

Farming Mode Drives Gut Microbiota Structure in Mandarin Fish: Diet Drives Functional Adaptation While Environment Drives Community Simplification

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Abstract

This study investigated the effects of farming modes (pond vs. industrial recirculating aquaculture system, RAS) and feed types (live bait vs. artificial feed) on the gut microbiota of mandarin fish (*Siniperca chuatsi*). Three experimental groups were set: pond farming with live bait (CE group), pond farming with artificial feed (CS group), and industrial RAS farming with artificial feed (GS group). All the groups were dominated by Fusobacteriota, Bacteroidota, Proteobacteria, and Firmicutes, but their abundances varied markedly. GS formed a simplified community dominated by *Cetobacterium* (64.94%) genus, unlike the balanced compositions of CE and CS. The CS exhibited the highest species richness (Chao1, ACE) and diversity (Shannon, Simpson), while the GS had the lowest diversity. Beta diversity analysis showed no significant difference between the CE and CS groups ($p = 0.36$), but a highly significant difference between the GS and CS groups ($p = 0.004$). LEfSe analysis identified 43 differential taxonomic units (from phylum to genus) between the GS and CS groups, far more than the 22 differential genera between the CE and CS groups. PICRUSt2 functional prediction indicated that artificial feed drove functional adaptation of gut microbiota (e.g., enriched nutrient transport and digestion pathways in the CS group), while the industrial environment induced simplification of microbial functions (e.g., focused genetic information processing pathways in the GS group). Collectively, this study demonstrates that farming environment plays a dominant role in shaping the gut microbiota of mandarin fish, leading to community simplification, while diet mainly regulates microbial functional adaptation.

Introduction

Siniperca chuatsi (*S.chuatsi*) is an important and valuable freshwater economic fish in China, with high market value due to its tender meat and delicious taste (Jianbo et al. 2024). With the continuous improvement of residents' living standards, the demand for high-value fish in the market continues to grow, and the consumption potential of *S.chuatsi* is becoming increasingly prominent. According to statistics, as of 2023, China's production of *S.chuatsi* has reached 477600 tons, an increase of 18.95% compared to 2022 (Liu Min et al. 2025). However, the traditional model of *S.chuatsi* farming is no longer suitable for current market demand. This model mainly relies on pond farming and feeding live fish feed (such as mud carp), but it has exposed many problems: On the one hand, due to the shortage of land resources, the area of pond aquaculture has gradually decreased, and the rental and operating costs of aquaculture have increased. Moreover, pond aquaculture has a low density and limited yield per unit area, which cannot meet the growing market demand. On the other hand, traditional *S.chuatsi* farming usually feeds live fish as bait. With the increasing demand, the supply of bait fish is unstable and the storage and transportation costs are high. Moreover, live bait fish are highly susceptible to carrying pathogens, greatly increasing the risk of disease transmission during the breeding process (Che et al. 2025). In addition, the environment of ponds is also quite complex, greatly affected by seasonal and weather changes, making it difficult to control during aquaculture. These factors collectively constrain the sustainable development of the *S.chuatsi* industry.

In order to break through the bottleneck of traditional aquaculture models, the *S.chuatsi* industry is actively transforming towards intensive recirculating aquaculture systems (RAS) and the application of compound feed. RAS can effectively improve production controllability and resource utilization efficiency, with sustainable environment, stable water quality, and controllable product quality (Jia et al., 2025). In fact, multiple aquatic economic species have achieved RAS, such as common carp (*Cyprinus carpio*) (Ma et al. 2023), shrimp (*Penaeus vannamei*) (Nugraha et al. 2023), and rainbow trout (*Oncorhynchus mykiss*) (Irani and Agh, 2020), *Micropterus salmoides* (Liu et al., 2025a) as well as *Salmo salar* (Schmidt et al. 2016), which have also verified the feasibility of this model. In addition, artificial feed can eliminate the dependence of fish on live bait. Studies have shown that the type of fish feed can affect their growth performance (such as weight gain rate, specific growth rate) and muscle nutritional composition (such as protein content, fatty acid composition) (Wei et al. 2023). For mandarin fish, artificial feeding can bring better feed conversion rate, physical indicators, fatty acid nutritional value, and meat texture, indicating that artificial feed can provide high-quality *S.chuatsi* through balanced nutritional supply (Niu et al., 2023; Zhang et al., 2024).

It is worth noting that the transformation of aquaculture models and feed types is essentially a systematic change in the living environment and nutrient intake structure of fish, which will inevitably affect the composition and function of their gut microbiota. At present, the differences in gut microbiota structure and health of *S.chuatsi* under factory farming environment and artificial compound feed feeding are not yet clear. During the long-term evolution of fish, the gut microbiota has formed a close symbiotic relationship with the fish body, participating in key physiological processes such as host food digestion, nutrient absorption, energy metabolism, growth and development, and immune regulation through various pathways (Nicholson et al. 2012; Flint et al. 2015). The gut microbiome can secrete abundant digestive enzymes (such as amylase, protease, cellulase, alkaline phosphatase, etc.), which degrade some large molecular substances into metabolites that are easily absorbed by the host, thereby significantly improving utilization efficiency (Mirghaed et al. 2018; Ringo et al. 2020). Therefore, in the process of shifting from traditional pond live bait farming to factory based feed farming for *S.chuatsi*, a series of questions have emerged: how will this transformation reshape the gut microbiota of mandarin fish? What adaptive changes will occur in its microbial community structure, function, and interaction with the host? In depth exploration of the response mechanism of gut microbiota in feed fed mandarin fish and industrial aquaculture models is of great theoretical and practical significance for optimizing aquaculture strategies, ensuring fish health, and ultimately achieving successful transformation and upgrading of the mandarin fish industry.

In recent years, the rapid development of omics technology has provided powerful tools for comprehensively analyzing the response of fish to feed and environmental factors. This study aims to use high-throughput sequencing technology of 16S rRNA gene to compare and analyze the differences in the structure and function of gut microbiota between pond farming and factory farming modes, as well as between artificially fed and live baited *S.chuatsi*. The research results are expected to reveal the microbial adaptation mechanism of *S.chuatsi* under new aquaculture models, providing theoretical basis

for the precise optimization of artificial feed and the formulation of factory based healthy aquaculture strategies, ultimately promoting the sustainable development of the *S.chuatsi* industry.

Materials and methods

Experimental samples collection

The experimental site is located in Huzhou City, China (28.3752°N, 116.9917°E). The experimental samples were collected from three farms: the Guledeng Aquatic Breeding Center base using a factory based circulating water aquaculture system and feeding artificial feed (GS group), Wu's Ecological Agriculture Co., Ltd. feeding live fish in the pond (CE group), and the Zhejiang Freshwater Fisheries Research Institute breeding base feeding artificial feed in the pond (CS group). The initial weight of mandarin fish fry in three farms is 2.62 ± 0.67 g, and the breeding period is 12 months. Firstly, anesthetize the fish, measure its total length and weight, then dissect it using high-temperature sterilized scissors and tweezers, and collect samples of intestinal contents in a 1.5mL centrifuge tube. Place the collected samples in liquid nitrogen for rapid freezing, and then transfer them to an ultra-low temperature freezer at -80°C for storage.

DNA extraction, Library Construction and Sequencing

Total genomic DNA was extracted using MagPure Soil DNA LQ Kit (Magan, D6356-02) following the manufacturer's instructions. DNA concentration and integrity were measured with NanoDrop 2000 (Thermo Fisher Scientific, USA) and agarose gel electrophoresis. Extracted DNA was stored at -20°C until further processing. The extracted DNA was used as template for PCR amplification of bacterial 16S rRNA genes with the barcoded primers and Takara Ex Taq (Takara). For bacterial diversity analysis, V3-V4 variable regions of 16S rRNA genes was amplified with universal primers 343F (5'-TACGGRAGGCAGCAG-3') and 798R (5'-AGGGTATCTAATCCT-3') (Nossa et al. 2010). The Amplicon quality was visualized using agarose gel electrophoresis. The PCR products purified with AMPure XP beads (Agencourt) and amplified for another round of PCR. After purified with the AMPure XP beads again, the final amplicon was quantified using Qubit dsDNA Assay Kit (Thermo Fisher Scientific, USA). The concentrations were then adjusted for sequencing. Sequencing was performed on an Illumina NovaSeq 6000 with 250 bp paired-end reads. (Illumina Inc., San Diego, CA; OE Biotech Company; Shanghai, China). The library sequencing and data processing were conducted by OE biotech Co., Ltd. (Shanghai, China). The raw data has been submitted to the NCBI database, with the submission accession number SUB16004442.

Analysis of Sequencing Data

Raw sequencing data were in FASTQ format. Paired-end reads were then preprocessed using Cutadapt software to detect and cut off the adapter. After trimming, paired-end reads were filtering low quality sequences, denoised, merged and detect and cut off the chimera reads using DADA2 (Callahan et al. 2016) with the default parameters of QIIME2 (2020.11) (Bolyen et al. 2019) to obtain a feature sequence

called ASV. All representative reads were annotated and blasted against Silva database (Version 138) using q2-feature-classifier with the default parameters. QIIME2 software was used for α and β diversity analysis. The microbial diversity in samples was estimated using the alpha diversity that include ACE, Chao1 (Chao 1984) and Shannon (Hill et al. 2003) and Simpson (Simpson 1997). The Euclidean distance matrix performed by R package was used for Principal coordinates analysis (PCoA) to estimate the similarities or differences between bacterial communities. Perform Adonis analysis using the compact_categorys.tpy tool in QIIME (1.9.1), and calculate the Adonis difference p-value using the Euclidean distance algorithm. A p-value of $p < 0.05$ is considered significant. LEfSe analysis reveals the composition of species differences between two or more biological communities.

Predictive Functional Analysis of Bacterial Communities

Use PICRUSt2 (2.3.0b0) (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) (Langille et al. 2013; Douglas et al. 2019) software predicts the composition of known microbial gene functions, in order to statistically analyze the functional differences between different groups. Perform functional prediction based on the 16S KEGG database and perform statistical analysis on the predicted KEGG results at three levels using t-test algorithm. Determine the microbial differences between the two groups based on significance ($p < 0.05$) and draw a heatmap of the different species.

Statistical analyses

All values were reported as means $\pm$ standard errors (SEM), and SPSS 22 was used for data analysis. An independent samples *t*-test was employed to identify the differences in growth performance between the two groups. Significance was determined at a threshold of $P < 0.05$.

Results

16S rRNA sequencing results

This study performed double ended amplicon 16S rRNA gene sequencing based on the NovaSeq Illumina platform. The raw reads data volume of the sequencing machine ranges from 78031 to 81744, the clean tags data volume after quality control ranges from 49176 to 72421, the valid tags data volume obtained by removing chimeras from clean tags ranges from 36883 to 65224, and the number of ASVs in each sample ranges from 132 to 310. In order to further analyze the distribution characteristics of ASVs among different samples, we created a flower chart that visually displays the number of ASVs within each sample and the ASVs shared among the three groups. The analysis results showed that the three groups shared 39 core ASVs (Fig. 1a). According to the taxonomic annotation, a total of 16 phylum, 32 classes, 72 orders, 112 families, 187 genus and 276 species were identified in the microbiota of the three groups. The identification of each group is shown in Fig. 1b. Circos described the correspondence between three aquaculture groups and four dominant bacterial phyla, especially the relationship with the characteristic expression sequence ASV (Fig. 1c).

Fig. 1 (a) The Venn diagram of ASV distribution in three groups, with the number of ASVs in each sample labeled in the figure (b) Statistical analysis of gut microbiota at different levels within each group

Gut bacterial composition

A bar chart illustrating the top 15 most abundant species at both the phylum and genus levels was generated for the three groups. At the phylum level, Fusobacteriota, Bacteroidetes, Proteobacteria, and Firmicutes consistently exceeded 5% relative abundance across all groups, though their distributions varied. Their respective abundances were 36.98%, 33.43%, 19.10%, and 9.96% in the CE group; 65.00%, 20.25%, 5.14%, and 9.25% in the GS group; and 20.49%, 46.99%, 19.07%, and 12.90% in the CS group (Fig. 2a). At the genus level, the number of taxa with relative abundance exceeding 5% varies among the three groups. The CE group contained the highest number (7 genera), predominantly *Cetobacterium* (36.86%), Muribaculaceae (10.84%), *Aeromonas* (8.90%), *Alloprevotella* (6.03%), *Bacteroides* (5.53%), Rikenellaceae_RC9_gut_group (5.61%) and *Plesiomonas* (5.35%). The CS group ranked second, with 6 genera exceeding the 5% threshold: *Cetobacterium* (20.34%), Muribaculaceae (16.33%), *Aeromonas* (16.41%), *Alloprevotella* (9.36%), *Bacteroides* (8.77%), and Rikenellaceae_RC9_gut_group (8.1%). The GS group had the fewest, with only two genera above this level: *Cetobacterium* (64.94%) and Muribaculaceae (7.27%) (Fig. 2b). The relative abundance of dominant bacterial genera shared by the three groups has undergone significant changes, with the GS group forming a community structure dominated by cetaceans, while the CE and CS groups maintain a more balanced composition among the main genera.

Fig. 2 (a) Bar chart of community structure for three groups (phylum) (b) Bar chart of community structure for three groups (genus)

Analysis of Alpha and Beta diversity of gut microbiota

Chao1, Ace, Shannon, and Simpson indices were used to analyze the alpha diversity of gut microbiota, which were used to evaluate species richness and diversity, respectively (Table 1). Among them, Chao1 and Shannon indices are mainly used to compare the overall richness of microorganisms among different groups. The results showed that the Chao1 index and Ace index were the highest in the CS group, and the richness indices of the CE group and GS group were similar. This suggests that changes in dietary factors and farming patterns may affect the richness of gut microbiota. In terms of diversity, the CS group also showed the highest Shannon and Simpson indices, indicating that the species distribution of its microbial community was the most uniform and the diversity was the highest. The Shannon index and Simpson index of the CE group were similar to those of the CS group, indicating high diversity. In contrast, the Shannon index and Simpson index of the GS group were the lowest, indicating that their community diversity and evenness were the most limited among the three groups. The T.TEST method was used to compare and analyze the differences between each two groups, and the results showed that there was a significant difference in Simpson index between the CE and GS groups ($p=0.048$), while there was no significant difference between other indices and groups ($p>0.05$) (Fig. 3a-d).

Table 1 alpha diversity of gut microbiota in three groups

	chao1	ace	shannon	simpson
CE group	208.31±26.93	208.02±28.00	4.51±0.35	0.86±0.027
GS group	211.94±22.96	211.92±23.80	3.49±0.45	0.71±0.06
CS group	262.43±17.04	264.45±16.76	4.79±0.42	0.87±0.04

In terms of β diversity, PCoA and PERMANOVA (Adonis) analysis based on Euclidean distance showed that there was no dispersion trend in the PCoA plot between the CE vs CS groups. The Adonis test further confirmed that there was no significant difference in microbial community structure between the two groups ($P=0.36$) (Fig. 3e). In the GS vs CS group, there is a trend of concentrated aggregation of GS and the p-value is 0.004 (Fig. 3h). There is a significant difference between the two groups.

Fig. 3 The diversity index of intestinal bacteria in three groups (a) Chao1 analysis (b) Ace analysis (c) Simpson analysis (d) Shannon analysis (e) PCoA analysis (f) PERMANOVA analysis. The asterisk above the bar chart indicates significant differences between groups ($p<0.05$)

Differences in gut microbiota caused by environmental and dietary factors

Use LEfSe analysis to compare differences between groups, including analysis of the contribution of different species to differences, annotation analysis of different species, and relative abundance of different species in each sample. There are 17 differences between the CE group and the CS group, including 3 orders (starting with o₋), 5 families (starting with f₋), and 9 genera (starting with g₋). The genus level results showed that Agathobacter, Oscillibacter, Moraxella, UCG_010, Olsenella, and Tyzzerla were highly expressed in CS, while Macellibacteroides, Reyranella and Crenobacter were highly expressed in the CE group (Fig. 4a). Comparing the GS and CS groups, there is a significant difference in the number of microorganisms between the two groups, with a total of 43, including 1 phylum (starting with p₋, Fusobacteriota), 1 class (starting with c₋, Fusobacteriia), 7 orders, 12 families, and 22 genera. Among them, 18 genera are highly expressed in CS group and 4 genera are highly expressed in GS group. The top 10 microorganisms with differences in genus level are: Cetobacterium, Moraxella, Olsenella, Alloprevotella, NK4A214_group, ASF356, Lachnospiraceae_NK4A136_group, Oscillibacter, Romboutsia and Turicibacter (Fig 4b).

Fig. 4 LEfSe analysis (a) CE vs CS groups (b) GS and CS groups. The colors of the bar chart represent their respective groups, and the length represents the LDA score, which represents the degree of significant differences in the impact of species between different groups.

Changes in intestinal microbial function caused by environmental and dietary

Picrust2 predictions for the two comparison groups. The results showed that there were significant differences between the CE group and the CS group in the transport and catabolism and digestive system of level2 (Fig 5a), which was dominant in the CS group, and there were 4 enriched pathways,

AMPK signaling pathway ($p=3.11e-03$), Glycosphingolipid biosynthesis-lacto and neolacto series ($p=1.61e-02$), Steroid degradation ($p=2.96e-02$), Cyanomino acid metabolism ($p=4.77e-02$) (Fig 5b). The change of food affected the functions of intestinal microbes in the artificial diet group in nutrient processing, transport and energy metabolism.

Another comparison group, GS and CS, showed fundamental differences in metabolism and genetic information processing ($p=1.3e-02$) at level1; Level2 difference significance top5 is Transcription ($p=9.32e-03$), Translation ($p=1.02e-02$), Replication and repair ($p=1.08e-02$), Biosynthesis of other secondary metabolites ($p=1.17e-02$) and Development and regeneration ($p=1.42e-02$) (Fig 5c); Looking further at the results of level3 pathway, the Heatmap shows the top15 with significant differences, among which the top6 with significant differences are Biosynthesis of cofactors ($p=1.9e-02$), Biosynthesis of amino acids ($p=2.9e-02$), Metabolic pathways ($p=3.1e-02$), Carbon metabolism ($p=3.3e-02$), Biosynthesis of secondary metabolites ($p=3.6e-02$) and Microbial metabolism in diverse environments ($p=4e-02$) (Fig 5d).

Fig. 5 (a) Bar chart showing the difference in KEGG functional prediction level 2 between the CE and CS groups. (b) Cluster top 15 heatmap of enriched pathways at level 3 predicted by KEGG function for CE and CS groups. (c) Bar chart showing the difference in KEGG functional prediction level 2 between the GS and CS groups. * representing $p<0.05$, **represents $p<0.01$. (b) Cluster top 15 heatmap of enriched pathways at level 3 predicted by KEGG function for GS and CS groups

Discussion

In this study, we investigated the diversity of gut microbiota in *Siniperca chuatsi* fed with live fish and artificial feed. The analysis of the distribution of community structures in three groups shows that at the phylum level, both groups are dominated by Fusobacteriota, Bacteroidota, Proteobacteria, and Firmicutes (Fig 2). Previous studies have also confirmed that these four common dominant microbial communities belong to aquatic organisms (Dalsgaard et al. 2014; Liu et al. 2016; Kanika et al. 2025). Fusobacteriota, it is quite common in both freshwater and saltwater fish, with high abundance in gut microbiota such as zebrafish, catfish, Rainbow trout, etc (Bledsoe et al. 2016; Michl et al. 2017; Stagaman et al. 2017). Bacteroidetes is characterized by metabolic flexibility (Tailford et al. 2015; Johnson et al. 2017). Similarly, Firmicutes can also participate in the fermentation of carbohydrates and the production of SCFAs (Mountfort et al. 2002; Tsuchiya et al. 2010; Dibaise et al. 2008). The abundance of the phylum Proteobacteria in the guts of carnivorous fish seems to have a role in the digestion and absorption of proteins (Rajili et al. 2010; Ray et al. 2012), and its abundance and composition changes are often regarded as important biomarkers of intestinal microecological balance (Shin et al. 2015). In the pond environment (CE and CS groups), the introduction of artificial feed in the CS group increased the abundance of carbohydrate treated bacterial communities (Bacteroidetes and Firmicutes), and the stability of Proteobacteria indicates that the protein source in the artificial feed can meet the needs of fish farming. Under the same artificial feed, the gut microbiota of *S.chuatsi* in pond and factory environments underwent significant changes, with a decrease in the proportion of Bacteroidetes,

Firmicutes, and Proteobacteria, and an increase in the proportion of Fusobacteriota. This trend of change may be a selection resulting from the combined effects of various factors, such as enclosed water environments and dim indoor lighting. At the genus level, the core bacteria of the three groups are *Cetobacterium*, which is a common probiotic in the intestinal tract of fish. Certain whale wax fungi are crucial for fish, especially those that consume plant-based diets or have low B12 levels, as they aid in protein fermentation and amino acid absorption to meet their growth and energy needs (Chen et al., 2022; Tsuchiya et al., 2010). However, the three groups showed significant differentiation in the dominant composition of other intestinal flora. The CE group had the most diverse dominant genera (7 genera), which showed that under the open pond environment and live bait feeding conditions, the intestinal microecology had a high microbial community richness, which was conducive to the host adapting to environmental changes and maintaining metabolic balance. Although the number of dominant genera decreased slightly (6 genera) in the CS group, the abundance of metabolically active bacterial genera such as *Aeromonas* and *Muribaculaceae* increased. Except for the genus *Cetobacterium*, the proportion of other top bacterial genera has increased compared to CE. For example, the genus *Muribaculaceae* is a beneficial bacterium that can inhibit the colonization of harmful bacteria, enhance intestinal barrier function, and over 73% of *Muridae* can participate in the synthesis of various amino acids (Chung et al., 2020; Li et al., 2024a). This indicates that CS group has a more stable colony structure and flexible colony regulation ability. The number of dominant genera in the GS group decreased to 2 genera, forming *Cetobacterium* as the absolute dominant bacterial genus. Although the abundance of the opportunistic pathogen *Aeromonas* genus (Guo et al. 2003) decreased, it also weakened the immune stimulation and competitive balance of the microbial community. Indicating that factory farming will lead to simplification of gut microbiota and decreased stability of the microbiota. Based on the above results, it can be concluded that the abundance changes in the gut microbiota structure are more significantly influenced by factory farming.

Alpha diversity analysis showed that the Chao1, ACE, Shannon and Simpson indexes of CS group were the highest, indicating that it was dominant in species richness, evenness and overall diversity, reflecting that the joint action of pond environment and artificial feed can support a more complex and balanced gut microbiota system. In contrast, the Shannon and Simpson indexes of GS group were lower, and the Simpson index was significantly different from that of CE group, indicating that although the total number of species was not significantly reduced, the distribution uniformity was significantly decreased, and the community structure tended to be single, which was consistent with the results of colony structure. The results of β diversity analysis showed a highly significant difference ($p < 0.01$) only between the GS group and the CS group, further confirming the decisive influence of aquaculture environment on the gut microbiota structure of *S. chuatsi*. This result is consistent with the analysis of Intestinal Microbial Community Structure, indicating that the role of environment may be stronger than the influence of feed. In the internal comparison of pond aquaculture, although there was no significant difference in the overall microbial composition (β diversity) between the CE group and the CS group, LEfSe analysis still identified 9 differential marker bacteria at the genus level. Most of the bacterial genera highly expressed in the CS group are beneficial bacteria. For example, *Agathobacter* is capable of

producing SCFAs, and its reduction simultaneously affects host metabolism and symbiotic microbiota (Hua et al., 2020; Liu et al., 2025b). *Oscillibacter* can participate in cholesterol metabolism and contribute to the reduction of cholesterol levels in human feces and plasma (Li et al., 2024b). Studies have shown that the reduction of inflammation in *Oscillibacter* is associated with a negative correlation between its levels and oxidative stress markers such as LDH, indicating its beneficial role in metabolic health (Konikoff and Gophna 2016; Martinez 2024). UCG-010 has a positive impact on certain specific diseases (Yang et al. 2021; Cui et al. 2024), and research has shown that the addition of fermented and distilled grains to feed can enhance the immune and antioxidant capacity of broiler chickens by increasing the abundance of microorganisms such as *Oscillospira* (Mei et al. 2025). The high abundance of *Macellibacter* species in CE was detected in a high-fat diet (Lim et al. 2022), indicating that the fat level of live feed feeding was higher than that of feed feeding. Compared to another comparison group, there were significant microbial differences between the GS group and the CS group, involving 43 taxonomic units from phylum to genus. It can be seen that the most iconic bacterial genus in the GS group is *Cetobacterium*. *Fusobacteria* is commonly found in the freshwater fish guts, and *Cetobacterium* is an important commensal bacterium (Navarrete and Paola 2012). Metabolites produced by *Cetobacterium* include, indeed, short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate, and vitamin B12, which can enhance fish health (Xie et al. 2021). The above results indicate that pond farming supports a more diverse gut microbiota, while factory farming screens out microbiota structures with relatively concentrated metabolic pathways and simplified functions.

Based on 16S KEGG functional prediction, there were differences in KEGG Level 2 between the CE group and the CS group. The CS group showed significant enrichment in transport and catabolism as well as digestive system related pathways, suggesting that the feeding of artificial feed may enhance the intestinal microbiota's adaptation to the transport, decomposition, and digestion of exogenous nutrients. The AMPK signaling pathway is a core hub for cellular energy homeostasis and metabolic regulation (Zhang et al. 2023; Min et al. 2024), the significant upregulation of this pathway ($p=3.11e-03$) in the CS group suggests that the gut microbiota is in a higher energy metabolism state, which may be a direct response to changes in available carbohydrates or energy density in artificial feed. The enrichment of glycosphingolipid biosynthesis lactose and neolactose series ($p=1.61e-02$) and steroid degradation pathway ($p=2.96e-02$) respectively indicate the adjustment of microbial membrane structure synthesis and lipid metabolism, reflecting the structural and metabolic adjustments made by the microbial community to adapt to the complex components of artificial feed. These changes may be related to the increased intake of complex components in feed, such as plant proteins and polysaccharides, prompting the microbiota to adjust their metabolic strategies to obtain energy more efficiently. The functional differences between GS group and CS group at levels 1 and 2 are mainly concentrated in genetic information processing. Specifically, the transcription, translation, replication, and repair pathways in Level2 together constitute the complete expression process of genetic information in organisms, from DNA to RNA and then to proteins. The above results suggest that the microbial cells in the GS group have more active gene expression and protein synthesis, which may be due to the relatively single and stable industrial environment, where dominant bacterial populations (such as the genus *Cetobacteria*)

need to be in a state of high-speed growth and stable reproduction to adapt to and dominate this niche. The differences in Biosynthesis of cofactors and Biosynthesis of amino acids are directly related to the enzyme activity and protein synthesis raw material supply of microbial cells (Graciano and Liu 2025). This indicates that the GS group microbiota has different abilities in autonomously synthesizing vitamins, coenzymes, and amino acids compared to the CS group, which may be related to the microbiota's adaptation to a single environment. The differences in metabolic pathways, carbon metabolism, and microbial metabolism in diverse environments suggest, from a more holistic perspective, that the two microbial communities have significant differences in core energy acquisition, carbon flow orientation, and metabolic network complexity. The GS microbial community may have a relatively single environmental resource (such as stable feed composition and simple organic matter in water), and its metabolic network is more focused on utilizing limited types of carbon and energy sources; The CS group microbiota, located in the "diverse environment" of ponds, may have stronger flexibility in their metabolic pathways to cope with changing external nutrient inputs.

Conclusion

This study aims to investigate the effects of aquaculture mode (pond/factory) and feed type (live bait/artificial feed) on the structure and function of gut microbiota in mandarin fish. The results indicate that in this study, changes in the aquaculture environment had a greater impact on the structure and function of gut microbiota than changes in feeding. In pond aquaculture, the gut microbiota of mandarin fish maintains high diversity and balanced community structure. Artificial feed can enrich the microbiota of farmed mandarin fish in nutrient transport, digestion and metabolism related pathways. The factory farming model (GS group) has led to significant restructuring and simplification of the gut microbiota, resulting in a singularity of the microbiota and a concentration of functions towards a single metabolic pathway such as genetic information processing. In contrast, the changes in the factory environment have brought about disruptive changes in the function of gut microbiota. In summary, the structural characteristics and functional expression of gut microbiota in mandarin fish are dominated by the aquaculture environment. In the future, intensive aquaculture of mandarin fish requires optimized environmental regulation strategies for factory based farming, supplemented by improved artificial feed formulas, in order to maintain the balance of the gut microbiota of mandarin fish and provide support for sustainable industrial development.

Declarations

Declaration of Competing Interest:

The authors report that they have no conflicts of interest in this work.

Ethics Approval:

The animal study protocol was approved by the Animal Experiment Committee of Zhejiang Institute of Freshwater Fishery (protocol code: ZJIFF20250317). No specific permission was required for the collection of mandarin fish.

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Author Contribution

Yayu Wang: Conceptualization, Methodology, Writing-Original draft. Jinpeng Zhang: Data curation. Shu Wang: Methodology. Cuiping Gong: Visualization. Dayan Hu: Writing-Reviewing and Editing, Validation. Rongfeng Wu: Sample collection.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

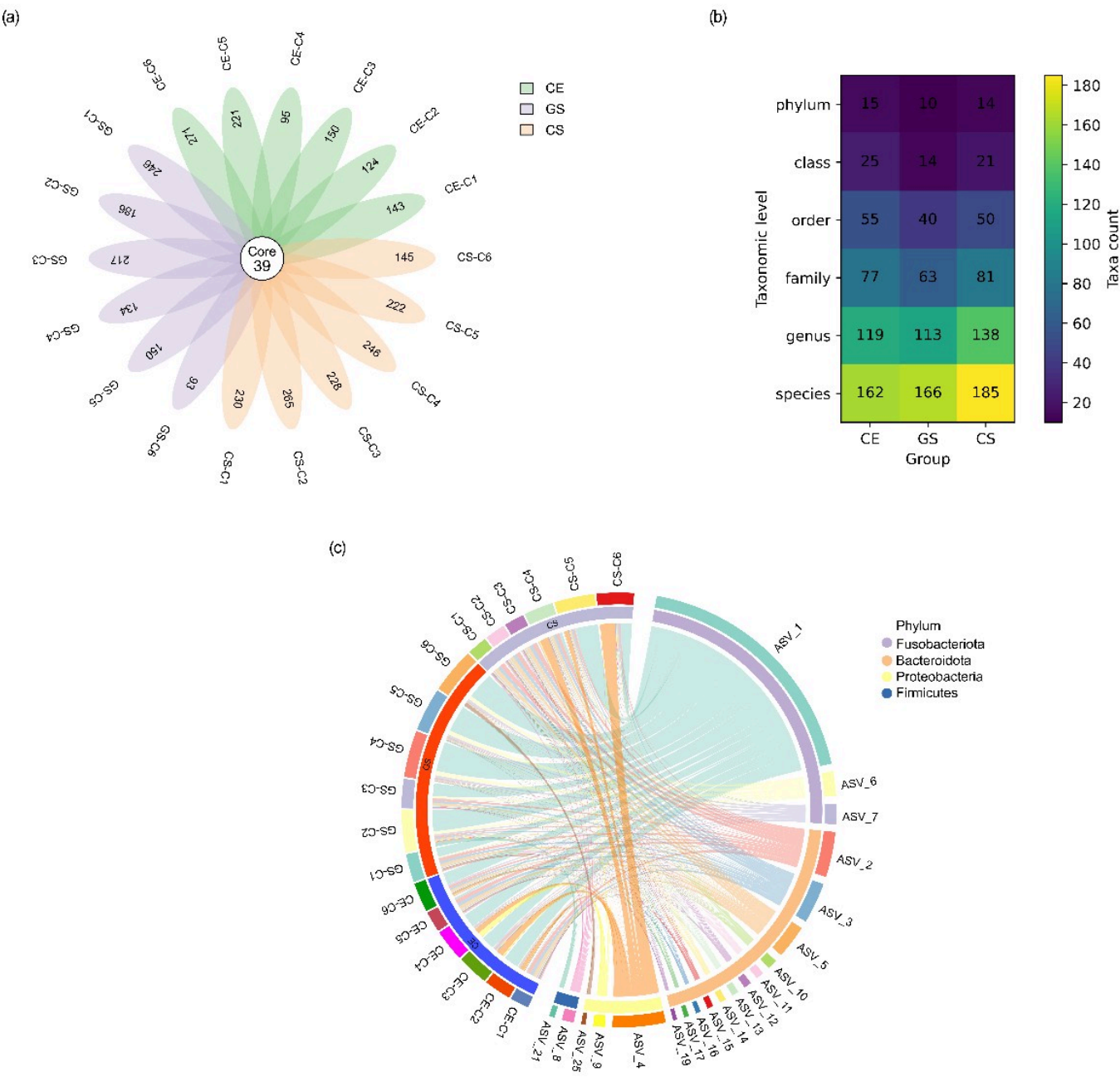


Figure 1

(a) The Venn diagram of ASV distribution in three groups, with the number of ASVs in each sample labeled in the figure (b) Statistical analysis of gut microbiota at different levels within each group

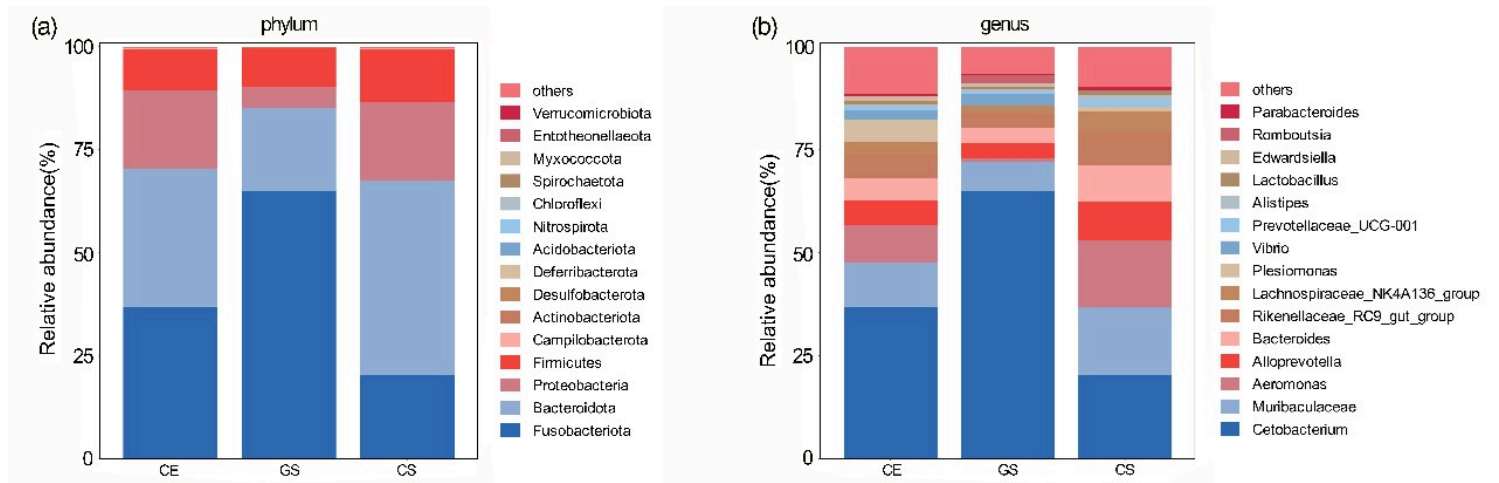


Figure 2

(a) Bar chart of community structure for three groups (phylum) (b) Bar chart of community structure for three groups (genus)

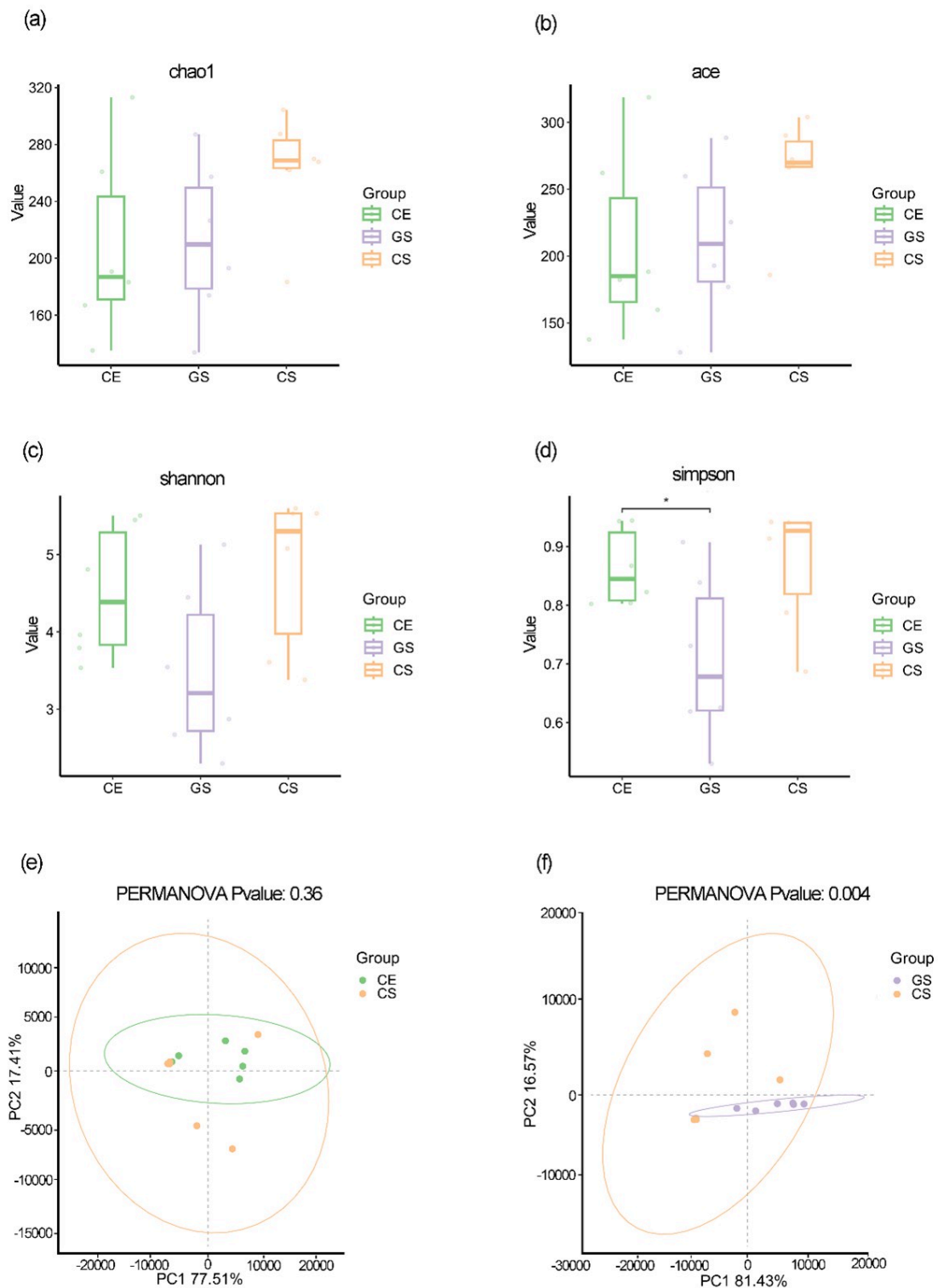


Figure 3

The diversity index of intestinal bacteria in three groups (a) Chao1 analysis (b) Ace analysis (c) Simpson analysis (d) Shannon analysis (e) PCoA analysis (f) PERMANOVA analysis. The asterisk above the bar chart indicates significant differences between groups ($p < 0.05$)

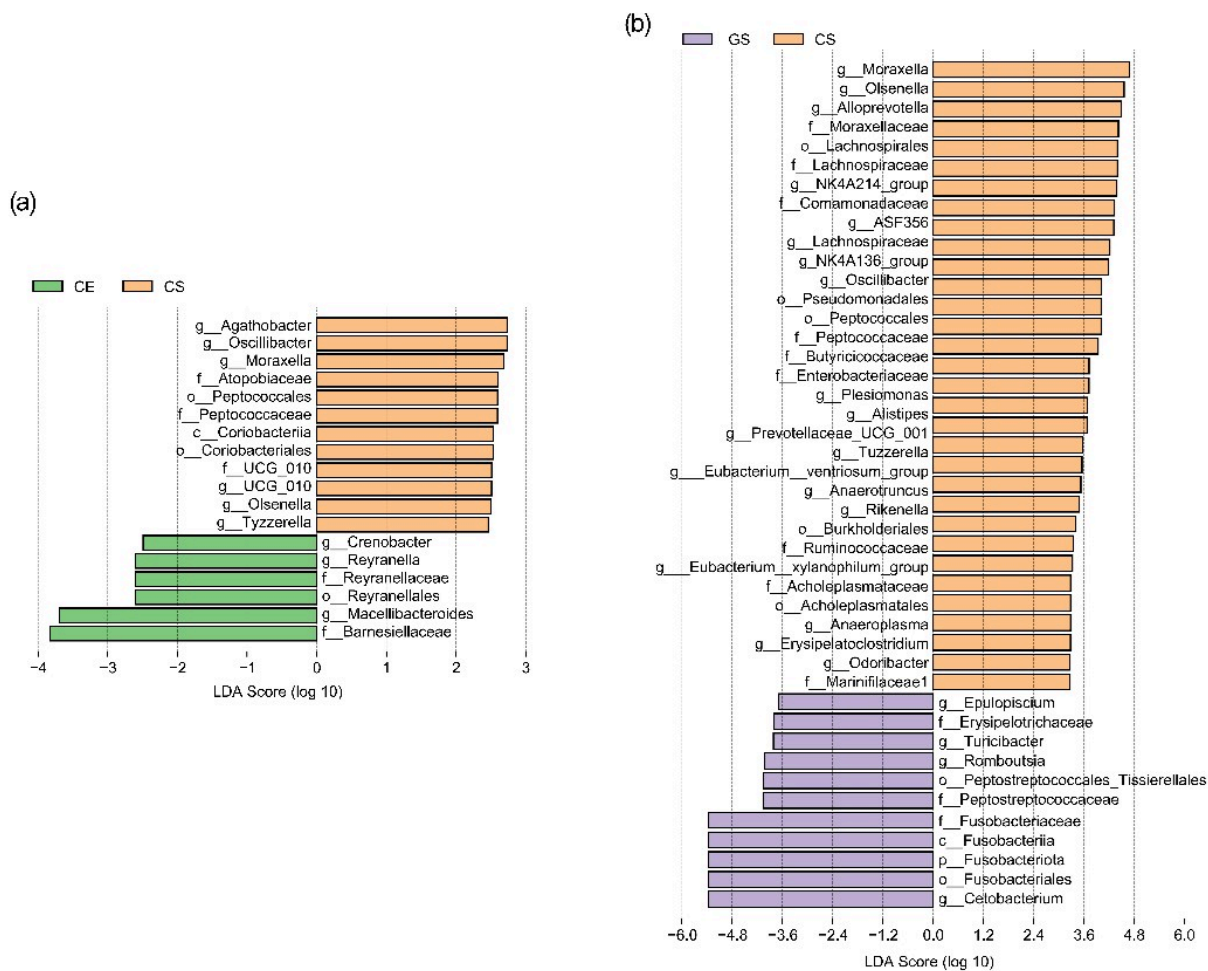


Figure 4

LEfSe analysis (a) CE vs CS groups (b) GS and CS groups. The colors of the bar chart represent their respective groups, and the length represents the LDA score, which represents the degree of significant differences in the impact of species between different groups.

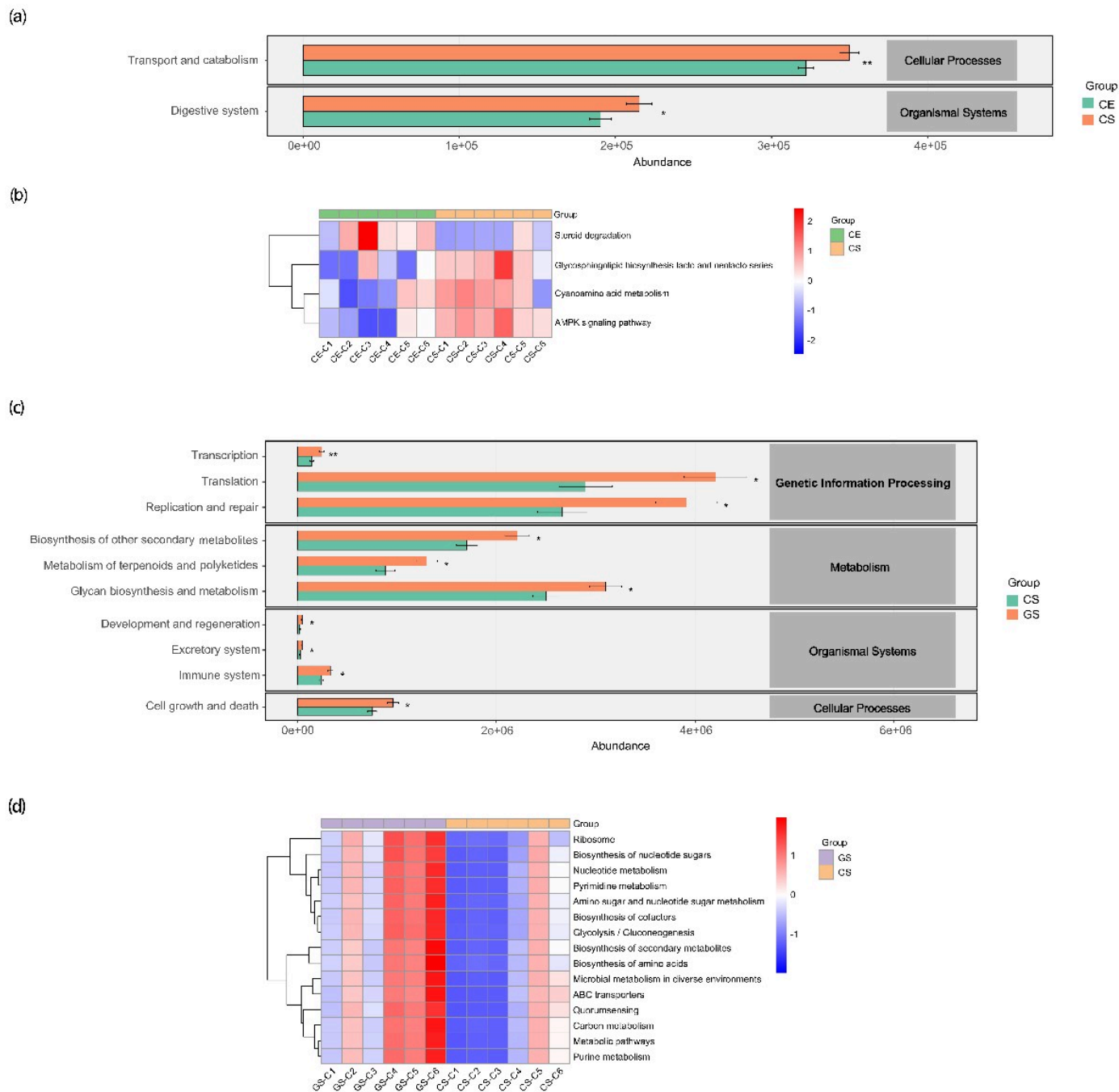


Figure 5

(a) Bar chart showing the difference in KEGG functional prediction level 2 between the CE and CS groups. (b) Cluster top 15 heatmap of enriched pathways at level 3 predicted by KEGG function for CE and CS groups. (c) Bar chart showing the difference in KEGG functional prediction level 2 between the GS and CS groups. * representing $p < 0.05$, ** represents $p < 0.01$. (d) Cluster top 15 heatmap of enriched pathways at level 3 predicted by KEGG function for GS and CS groups