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Variation in the gut microbiota during the early developmental stages of common carp (*Cyprinus carpio* L.) and its correlation with feed and pond water microflora

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Abstract

Background Fish gut microbiota undergo dynamic changes under the influence of many factors and play an important role in the nutrition, immunity and development in fish. Although common carp (*Cyprinus carpio* L.) is an economically important freshwater fish, there are few reports on its gut microbiota changes at different early developmental stages. In the present study, the gut microbiota of common carp during the early developmental stages and its correlation with the feed and pond water flora were studied using the Illumina MiSeq sequencing platform.

Results The results showed that the gut microbiota of common carp underwent continuous and mild changes over the development process, and the pond water environment might provide bacterial resources and have a certain influence on the changes in the gut microbiota of common carp. However, host selection pressure played a more important role in shaping the gut microbiota. Although the gut microbiota was affected by many factors, the presence of core microbiota indicated that some bacterial species adapt to the gut microenvironment of common carp and played a role in its growth process.

Conclusions The dynamic changes of gut microbiota of carp in early development stage were related to the feed, water environment and host selection. The results of this study provide a theoretical basis for healthy farming and disease prevention of common carp.

Keywords Common carp (*Cyprinus carpio* L.), Early developmental stage, Gut microbiota, Illumina MiSeq sequencing

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Introduction

The vertebrate gut microbiota has been widely studied in recent years, and a series of studies have shown that the host gut microbe systems are large and constantly changing [1]. As ancient vertebrates, fish were initially thought to be aquatic animals without stable endogenous microorganisms. However, with the development of DNA sequencing and bioinformatics technology, relevant studies on the gut microbiota of various fish have been reported, which have shown that changes in the fish gut microbiota are a complex process [2]. After hatching, the microbiota can colonize in gut, and the composition, abundance and diversity of the fish gut microbiota also change with development [3]. The specialized gut microbiota and related gut morphology enable fish species to tolerate resource fluctuations differently [4]. Fish gut microbiota has a variety of functions, including nutritional effects [5], barrier effects [6], immune effects [7], influence on fish disease outbreaks [8], promotion of host development [9] and other functions [10]. Factors that affect the gut microbiota of fish include developmental stage [11], dietary composition [12, 13], habitat [14]and the surrounding environment [15–17].

Studies on fish gut microbiota have also revealed the effects of gut microbiota on fish growth and metabolism [18], reproduction [19] and behavior [20], providing new insights for the improvement of related fish farming issues [21]. Therefore, it is of great significance to explore the patterns of fish gut microbiota change. As one of the factors affecting the gut microbiota of fish, developmental process has been reported in a variety of species, including zebrafish (*Danio rerio*) [22, 23], cardinalfish (*Ostorhinchus fasciatus*) [24], channel catfish (*Ictalurus punctatus*) [25], Atlantic cod (*Gadus morhua*) [26], Nile tilapia (*Oreochromis niloticus*) [27, 28], grass carp (*Ctenopharyngodon idella*), Chinese perch (*Siniperca chuatsi*), and southern catfish (*Silurus meridionalis*) [29, 30]. Studies have shown that, compared with other developmental stages, the larvae and juveniles of common carp are more susceptible to factors such as living environment (mainly pond water and pond bottom sediment), feed conversion [31] and disease outbreak, resulting in considerable changes in the gut microbiota [27]. The composition and succession of the gut microbiota in larval and juvenile common carp affect their nutrition, immunity, growth and development, which has important research value and significance [31]. Bakke et al. reported the composition of gut microbiota during the development of juvenile Atlantic cod, and found that gut microbiota changes with age, which may be caused by different selection pressures during gut system development [26]. Giatsis et al. reported the correlation between intestinal flora and environmental factors during the growth of juvenile tilapia, and found that compared with the microorganisms in feed, the flora in water environment is more similar to intestinal flora, and there are more OTUs [27]. Meanwhile, Giatsis et al. elucidated the changes in gut microbiota during the development of juvenile tilapia in different aquaculture systems, and found significant differences in gut microbiota composition among different aquaculture systems [28].

Recently, many factors have been reported to influence the gut microbiota of common carp, including intestinal tapeworms [32], deltamethrin [33], and dechlorane [34]. However, there are few reports on the influence of the early developmental stage of common carp on the composition of the gut microbiota. In this study, 9 time points during the early development of common carp were selected to study the composition of the gut microbiota at different stages and its correlation with the microbial flora in feed and pond water. Our study provides evidence for changes in the gut microbiota of common carp during early development, highlighting the influence of fish developmental stages on the gut microbiota [35].

Materials and methods

Incubation and rearing of experimental fish

All the larvae and juvenile fish used in the study were obtained from the Jinan Agricultural Technology Extension Service Center, where the fertilization, hatching and breeding of the broodstock were completed. Ten pairs of healthy common carp broodstock were selected and injected with chorionic gonadotropin and luteinizing hormone-releasing hormone A2 under the pectoral fin to promote egg spawning and fertilization. The common carp broodstock injected with hormones were placed in a breeding pond with sterilized brown flakes as substrate for oviposition.

The fertilized eggs were incubated naturally in the pond. Most larvae were fed cooled sterilized soy milk on the second day after hatching, fine-grain flour feed on the seventh day after initial feeding, large-grain flour feed on the 28th day after initial feeding, and conventional commercial feed on the 50th day after initial feeding until the end of sampling.

Sampling of the fish, pond water and feed

Fertilized eggs, larvae and juveniles of common carp at different stages of development were collected from the pond (Fig. 1A). About 8 h after fertilization of the fish eggs, they were quickly treated with 0.1% benzalkonium bromide for 1 min, washed 3 times with 0.68% NaCl disinfection solution for 3 min each time, and frozen at -80℃ for later use. Common carp larvae that were in the yolk sac stage approximately 24 h after hatching and larvae and juvenile fish on the 1,3,7 and 14 days after initial feeding were rapidly treated with 0.1% benzalkonium bromide for 1 min. Then like fish eggs, juvenile fish

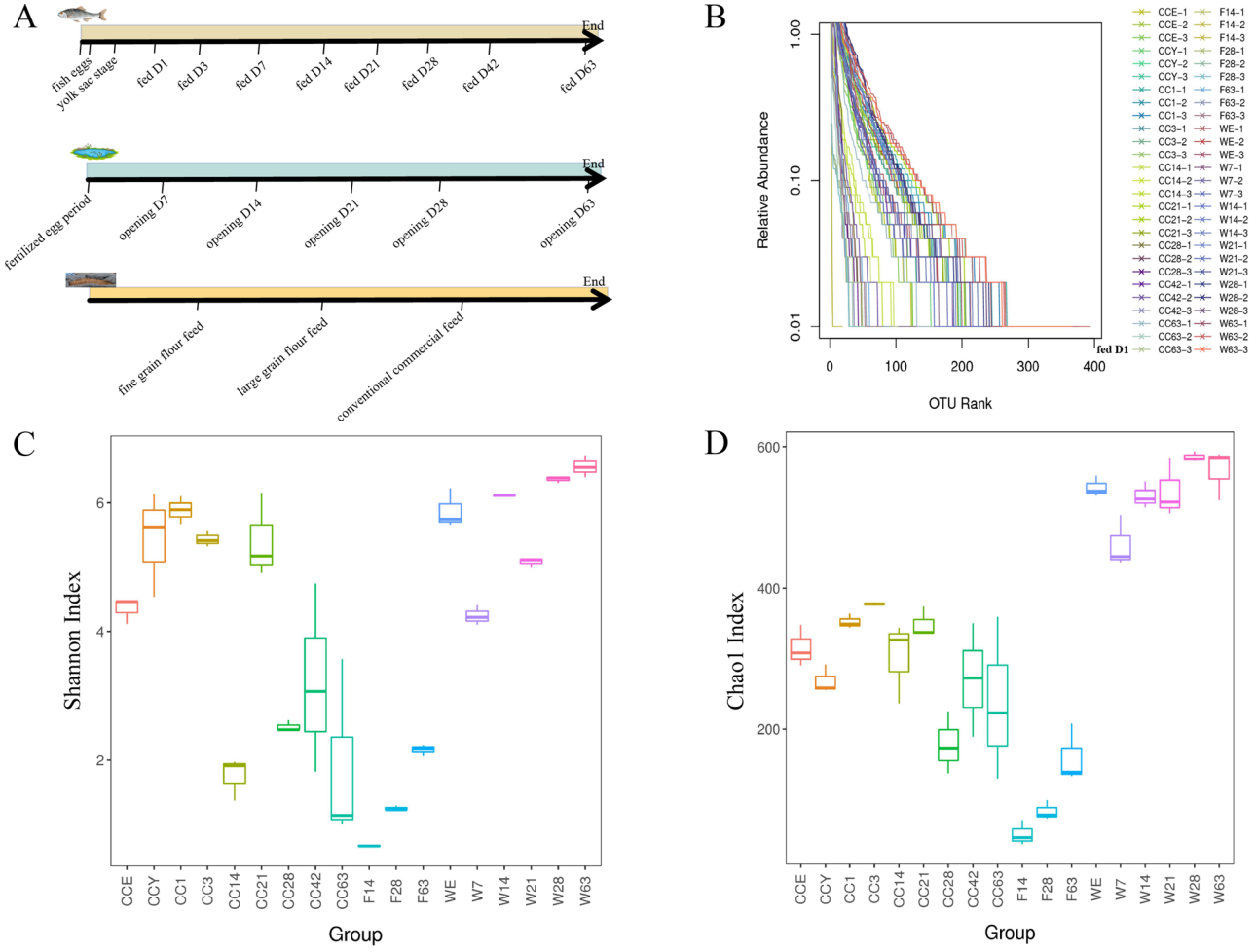

Fig. 1 Diversity and abundance of the gut microbiota during the early developmental stage of common carp vary with the development process. (**A**) Timeline for sampling of the fish, pond water and feed. (**B**) Rank–abundance distribution curves of different samples. The X and Y axes denote the OTU rank and the relative abundance of the corresponding OTU, respectively, and each curve corresponds to one sample. (**C**) Box plots of intergroup differences in the Shannon index. The X and Y axes denote the sample group and the Shannon index, respectively, and each boxplot shows the smallest, first quartile, median, third quartile and largest values of the Shannon index. (**D**) Boxplots of intergroup differences in the Chao1 index. The X and Y axes denote the sample group and the Chao1 index, respectively, and each boxplot shows the smallest, first quartile, median, third quartile and largest value of the Chao1 index

washed three times with sterilized 0.68% NaCl solution for 3 min each time and frozen for use at -80℃. Larval and juvenile carp on the 21, 28, 42, and 63 days after initial feeding were cleaned with 0.68% NaCl and their body surface was wiped with 75% ethanol, and then anesthetized by immersion in a 100 mg/L solution of MS222 (Sigma). The intestinal tract was dissected aseptically and frozen at -80 °C for use after the intestinal contents were collected.

Pond water was taken at the key time points of larval and juvenile common carp development, which were the fertilized egg stage, soy milk pouring period (7 days after initial feeding), pond phytoplankton foraging period (14 days after initial feeding), flour feeding period (21 days after initial feeding), pond settling period (28 days after initial feeding), and feed feeding period (63 days after initial feeding). 1500 ml water was extracted from 0.5 m below the surface at three different positions in the pond and taken to the laboratory in a sterilized bottle. The bacteria were recovered by centrifugation at 4 °C and 13,000 rpm for 15 min, and frozen at -80 °C.

In the feed conversion period, fine-grain flour feed, large-grain flour feed and conventional commercial feed were collected, divided into sterilization tubes and frozen for use at -80℃. The statistics for the different samples are shown in Table 1.

Extraction, amplification, purification and sequencing of bacterial genomic DNA

The work was performed by Suzhou Jinweizhi Biotechnology Co., Ltd. Bacterial genomic DNA was extracted using a Tiangen soil genome extraction kit, and DNA

Table 1 Statistical table of sample names

Sample name	Sample description	Sample name	Sample description
CCE-1/2/3	Carp egg samples 1/2/3	F14-1/2/3	Feed samples for 14 days of open feeding are 1/2/3
CCY-1/2/3	Samples of carp in yolk sac stage 1/2/3	F28-1/2/3	Feed samples 1/2/3 for 28 days of open feeding
CC1-1/2/3	Carp samples 1/2/3 that were ingested for 1 day	F63-1/2/3	Samples of open feed for 63 days were 1/2/3
CC3-1/2/3	Samples of carp fed for 3 days were 1/2/3	WE-1/2/3	Pond water samples at egg stage 1/2/3
CC14-1/2/3	Samples of carp fed for 14 days were 1/2/3	W7-1/2/3	Open mouth ingestion 7 Tianchi pond water samples 1/2/3
CC21-1/2/3	1/2/3 carp samples taken after 21 days of open feeding	W14-1/2/3	Open ingestion 14 Tianchi pond water samples 1/2/3
CC28-1/2/3	Samples of carp fed for 28 days were 1/2/3	W21-1/2/3	Open mouth ingestion 21 Samples of Tianchi Pond water 1/2/3
CC42-1/2/3	Samples of carp fed for 42 days at the opening were 1/2/3	W28-1/2/3	Open feeding 28 Tianchi Pond water samples 1/2/3
CC63-1/2/3	Carp samples 1/2/3 after 63 days of open feeding	W63-1/2/3	Open feeding 63 Samples of Tianchi Pond water 1/2/3
CCE	Carp egg samples merged	F14	Feed samples for 14 days of open feeding were combined
CCY	Carp samples in yolk sac stage were combined	F28	Feed samples for 28 days of open feeding were combined
CC1	Carp samples after opening for 1 day were merged	F63	Open feeding 63 days of feed samples combined
CC3	Carp samples after 3 days of open feeding were merged	WE	Pond water samples merge during egg stage
CC14	Carp samples after 14 days of open feeding were merged	W7	Open feeding 7 Tianchi pond water samples merged
CC21	Carp samples after 21 days of open feeding were merged	W14	Open feeding 14 Tianchi pond water samples merged
CC28	Carp samples after 28 days of open feeding were merged	W21	Open mouth feeding 21 Tianchi pond water samples merged
CC42	Carp samples after 42 days of open feeding were merged	W28	Open feeding 28 Tianchi pond water samples merged
CC63	Carp samples were merged after 63 days of open feeding	W63	Open feeding 63 Tianchi pond water samples combined
CC	All carp samples were combined	W	All pond water samples are consolidated
F	All feed samples were combined		

concentration and quality were determined by a Qubit 2.0 fluorometer. PCR amplification was performed with the forward primer CCTACGGRRBGCASCAG-KVRVGAAT and reverse primer GGACTACNVGGGT-WTCTAATCC targeting the V3–V4 variable regions of the 16 S gene. The quality of the amplification product was assessed using an Agilent 2100 bioanalyzer, and the library concentration was detected by a Qubit 2.0 fluorometer and PE300 sequencing was performed by the Illumina MiSeq sequencing platform.

Bioinformatics analysis of sequencing data

The forward and reverse reads obtained by Paired-end sequencing were first assembled and connected in pairs, and then the sequencing data were quality-controlled. Sequences less than 200 bp in length and chimeric sequences were removed, and the final sequences were assigned to operational taxonomic units (OTUs). VSEARCH was used to perform cluster analysis of sequences with 97% similarity as the threshold (the 16 S rRNA reference database used for comparison was SILVA), and the representative OTU sequences were analyzed by species taxonomy using the RDP classifier Bayesian algorithm.

Based on the OTU analysis results, the data were analyzed informatically. A rank– abundance plot was constructed using R language, and a rarefaction curve was plotted using QIIME. The abundance of flora was calculated using the ACE (http://www.mothur.org/wiki/ACE) and Chao1 (http://www.mothur.org/wiki/Chao) indices. The flora diversity was calculated with the Shannon (http://www.mothur.org/wiki/Shannon) and Simpson (http://www.mothur.org/wiki/Simpson) indices. Good's coverage (http://www.mothur.org/wiki/Coverage) was used for sequencing depth calculation, and the software for analysis was QIIME.

Statistical analysis

Statistical significance was assessed using one-way analysis of t-tests in GraphPad Prism 6. All data were normally distributed, and $P<0.05$ was considered statistically significant.

Results

The diversity and abundance of the gut bacterial community in common carp at the early developmental stage

In this study, common carp at the fertilized egg stage, yolk sac stage, and 1, 3, 14, 21, 28, 42 and 63 days after initial feeding were selected as samples (Fig. S1A-K). Genomic DNA was extracted from common carp, feed and pond water samples under different treatments

(Table 1). The concentration and quality of genomic DNA in the samples was measured and met the requirements (Table S1). The final effective sequences were obtained by removing primers and linker sequences, bases with mass values less than 20 at both ends, sequences with lengths less than 200 bp, and chimeric sequences (Table S2). The length statistics of effective sequences showed that most of the effective sequences ranged from 430 to 470 bp, among which the number of sequences between 435 and 445 bp and 455–465 bp was the highest (Fig. S2A).

We classified the valid data as OTUs, and the number of OTUs varied greatly among different samples. The number of species in feed samples was the lowest, followed by fish samples, and the number of species in pond water samples was the highest (Table S3). The rarefaction curve was plotted by taking the number of effective sequences of samples as the abscissa and the OTU type classified by effective sequences as the ordinate (Fig. S2B). With the increase in the number of samples, the rarefaction curve of all samples tended to flatten off, indicating that the amount of sequencing data of samples was reasonable and could reflect the composition of bacteria in the samples. Based on the OTU analysis results, informatics analysis was conducted, and a rank– abundance plot (Fig. 1B) was constructed. The curve width of the feed group was narrower, and the declining trend was faster. The curve width of pond water samples was larger, and the declining trend was slower. The carp samples were intermediate between the feed group and the pond water group in terms of declining trend and curve width. The results showed that the species abundance of pond water samples was the highest, the species evenness of the feed group was the highest, and the carp samples were in the intermediate position. We also calculated alpha-diversity index statistics for different samples (Table S4) and constructed boxplots of Shannon index and Chao1 index differences among sample groups (Fig. 1C and D). The results showed that the diversity and abundance of the samples varied with the development process.

The gut microbiota of juvenile common carp changed during development

To investigate the changes in the gut microbiota during the early development of common carp, we analyzed the taxonomic changes in the gut microbiota at the phylum, genus and species levels during development from fertilized eggs to 63 days after initial of feeding. At the taxonomic level of phyla (Fig. 2A), Proteobacteria, Cyanobacteria, Firmicutes and Actinobacteria occurred in all fish samples, but their relative abundances changed with the development process. For example, Proteobacteria and Firmicutes increased first and then decreased twice in a row with common carp development. The species with high abundances also changed at different developmental stages. For example, from Bacteroidetes and Proteobacteria at the CCE and CCY stages to Proteobacteria at CC1, CC3, CC14, from Proteobacteria and Cyanobacteria at CC21, to Proteobacteria and Fusobacteria at CC28. There were also some differences in the number of taxa in different developmental stages. The CC1 and CC3 stages had the most taxa (20 taxa each), the CC14 and CC28 stages had the fewest taxa (12 and 11 taxa, respectively), and the remaining stages had 13–15 taxa.

At the genus level (Fig. 2B), only *Pseudomonas* occurred in all fish samples, but the relative abundance of *Pseudomonas* varied greatly at different developmental stages, showing a first increasing and then decreasing trend two consecutive times. Excluding unclassified and ambiguous taxa, the relative abundances of most genera varied with developmental stage.

At the species level (Fig. 2C), there were different types of gut microbiota at different stages, with relatively few species at CCY, CC63 and CC28. Moreover, the species with high abundance varied in different developmental stages. For example, *Sphingomonas* sp. LYH-20 and *Chroococcopsis gigantean SAG* 12.99 occurred in the CCE stage. The species found in the CCY stage was *Gyrodactylus salaris* (gyrodactylosis fluke), and those found in the CC1 stage were *Shewanella putrefaciens*, *Comamonas testosteroni*, *Comamonas aquatica*, *Oryza meyeriana* and *Exiguobacterium undae*. The results showed that the composition of the gut microbiota changed with development.

The gut microbiota of juvenile common carp was more affected by host selection pressure

Subsequently, the similarities and differences of the samples were analyzed. The heatmap analysis was conducted based on the 30 OTUs with the highest abundance (Fig. 3A). It was found that the similarity among pond water samples was high, and the similarity between CCE and CCY samples was high, and these samples clustered together with pond water samples. The similarity among feed samples was also high, and CC1 and feed samples were clustered together. The remaining fish samples were more similar and clustered together. Subsequently, we conducted UPGMA tree analysis (Fig. S3), weighted UniFrac distance matrix analysis (Fig. 3B), principal coordinate analysis (PCoA) (Fig. 4A), and non-metric multidimensional scaling (NMDS) analysis (Fig. 4B) for the common carp-related samples. UPGMA tree analysis showed that the difference between the microbiota in the gut, pond water and feed was reflected in the fact that the common carp samples were clustered with pond water samples and then with feed samples. Except for 9 samples from CC28, CC42 and CC63, the common carp samples were clustered first within groups

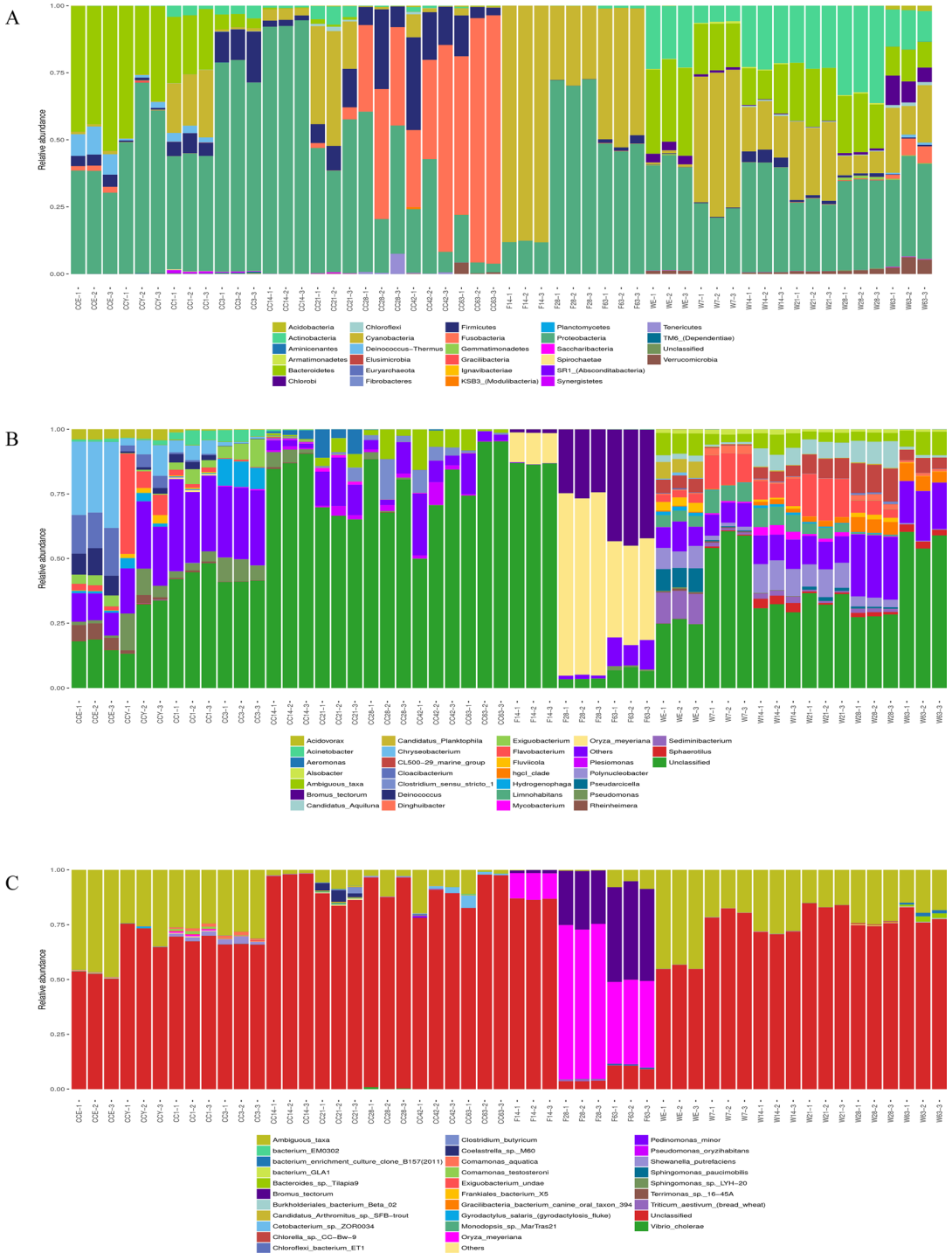

Fig. 2 Changes in the gut microbiota of juvenile common carp during development. (**A**) Bar graph of species distribution in different samples at the phylum level. The X axis denotes the sample name and the Y axis denotes the relative abundance of different phyla. (**B**) Bar graph of species distribution in different samples at the genus level. The X axis denotes the sample name and the Y axis denotes the relative abundance of different genera. (**C**) Bar graph of species distribution in different samples at the species level. The X axis denotes the sample name and the Y axis denotes the relative abundance of different species

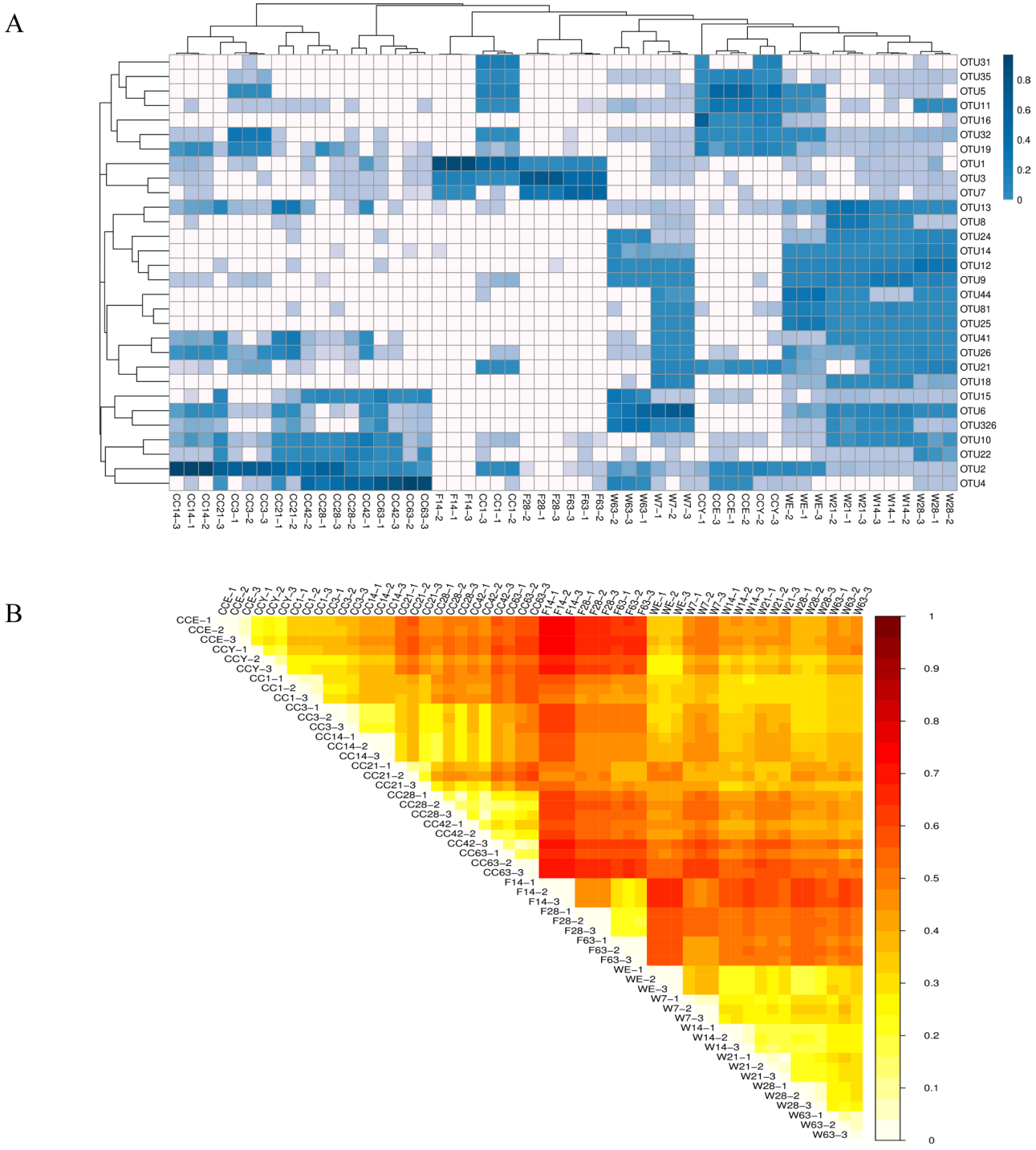

Fig. 3 Difference of the microbiota between common carp samples and other samples is greater than that between samples from different common carp developmental stages. (**A**) Heatmap of OTU abundance. The tree at the top of the figure is the sample cluster tree, the tree on the left of the figure is the species cluster tree, and the color of each square in the heatmap corresponds to the relative abundance value of the species. (**B**) Heatmap of the distance matrix with weighted UniFrac distances. The color of the cross block represents the degree of difference between the compared samples, and the deeper the color is, the larger the difference

and then between groups. On the one hand, the differences between groups were greater than the differences within groups. On the other hand, the common carp samples were different from the pond water samples and feed samples, reflecting the difference between the carp samples and other nonbiological samples. The weighted UniFrac distance matrix heatmap (Fig. 4C) showed that the differences between the common carp samples and

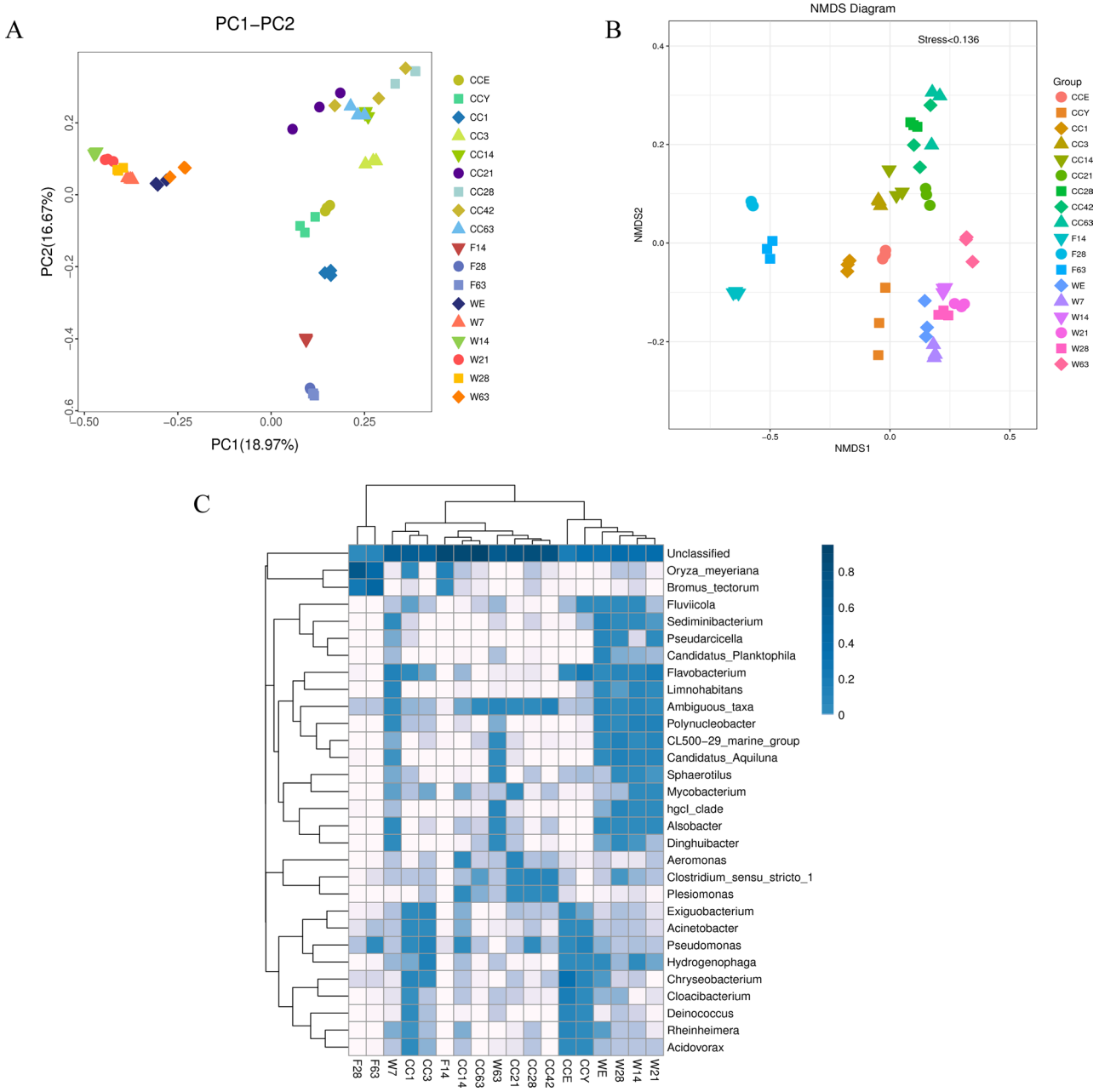

Fig. 4 Differences of the microbiota between common carp samples and environmental samples. (**A**) PCoA plot. The X and Y axes denote the two main coordinates and the contribution of the two coordinates separately, each plot denotes one sample, the distance of the plots denotes the similarity of the microbiota in different samples, and the smaller the distance is, the greater the similarity. Each sample group is represented by same figure with the same color and shape. (**B**) NMDS plot. Each plot denotes one sample, the distance of the plots denotes the difference between the compared samples, and each sample group is represented by the same figure with the same color and shape. The NMDS reflects the degree of difference between the samples when the stress < 0.2. (**C**) Heatmap of sample groups at the genus level. The tree at the top of the figure is the sample cluster tree, the tree on the left of the figure is the genus cluster tree, and the color of each square in the heatmap corresponds to the relative abundance value of the genus

other samples were significantly higher than the differences among the common carp samples, indicating that the differences between the common carp samples and other samples were more significant than the differences among different developmental stages. PCoA and NMDS analysis showed that pond water samples were clustered together, feed samples were clustered together, and carp samples were basically clustered together. However, the CCE, CCY and CC1 samples were more concentrated and closer to the pond water samples, reflecting the difference between the carp samples and environmental samples. The most likely reason for this difference was host selection pressure, which is consistent with previous reports [29].

The comparative analysis between the common carp samples and the pond water samples showed that the gut microbiota of larval and juvenile common carp was different from that of pond water (Table S5). Common carp samples, pond water samples and feed samples were further combined, and intergroup analysis of similarities (ANOSIM) showed that the composition of microbiota between common carp samples and pond water samples and between carp samples and feed samples were significantly different (Fig. 5A-C), indicating that the construction of the gut microbiota of fish was more influenced by the host during development. Thus, the microbiota structure was related to the host development stage, and environmental factors, including the pond water and feed, had less influence on the fish gut microbiota than the host. This result was consistent with the research of gibel carp [36]. To further explore the differences between groups of common carp samples at adjacent developmental stages, we analyzed the differences in the five bacterial genera with the largest differences between groups at two adjacent developmental stages (Fig. 6A-H).

Core microbiota in the intestinal tract of juvenile common carp during development

A petal diagram of common bacteria in all common carp samples were made (Fig. 7A). There was only one common OTU in all common carp samples, which was annotated to the class Gammaproteobacteria, but the abundance in different samples varied significantly (one-way ANOVA, $P<0.0001$). This bacterium played an important role in juvenile common carp, but it could not be identified to the species level, so it was difficult to determine its specific role in the development, nutrition and immunity of common carp. As the differences within groups were significantly smaller than the differences between groups, the results of the petal diagram of carp samples at each sampling time point (Fig. 7B) showed that there were 26 OTUs, among which 3 were annotated to species, 10 to genus, 11 to family, 1 to order, and 1 to class. The number of reads with common OTUs accounted for 49.1% of the total. *Shewanella putrefaciens* [37]was one of the common microorganisms with different abundances in samples at different times. CC1 and CC3 had the highest *S. putrefaciens* abundances. However, this bacterial species was not detected in feed samples, and only pond water sample W63 contained a very small amount of this bacterium, indicating that *S. putrefaciens* occurs in fish samples, and its relative abundance changed continuously during development. As *S. putrefaciens* was present as the core bacterium in this study, it must play an important role in the early development of juvenile common carp. The petals diagram of OTUs in the pond water samples (Fig. 7C) showed that there are 62 species of OTUs in all the pond water samples. The average number of reads contained in 62 OTUs accounted for 48.5% of the total reads, and there were some differences among different samples, ranging from 28.1 to 68.1%. There are 15 common OTUs annotated to species, 21 to genus, 20 to family, 4 to order, and 2 to class.

Discussion

The gut microbiota plays an important role in the nutrition, immunity and development of fish. However, there are few reports on the changes of gut microbiota at early developmental stages of common carp. Thus, the gut microbiota of common carp during the early developmental stages and its correlation with the feed and pond water flora were studied in the present study, which may be helpful for the disease prevention and healthy farming of common carp.

The results showed that the gut microbiota of common carp changed continuously and mildly during the early stages of development. Similar results were found

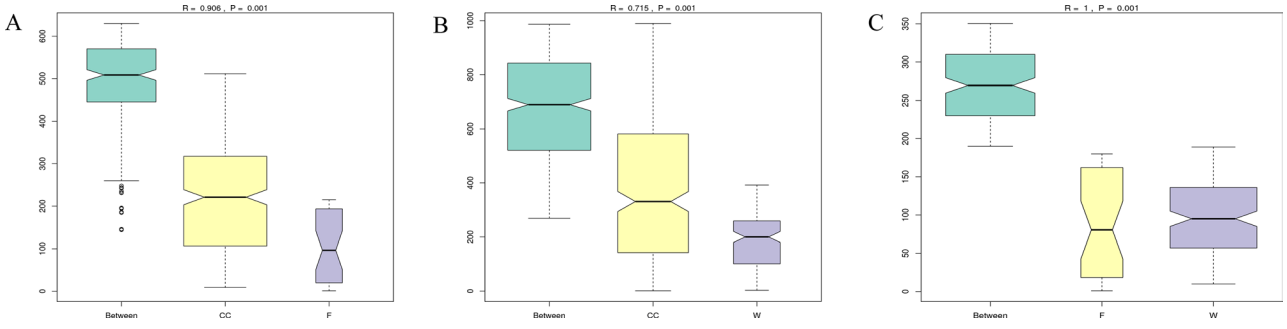

Fig. 5 Differences in the bacterial composition of common carp samples and pond water samples, and common carp samples and feed samples. (**A**) ANOSIM of CC (common carp) and F (feed). "Between" on the X-axis represents the results of CC and F, and "CC" and "F" on the X-axis represent the intragroup results separately. (**B**) ANOSIM of CC and W (water). "Between" on the X-axis represents the results of CC and W, and "CC" and "W" on the X-axis represent the intragroup results. (**C**) ANOSIM of F and W. "Between" on the X-axis represents the results of F and W, and "F" and "W" on the X-axis represent the intragroup results. The intergroup difference was larger than the intragroup difference if the value of R was near 1, and the statistics were significant if the value of P was smaller than 0.05

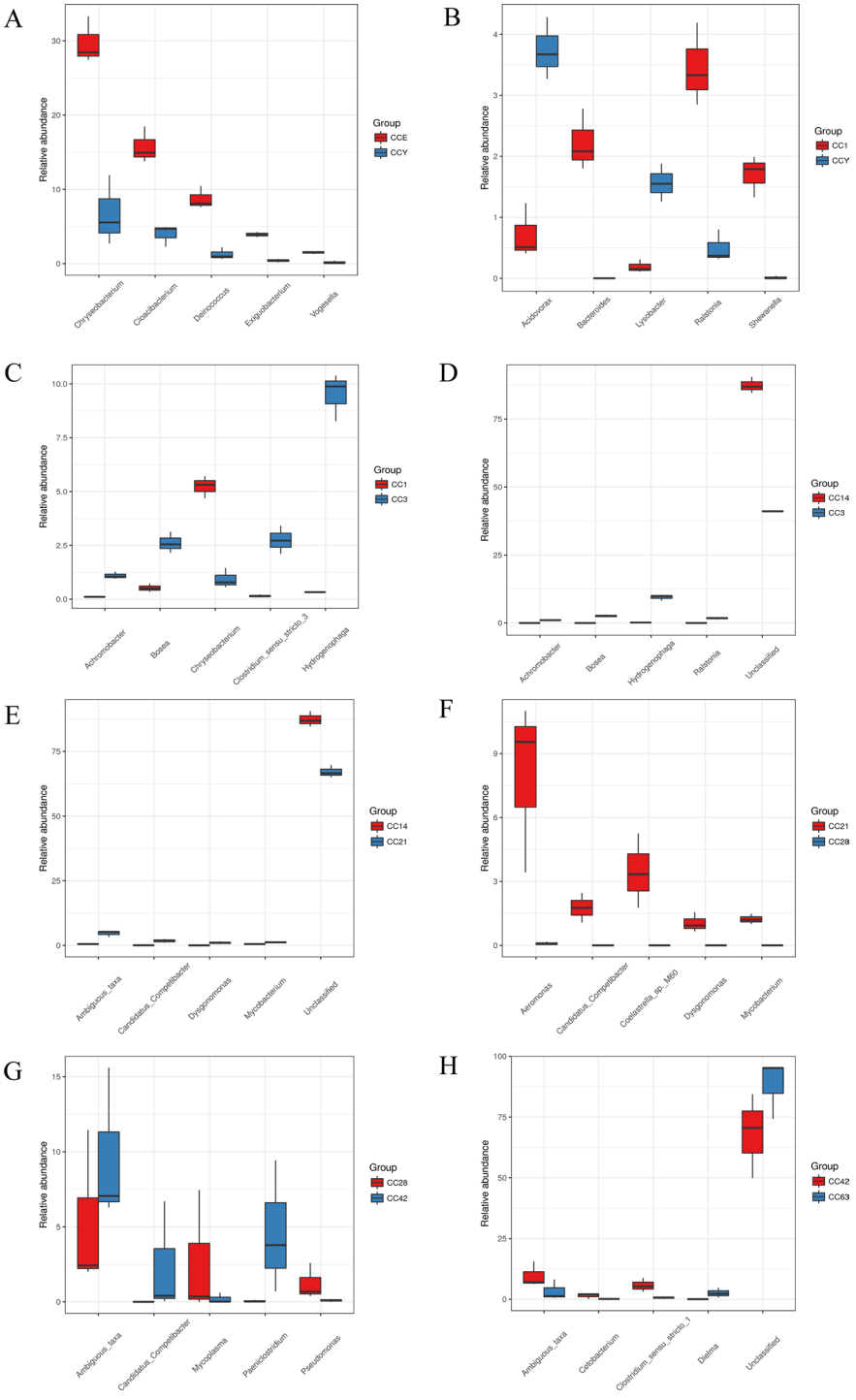

Fig. 6 Difference in the five most abundant genera found in common carp samples between two adjacent developmental stages. (**A**-**H**) Difference in CCE vs. CCY, CCY vs. CC1, CC1 vs. CC3, CC3 vs. CC14, CC14 vs. CC21, CC21 vs. CC28, CC28 vs. CC42 and CC42 vs. CC63. Each figure shows the five genera with the maximal differences, the X axis denotes the name of genus, and the Y axis denotes the relative abundance of the genus

in Atlantic salmon (*Salmo salar*) [38] and gibel carp (*Carassius auratus gibelio*) [36], however, the changes of gut microbiota tended to be stable in the adult stages of gibel carp and zebrafish [39]. Bledsoe et al. found that during early development the gut microbiota diversity of channel catfish showed a significant difference between 3 days and 65 days after hatching and between 65 days and 125 days after hatching [25]. Giatsis et al. also found that there were significant differences in the gut microbiota of the juvenile at different developmental stages in a

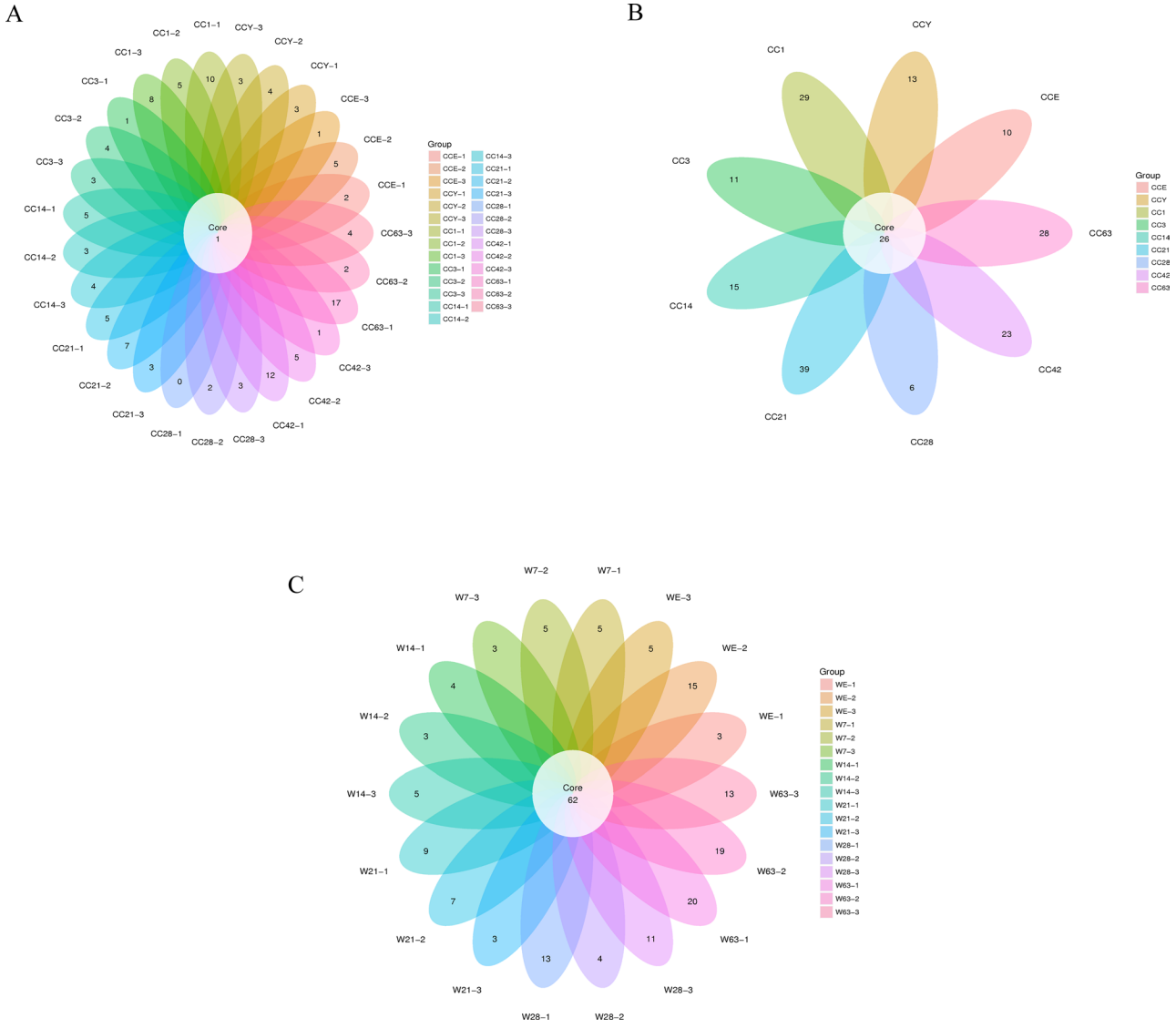

Fig. 7 Core microbiota in the intestine of juvenile common carp during development. (**A**) Petal diagram of OTUs in common carp samples. Each petal denotes one sample, the number in the petal denotes the number of OTUs occurring only in that sample, and the number at the center of the petals denotes the number of OTUs common to all samples. (**B**) Petal diagram of OTUs in common carp samples. Each petal denotes a pooled sample of one group, the number in the petal denotes the number of OTUs occurring only in that sample group, and the number at the center of the petals denotes the number of OTUs common to all sample groups. (**C**) Petal diagram of OTUs in pond water samples. Each petal denotes one sample, the number in the petal denotes the number of OTUs occurring only in that sample, and the number at the center of the petals denotes the number of OTUs common to all samples

circulating aquaculture system [28]. These results showed that the gut microbiota of juvenile fish had a series of dynamic changes along with the development process.

Proteobacteria, Cyanobacteria, Firmicutes and Actinomycetes were present in all samples of common carp, but their relative abundance changed with the development process of fish. This finding is similar to the composition of gut microbiota in the early developmental stages of Atlantic salmon (*Salmo salar*) [38] and pikeperch (*Sander lucioperca*) [40], indicating that Proteobacteria is a necessary part of the gut microbiota in the early development process of fish. According to the results of PCoA and NMDS analysis, the microflora composition of CCE, CCY and CC1 samples of common carp was more similar to that of pond water samples. 66.5% of OTUs in CCE also appeared in WE, and the number of reads contained in CCE accounted for 95.9% of the total reads, indicating that the water environment played an important role in the gut microbiota of common carp. The results were consistent with the reports of many kinds of other fishes [29, 41, 42].

In the early development process of common carp, the abundance of Cyanobacteria showed a trend of first increasing and then decreasing. Meanwhile,

Cyanobacteria were the main bacterial species in the feed, indicating that the feed flora have an impact on the gut microbiota of fish, and have an important impact on the health of fish [43]. However, the host selection plays a dominant role with the development of common carp, and the gut microbiota composition of common carp gradually shows significant differences from the environmental flora. This finding is also consistent with the development trend of intestinal bacterial communities in Southern Catfish [44, 45].

Conclusions

The gut microbiota of common carp changed dynamically with the development of juvenile fish, and the changes continued until at least 63 days after initial feeding. The early gut microbiota may come from external environment. The similarity between gut microbiota and water environment flora decreased with the development process, and there was a certain core flora in the gut of common carp, indicating that the gut of common carp had a selective effect on environmental flora, and host selection pressure was an important factor affecting gut microbiota. Therefore, the study provided basic data for the changes of intestinal flora in the early stage of carp development. Furthermore, the fish microbiota can be changed through adding of related prebiotics in the early stage of development, which may treatment for fish related diseases and improve fish health [21, 45, 46].

Abbreviations

OUT	Operational Taxonomic Units
PCoA	Principal Coordinate Analysis
NMDS	Non-metric Multidimensional Scaling
Anosim	Analysis of similarities

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

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Not applicable.

Author contributions

Jiahui Zhang: Performed the experiments, Analyzed the data, Writing – original draft. Yu Liu: Performed the experiments, Analyzed the data. Shijuan Shan: Performed the experiments, Funding acquisition. Cong Xu: Analyzed the data, Writing – review & editing. Liguo An: Conceptualization and design of study, Analyzed the data. Guiwen Yang: Writing – review & editing, Funding acquisition. Lei Wang: Conceptualization and design of study, Performed the experiments, Analyzed the data, Writing – original draft. Hua Li: Conceptualization and design of study, Analyzed the data, Writing – original draft, Writing – review & editing, Funding acquisition.

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Data availability

The NCBI Sequence Read Archive database has been logged to PRJNA1167471 for this raw data.

Declarations

Ethics approval and consent to participate

All animal experiments in this article were performed in accordance with relevant guidelines, which was approved by the Animal Experimental Ethics Committee of Shandong Normal University. The study was carried out in compliance with the ARRIVE guidelines. All common carp used in this article obtained the informed consent of Jinan Agricultural Technology Extension Service Center and could be used in this study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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