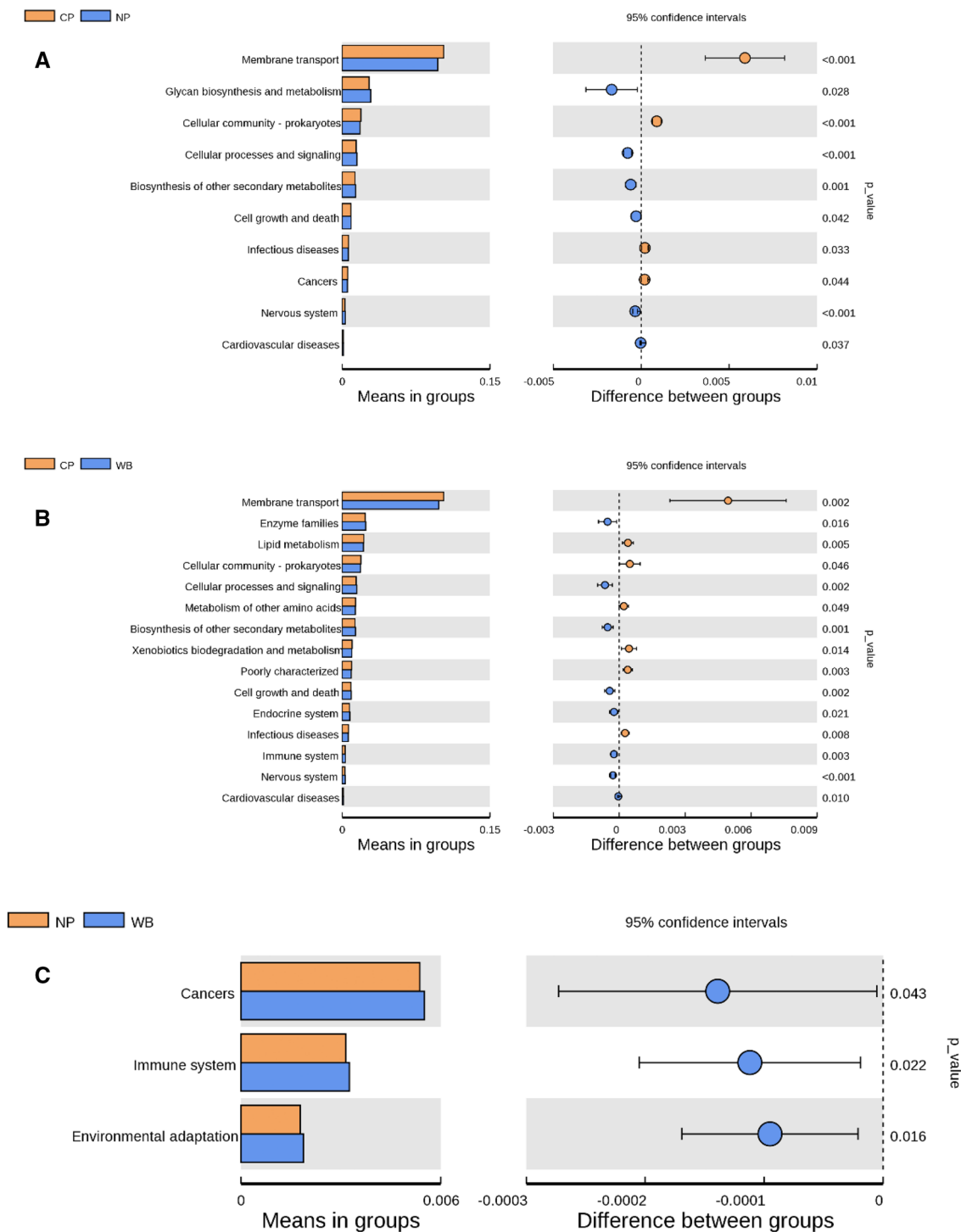


Fig. 8 Predicted microbial gene functions with significant differences among CP group, NP group and WB group by *T* test ($P < 0.05$). *CP* commercial pig, *NP* domestic native pig, *WB* wild boar. **a** Compari-

son of microbial functions between CP and NP group (NP); **b** comparison of microbial functions between CP and WB groups; **c** comparison of microbial functions between NP and WB groups

similar characteristics existed in the fecal microbial community of the three groups of animals. The total abundance of *Firmicutes* and *Bacteroidetes* was also more than 90%, but at the genus level, *Streptococcus* and *Lactobacillus* were

more abundant in CP and NP groups, whereas unidentified *Christensenellaceae* was more abundant in WB group. It was reported that microbial diversity may be a generally good indicator of a healthy gut (Sommer et al. 2017a, b).

Lower bacterial diversity has been reproducibly observed in sows and neonatal piglets associated with porcine epidemic diarrhea virus infection (Song et al. 2017) and people with inflammatory bowel disease (Manichanh et al. 2006) and celiac disease (Scher et al. 2015). The association between reduced diversity and disease indicated that the wild boar gut ecosystem with more abundant species may be more robust against environmental influences, as functionally related microbes in an intact ecosystem can compensate for the function of other missing species.

Principal coordinate analysis (PCoA) and NDMS analysis show that the bacterial communities of the three groups were different from each other. Among all 15 detected phyla, both *Firmicutes* and *Bacteroidetes* were the major phyla in the feces of three groups. This result is in agreement with the previous studies (Leser et al. 2002; Kim et al. 2012; Alain et al. 2014; Yang et al. 2014). Recent studies show that the characteristics and enterotypes of gut microbiota may be determined by a dominant presence of *Bacteroides*, *Prevotella* or *Ruminococcus* (Stearns et al. 2011). *Acidobacteria* were not detected in the feces of CP and WB, while *Fusobacteria* were not detected in CP, which may be due to their diet structure, living environment or detection accuracy, which requires us to further study. It is notable that the proportion of *Bacteroidetes* was higher in WB group. It had been reported that *Bacteroidetes* were beneficial to the normal development of gastrointestinal tract (Thomas et al. 2011). For example, they can produce butyrate, which is a final product of colonic fermentation with antineoplastic properties and maintaining intestinal health (Kim and Milner 2018).

By comparing the composition of bacterial community, we found that the structure of fecal bacteria had a certain similarity at phylum level among the three groups. However, significant differences in composition and abundance were found at the sublevels. A significant difference at the family level emerged among the three groups in this study. The proportion of *Ruminococcaceae* (38.21%), *Prevotellaceae* (11.30%) and *Christensenellaceae* (10.1%) in WB group were higher than in NP and CP groups. This may be due to their diet. As shown in Table S1, the wild boar may have a higher cellulose content and lower carbohydrates or fat content in the diet. Daniel et al. measured the change in fecal bacterial communities in mice which were fed a carbohydrate or high-fat diet for 12 weeks. The high-fat diet caused shifts in the diversity of dominant gut bacteria and caused a decrease in species of the *Ruminococcaceae* family (Daniel et al. 2014). Diet is one of the most important factors that influence the composition and diversity of the intestinal microbiota (Senghor et al. 2018). Thus, the gut microbiota can be modulated by changing in diet habits, modifying dietary components (fats, proteins and carbohydrates), introducing probiotics and prebiotics (Houghton

et al. 2016). The CP group had more *Streptococcaceae* and *Lactobacillaceae* than WB and CP group (Fig. 2c). *Ruminococcaceae* and *Prevotellaceae* in WB group may be related to the degradation of cellulose and the production of anti-inflammatory substances like short-chain fatty acids (SCFA) (Hayashi et al. 2007; Lamendella et al. 2011; Canfora et al. 2015). As the end products of dietary fiber fermentation, SCFAs are essential for maintaining the normal metabolism of colon mucosa, regulating colonocyte growth and proliferation (Rossi et al. 2010) and increase beneficial bacteria number (Duncan et al. 2009). SCFAs could also act as energy substrates for colonocytes, modulate satiety and alleviate inflammation (Koh et al. 2016). For example, the dietary supplementation of sodium butyrate (1 g/kg feed) significantly decreased diarrhea incidence of weaned piglets and enhanced the immune function by increasing the serum IgG concentration as well as the IgA⁺ cell count in jejunum, thus reducing the adverse effects of weaning stress, and maintained the integrity of intestinal mucosa (Fang et al. 2014). Therefore, it is necessary to investigate the correlation between intestinal microbes and SCFAs in wild boars' gut in future research.

The abundance of unidentified *Christensenellaceae*, *Phascolarctobacterium* and *Megasphaera* in WB group was higher than those in CP and NP groups. *Christensenellaceae* and *Phascolarctobacterium* could reduce the probability of obesity in mammal (Stenman et al. 2016; Muñiz Pedrego et al. 2018). This may be consistent with the characteristic of the high lean meat rate and low-fat content of wild boar (Zhang et al. 2015). Furthermore, *Megasphaera* bacteria can convert lactic acid into propionic acid and, thus, reduce the risk of acidosis in mammals (Henning et al. 2010). This may be quite meaningful to wild boars, because they often have higher level of physical activity than domestic pig, e.g., preying or escaping predators. *Streptococcus* and *Lactobacillus* were the most abundant genera in CP group (Figs. 2c, 3a). The relative abundance of *Lactobacillus amylovorus* (Fig. 6a) was remarkably high in NP group. This may be explained by the relatively high starch dietary structure of domestic native pig. Of note, *Streptococcus gallolyticus* (pathogenic microorganism) was mainly present in CP group. However, the presence or absence of pathogenic organism may not necessarily indicate that the occurrence of the disease unless the number proliferate enough to overwhelm the general microbial population in the GIT, or in a specific area of the GIT (Pluske et al. 2018). Gut health should be on supporting the animal to regulate shifts in the intestinal microbiome such that rapid population swings are avoided and equilibrium can be maintained. In this study, the fecal bacterial community structure can only partially reflect the condition of the intestinal microflora and cannot reflect the status of all microorganisms in the intestine. The microbial community of the gastrointestinal tract exhibits many

important physiological functions in vertebrates (Viaud et al. 2013), such as immunity, anti-inflammatory and anti-cancer activity. The microbiota controls homeostasis in gut through a variety of substances, e.g., lipopolysaccharides, flagellins, peptidoglycans and formylated peptides (Bengmark 2013).

Microbial functional analysis (Tax4Fun) showed that carbohydrate metabolism, replication and repair, membrane transport, amino acid metabolism and translation were the most abundant predicted microbial gene functions in the present report (Fig. S3). This result is consistent with previous reports about pigs (Zhao et al. 2015; Ke et al. 2019). It is notable that the predicted microbial gene functions of the three groups vary from each other. We observed that the predicted microbial gene functions relating to cell growth and death, transport and catabolism, cell motility, energy metabolism, immune system, environmental adaptation and endocrine system were more abundant in WB group, which may be corresponding to wild boar's field adaptability and disease resistance. We also found that CP group contained more predicted functions involved in infectious disease and drug resistance than other groups. This result suggested that the pathogen invasion pathways may be enriched in commercial pig gut which is consistent with the higher abundance of *Streptococcus gallolyticus* in the previous results. In addition, the commercial pig feces contained more predicted microbial gene functions about drug resistance than the other two groups, which may be due to the antibiotics supplemented in the fodder. Meanwhile, a higher abundance of predicted functions related to carbohydrate metabolism and lipid metabolism was found in CP group, which may indicate that commercial pig gut contains more bacteria with greater metabolic capacity, and these bacteria may produce more energy for the hosts; this may be related to the good production performance of the hybrid pigs. The production performance of hybrid breed can be affected by many factors such as breed or diet which affect the composition and structure of the gut bacterial community. The commercial pig of hybrid breed is a combination of characteristics of different breeds of pigs and has great advantages in production performance such as fattening or intramuscular fat (LiGang et al. 2009; Perevoyko 2015; Šprysl et al. 2018). It is reported that the effect of dietary fat saturation and dietary vitamin A level concentration on intramuscular fat content and fatty acid profile of subcutaneous backfat is variable according to pig genotype (Olivares et al. 2009). In the production of commercial pigs, dietary strategies are equally important for pork breeds, and a number of dietary strategies have also been carried out to enhance intramuscular fat content in swine production. It has been reported that strategies that aim to increase tissue fat saturation lead to a higher intramuscular fat content (D'Souza et al. 2003)

and carcass fatness (Sanz et al. 2000). Despite the remarkable similarity of microbiomes in feces and large intestine, feces cannot fully represent the microbial profiles of GITs. In further study, other modern sequencing and analytical methods, e.g., whole-genome shotgun sequencing and third-generation sequencing, meta-transcriptomics and metabolomics, can be applied to obtain deeper insight into the composition and effects of gut microbiota, as well as their correlation with the host's physiological activity.

Conclusion

The composition, structure and function prediction of wild boar's fecal bacterial community were investigated in this work. In addition, we further compared these features with commercial pigs and domestic native pigs. Fifteen bacterial phyla were found in all fecal samples, with *Firmicutes*, *Bacteroidetes* and *Spirochaetes* predominating. Wild boar had the most diverse fecal bacterial community probably due to their complex diet structure. Although the three groups shared a large size of OTUs comprising a core microbiota community, a strong distinction in composition of gut microbiota occurred at family and genus levels. Wild boar group had more proportion of *Bacteroidetes*, *Ruminococcaceae*, *Prevotellaceae*, *Christensenellaceae* and unidentified *Christensenellaceae* than domestic native pigs and commercial pigs. At the genus level, the proportion of unidentified *Christensenellaceae* was remarkably higher in wild boar group, while commercial pig and domestic native pig groups had a higher abundance of *Streptococcus* and *Lactobacillus*. Furthermore, predicted bacterial gene functions in fecal content of wild boar were significantly enriched in categories associated with amino acid metabolism, cell growth and death, cell motility, energy metabolism, immune system, environmental adaptation, while the fecal content of commercial pigs contained more bacterial gene functions related to carbohydrate metabolism, drug resistance, aging, infectious diseases, lipid metabolism, and endocrine and metabolic diseases. We observed that the gut microbial ecosystem of the wild boar was different from the ecosystem of the commercial pig and the domestic native pig. It suggested that the diet may be one of the factors that lead to a more diverse and abundant microbiome to maintain animal survival and improve food effectiveness.

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