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Bacterial composition, abundance and diversity in fish polyculture and mussel–fish integrated cultured ponds in China

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Abstract

Bacterial community and abiotic environmental parameters in twelve freshwater aquaculture ponds were analysed. According to the major component of stocked animals, the ponds were grouped into four types: black carp *Mylopharyngodon piceus*, largemouth bass *Micropterus salmoides*, yellow catfish *Pelteobagrus fulvidraco* and pearl mussel *Hyriopsis cumingii* ponds. Each type of pond was stocked with three species of Chinese carps (silver carp, bighead carp and gibel carp) to form a unique mode of fish polyculture or mussel–fish integrated culture. The bacterial composition was identified using 16S rDNA sequencing. Totally, 3701 and 11 150 operational taxonomic units (OTUs) were identified from the water and sediment samples respectively. The number of OTUs, abundance-based coverage estimator, Chao1 index and Shannon diversity index were lower in the water column than in the sediment, suggesting that diversity and stability of bacterial community were higher in the sediment. In the water column, *Proteobacteria*, *Actinobacteria* and *Bacteroidetes* dominated at the phylum level, and 26 dominant genera were identified. In the sediment, *Proteobacteria*, *Chloroflexi*, *Bacteroidetes*, *Acidobacteria* and *Nitrospirae* dominated at the phylum level, and 25 dominant genera were identified. Bacterial compositions between the ponds with different aquaculture modes were similar at the phylum levels, but varied at the genus levels. The bacterial composition in the ponds was correlated with hardness, ammonia and total nitrogen in the water column. This study indicates that the type of aquaculture mode is a factor regulating the microbial community, which provides an insight towards microbial management through probiotic manipulation in pond culture.

Keywords: bacterial community, diversity, water column, sediment, fish polyculture, integrated culture

Introduction

Aquaculture has been expanded rapidly in the world in the past four decades to meet the increasing demand for food proteins (FAO 2014). Pond aquaculture greatly contributes to the production of freshwater fish due to the advancement of intensive aquaculture technology. In intensive pond culture, fish are stocked at high densities and fed with formulated feed, and aeration and water exchange are frequently used to maintain excellent water quality (Xie, Qin, Yang, Wang, Wang & Li 2013). However, the heavy loading of formulated feed to fish ponds has caused serious nutrient (nitrogen and phosphorus) wastes due to low nitrogen retention efficiency (<45%) in warm water fish fed formulated feed (Chai, Ji, Han, Dai & Wang 2013). Accumulation of nutrient wastes in ponds can be worsened with the age of the pond used for fish farming, and management of nutrient wastes is the key to improve fish production as well as to reduce aquaculture pollution.

The structure of food web in aquaculture systems is generally simplified to optimize nutrient flux efficiency from food input to uptake of farmed animals. In intensive fish ponds, formulated feed is

the main source of nutrient input. The uneaten food and fish faeces can be utilized by filter-feeding animals (e.g. silver carp, bighead carp and mussels) and microbial organisms (e.g. algae and bacteria) as nutrients (Schneider, Sereti, Eding & Verreth 2005). The wastes accumulation in fish ponds depends on feed input, feed utilization efficiency and wastes utilization efficiency of the filter-feeding animals and microbes. Measures need to be taken before concentration of wastes reach a critical point that negatively affect growth and health of fish.

Bacteria are principal degraders of organic compounds in terrestrial and aquatic ecosystems. Fish production in ponds is dependent on microbial community as microbial metabolism can affect the situation of dissolved oxygen, organic compounds and hazardous toxicity (Moriarty 1997), and fish diseases occur when pathogenic organisms prevail (Murray & Peeler 2005). The function of adding microbial products to improve water quality in aquaculture systems is controversial although commercial microbial products have been widely used in aquaculture practice (Tang, Dai, Li, Qin & Wang 2016). Understanding bacterial diversity becomes important to improve the application of microbial products as the native bacterial community can sway the abundance and function of any microbes added to the system. The microbial composition has been studied in the water column and sediment of shrimp ponds (Li, Zhang, Juck, Fortin, Greer & Tang 2010; Tang, Tao, Tan, Mu, Peng, Yang, Tong & Chen 2014; Zhang, Sun, Liu, Xuan, Jiang, Pan, Zhang, Gong, Lu, Yu, Kumar, Hu, Cao, Xue & Gong 2016), freshwater crab ponds (Liu, Zhou, He, Yao & Ringo 2013), seawater fish ponds (Zeng, Ma, Wei, Jiao, Tang, Wu & Jian 2010; Pereira, Salvador, Arrojado, Silva, Santos, Cunha, Gomes & Almeida 2011) and lobster tanks (Bourne, Young, Webster, Payne, Salmon, Demel & Hall 2004; Payne, Hall, Sly & Bourne 2007). However, knowledge in bacterial diversity in freshwater polyculture and integrated ponds is scarce (Zhou, Wang, Tang & Dai 2013; Huang, Liu, Li & Wang 2014).

Black carp (*Mylopharyngodon piceus*), largemouth bass (*Micropterus salmoides*), yellow catfish (*Pelteobagrus fulvidraco*) and pearl mussel (*Hyriopsis cumingii*) are commercially important for pond culture in China (Wang, Wang, Qin, Wang & Zhu 2009; Chiu, Li, Guo, Bai, Fedor & Naylor 2013). In a natural habitat, black carp and largemouth bass are carnivore, and feed on molluscs or small fish respectively. Yellow catfish is an omnivore and feed on aquatic insects and detritus. Pearl mussel is also an omnivorous filter but feed on microalgae and detritus. In commercial farming ponds, black carp and yellow catfish are fed with formulated feed, and largemouth bass are fed with either raw fish or formulated feed. Pearl mussel

under the water surface and were transferred into 1-L sterile glass bottles. The sediment cores were collected with a Plexiglas tube sampler (Zhou *et al.* 2013) and were transferred into a 250-mL sterile glass bottle. Only the top 10 cm of the sediment core was used for microbial and chemical analyses. At each sampling site, temperature, pH and dissolved oxygen (DO) in the water column were measured with a YSI 63 pH meter and a YSI 550A DO meter (Yellow Spring Inc., Ohio, USA) respectively. Secchi depth was measured with a Secchi disc. The water and sediment samples were labelled as BCW and BCS for the black carp ponds, MSW and MSS for the largemouth bass ponds, PFW and PFS for the yellow catfish ponds, and FMW and FMS for the pearl mussel ponds respectively. The water and sediment samples were stored in a portable freezer at 0°C and transported to the Laboratory of Aquatic Ecosystem and Aquaculture at Zhejiang University (Hangzhou, China), and were stored at 4°C in a refrigerator. The bacterial DNA extraction and chemical analysis were finished within 3 days.

Extraction and sequencing of bacterial 16S rDNA

A 100 mL subsample was taken from each water sample and was filtered through an 8-μm cellulose acetate membrane (diameter 47 mm, Xingya Purification Material Factory, Shanghai, China) to remove large particles. The liquid was further filtered through a 0.2-μm Whatman 111106 nuclepore track-etched membrane (diameter 47 mm, Whatman, Maidstone, UK). The bacterial genomic DNA on the nuclepore membrane was extracted with E.Z.N.A.® Water DNA kit (Omega Bio-Tek, Norcross, USA). The 16S rDNA variable V3-V4 region was amplified with the primer pair 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). The PCR was performed at ABI GeneAmp® 9700 with TransStart Fastpfu DNA Polymerase (TransGen Biotech, Beijing, China). The 20-μL PCR mixture comprised 4 μL 1× FastPfu Buffer, 2.5 mM dNTPs, 5 μM each of forward and reverse primers, 1 unit FastPfu Polymerase (2.5 unit μL^{-1}) and 10-ng DNA template. After an initial denaturation at 95°C for 3 min, 27 cycles each including 30 s at 95°C, 30 s at 55°C and 45 s at 72°C were performed. A final extension step was 10 min at 72°C. All the amplifications were checked using electrophoresis with 2% agarose gels. The bands were extracted and purified with the AxyPrepDNA Gel (Axygen, CA, USA). Pyrosequencing of the amplifications was performed on the Illumina MiSeq platform (Shanghai Majorbio Bio-Pharm Technology Co., Ltd, Shanghai, China).

All the raw reads were treated with the following processes. For quality control, the reads which contained one or more ambiguous bases ('N') were removed. Illumina sequencing generated a pair of reads from the two ends (paired-end reads) for one DNA fragment. A self-written script was developed to complementarily reverse one of the paired-end reads and then compared with each other. Then, the tag sequences were sorted into different individual files according to the barcodes of all samples.

Sequencing data were processed using read trimming and identification of V3-V4 sequences, and the chimeric sequences were identified and removed using UCHIME. Operational taxonomic units (OTUs) were identified with a cut-off of 97% identity. The reads from filtered OTUs were processed using Quantitative Insights into Microbial Ecology (QIIME) to construct a representative sequence for each OTU. All samples were rarefied to the size of the sample containing the fewest sequences. The representative sequences were assigned at different taxonomic levels (phylum to genus) to the SILVA data set of bacteria following the Bayesian approach and cut-off of 97%. The species abundance [abundance-based coverage estimator (ACE) and Chao1] and diversity (Shannon diversity index and Simpson's diversity index) of bacteria in each sample were calculated with QIIME. The Venn diagram with shared and unique OTUs was used to depict the similarity and difference among the bacterial communities.

In each pond, 1 g of sediment sample was used for DNA extraction. The bacterial genomic DNA in sediment was extracted with Ezup Column Soil DNA Purification Kit (Sangon Biotech, Shanghai, China). The 16S rDNA variable V3-V4 region was amplified with the primer pair 338F (5′-ACTCCTA CGGGAGGCAGCA-3′

oxygen demand (BOD_5) and chemical oxygen demand (COD_{Mn}) of the water samples were analysed with the methods described in APHA (2005). Nitrate was measured with the method described in Parsons and Takahashi (1973). Hardness was measured using titration with ethylenediaminetetraacetic acid **(**EDTA). The TN and TP in the sediment samples were measured with the method described in Bao (2008).

Calculation and statistical analysis

The differences in abiotic parameters of the water and sediment samples, sequence information (number of sequence and OTU) and bacterial diversity index among the ponds with different aquaculture modes were examined with one-way ANOVA respectively. Duncan's test was used to examine the differences between the ponds with different aquaculture modes. The differences in sequence information and bacterial diversity index between the water and sediment samples were examined with paired *t*-test. The ANOVA and *t*-test were performed using the software SPSS (version 21.0), and significant level was set at $P < 0.05$. The relationships of bacterial community between the water and sediment samples were examined using principal component analysis (PCA), and the relationship between bacterial community and abiotic parameters was examined using redundancy analysis (RDA). Prior to the PCA and RDA, abundance of bacterial OTU was standardized using Hellinger transformation. Forward selection for RDA was performed, and the abiotic parameters with variance inflation factors (VIF) <5 were selected. Only the parameters with significance ($P < 0.05$) were chosen by the Monte Carlo permutations. After the forward selection, variation partitioning was conducted to discriminate the influence of each significant parameter using partial RDA (pRDA). The PCA, RDA and pRDA were performed using the vegan package in R (Dixon 2003).

Results

Bacterial composition, abundance and diversity

Totally, 191 384 and 127 576 effective sequences of 16S rDNA were identified from the water and sediment samples respectively. The reads sequences were more in the water column (15 949 ± 5425, $n = 12$) than in the sediment (10 636 ± 3599, $n = 12$, *t*-test, $P < 0.05$). The OTU table was rarefied at 7180 sequences per sample (the minimum number of the sequences among all the samples). Bacterial richness and diversity are shown in Table 1. At 97% gene similarity, a total of 305, 383, 227 and 319 OTUs were identified in the BCW, FMW, MSW and PFW samples respectively. The number of OTUs, ACE and Chao 1 index were lower in the MSW sample than in the BCW, FMW and PFW samples (Duncan's test, $P < 0.05$). At 97% gene similarity, a total of 981, 667, 1028 and 1041 OTUs were identified in the BCS, FMS, MSS and PFS samples respectively. The Chao 1 and ACE were lower in the FMS sample than in the PFS sample (Duncan's test, $P < 0.05$).

The number of OTUs (308 ± 69, $n = 12$), ACE (404 ± 104, $n = 12$), Chao1 index (409 ± 107, $n = 12$) and Shannon diversity index (5.8 ± 0.7, $n = 12$) of the water column were lower than those (929 ± 237, 1220 ± 289, 1224 ± 281, 8.3 ± 0.8, $n = 12$) of the sediment (*t*-test, $P < 0.05$). Simpson diversity index (0.98 ± 0.01, $n = 12$) of the water column was higher than that (0.94 ± 0.05, $n = 12$) of the sediment (*t*-test, $P < 0.05$).

The Venn diagrams are shown in Figure 1. In the water samples, 213 common OTUs were identified, and the number of special OTUs was 58, 173, 26 and 99 in the BCW, FMW, MSW and PFW samples respectively. In the sediment samples, 1035 common OTUs were identified, and the number of special OTUs was 109, 131, 79 and 126 in the BCS, FMS, MSS and PFS samples respectively. The bacterial communities in the water column were clustered together, while the bacterial communities in the sediment dispersedly distributed (Fig. 2). The bacterial communities in the sediment of the pearl mussel ponds separated from those of the fish ponds. The horizontal axis and vertical axis explained the 36.1% and 13.1% information respectively.

At the phylum level, *Proteobacteria*, *Actinobacteria* and *Bacteroidetes* shared 67%–77% of the total OTUs in the water column of all the ponds, while the other OTUs were mainly classified to *Cyanobacteria*, *Verrucomicrobia*, *WCHB1-60*, *Chloroflexi*, *Planctomycetes*, *Chlorobi*, *Candidate_division_TM7* and *Firmicutes* (Fig. 3a). At the genus level, totally 26 dominant genera (the genus with percentage >1%) were identified, and the relative abundance of *CL500-29_marine_group*, *Sporichthyaceae_unclassified*, *Alpinimonas* and *Zymomonas* was significantly different among the ponds (Fig. 3b). The

Table 1 Bacterial indices of water and sediment samples in the fish polyculture and mussel–fish integrated culture ponds (mean ± SD, $n = 3$)

Sample	OTUs*	ACE†	Chao 1 index‡	Shannon diversity index	Simpson diversity index
BCW	305 ± 28	392 ± 50	396 ± 53	5.8 ± 0.6	0.95 ± 0.05
FMW	383 ± 72	509 ± 142	520 ± 152	6.2 ± 0.3	0.96 ± 0.01
MSW	227 ± 12	300 ± 23	306 ± 11	3.6 ± 0.4	0.92 ± 0.07
PFW	319 ± 38	415 ± 50	417 ± 45	5.1 ± 0.7	0.93 ± 0.08
BCS	981 ± 255	1295 ± 284	1287 ± 299	8.3 ± 1.1	0.99 ± 0.01
FMS	667 ± 29	874 ± 31	901 ± 23	7.7 ± 0.2	0.99 ± 0.00
MSS	1028 ± 271	1329 ± 330	1319 ± 309	8.7 ± 0.7	0.99 ± 0.00
PFS	1041 ± 168	1381 ± 155	1387 ± 182	8.4 ± 0.9	0.99 ± 0.02

BCW, FMW, MSW and PFW represented the water samples from black carp ponds, pearl mussel ponds, largemouth bass ponds and yellow catfish ponds respectively. BCS, FMS MSS and PFS represented the sediment samples from black carp ponds, pearl mussel ponds, largemouth bass ponds and yellow catfish ponds respectively.

*OTU: operational taxonomic units that were determined with a 3% width.

†ACE: abundance-based coverage estimator.

‡Chao1: richness estimates.

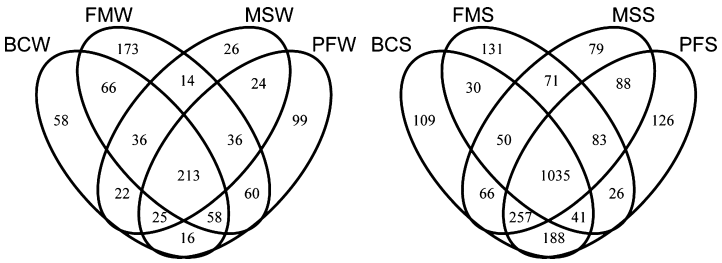


Figure 1 Venn of bacterial communities (on OTUs at 3% distance) in the water column and sediment of the fish polyculture and mussel–fish integrated culture ponds.

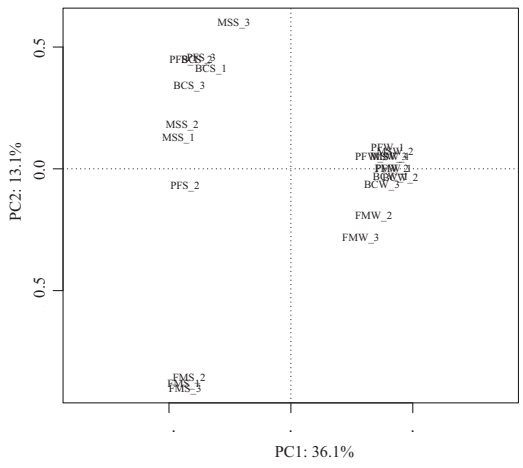
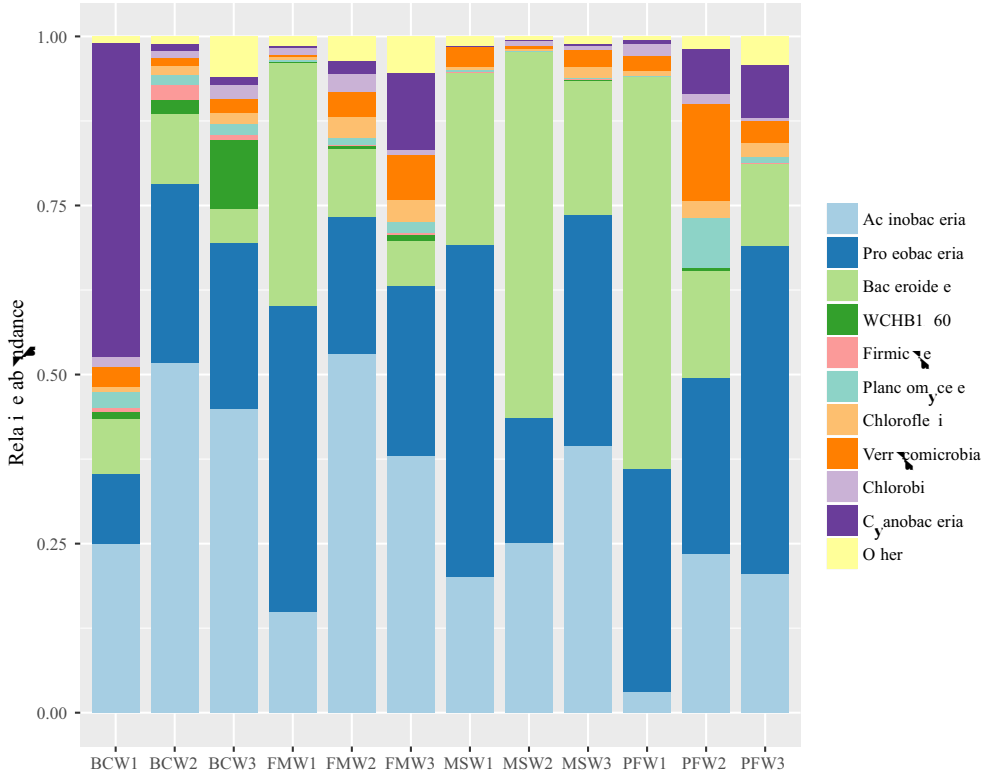


Figure 2 Principal component analysis (PCA) on bacterial communities in the fish polyculture and mussel–fish integrated culture ponds.

percentage of *CL500-29_marine_group* was higher in the FMW sample (14.4%) than in the PFW sample (1.9%, Duncan's test, $P < 0.05$). The percentage of *Sporichthyaceae_unclassified* was higher in the BCW sample (1.7%) than in the MSW (0.25%) and PFW samples (0.17%, Duncan's test, $P < 0.05$). The percentage of *Alpinimonas* was higher in the BCW sample (8.1%) than in the FMW (0.32%), MSW (1.6%) and PFW samples (2.3%, Duncan's test, $P < 0.05$). The percentage of *Zymomonas* was higher in the MSW sample (2.1%) than in the FMW (0.33%) and PFW samples (0.44%, Duncan's test, $P < 0.05$). In the ponds with the same aquaculture mode, the percentage of *Flavobacterium* varied greatly in the FMW (FMW1, 18.6%; FMW2, 0.27%; FMW3, 0.14%), MSW (MSW2, 42.9%; MSW1, 9.3%; MSW3, 5.8%) and PFW (PFW1, 52.2%; PFW2, 3.8%; PFW3, 2.4%) samples. The percentage of *Rhodobacteraceae_unclassifie*d was 13.9%, 0.22% and 0.05% in the FMW1, FMW2 and FMW3 samples respectively. The percentage of *Cyanobacteria_norank* was 45.3%, 0.75% and 0.48% in the BCW1, BCW2 and BCW3 samples respectively. The percentage of *Microcystis* was 7.7%, 0.02%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Rela i e ab ndance
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MSW3
PFW1
PFW2
PFW3
Ac inobac eria
Pro eobac eria
Bac eroide e
WCHB1 60
Firmic e
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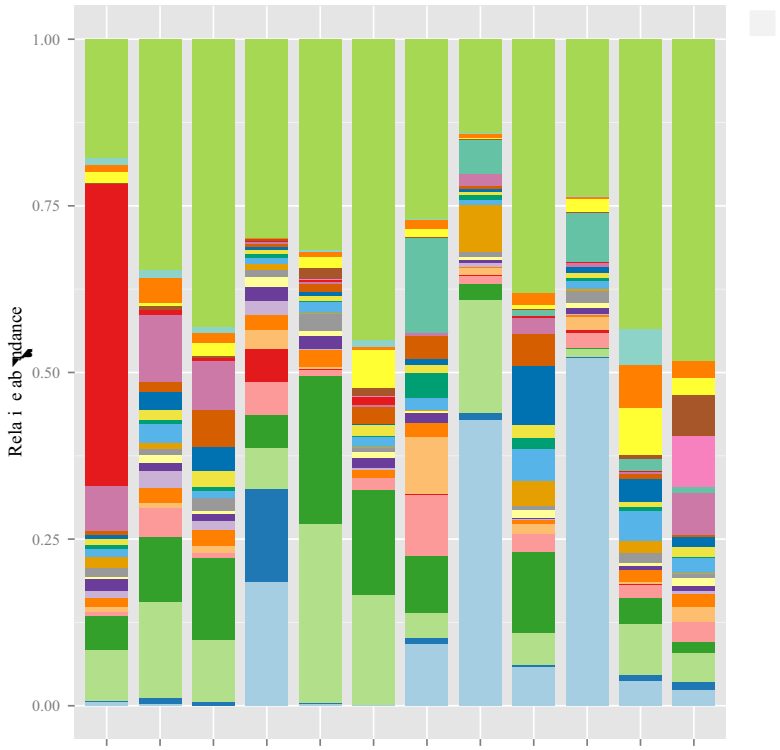
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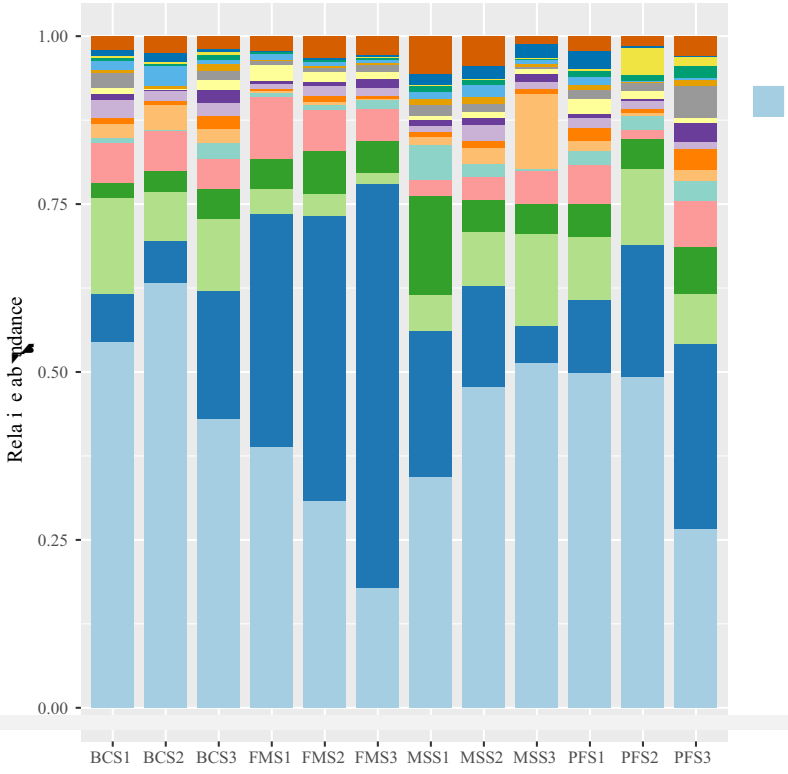
and 0.02% in the PFW3, PFW1 and PFW2 sam-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BCS1
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FMS3
MSS1
MSS2
MSS3
PFS1
PFS2
PFS3

Table 2 Parameters of abiotic environment in water column of the polyculture and integrated culture ponds (mean ± SD, $n = 3$)

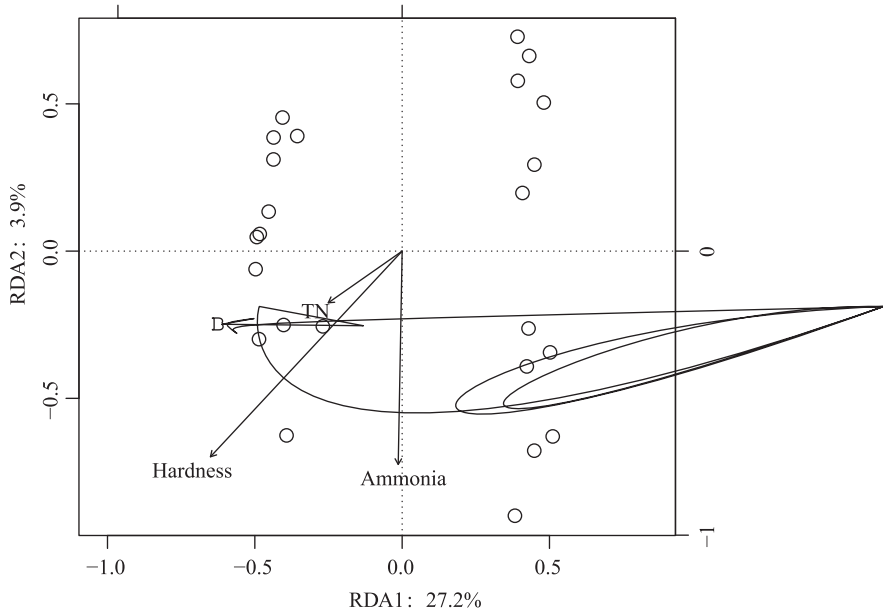
Sample	BCW	FMW	MSW	PFW
Water temperature (°C)	25.9 ± 0.9^{b}	23.2 ± 0.2^{a}	26.2 ± 0.4^{b}	26.2 ± 0.4^{b}
Secchi depth (cm)	38.7 ± 14.6	22.7 ± 2.1	29.7 ± 4.9	23.7 ± 17.2
pH	7.4 ± 0.3	8.3 ± 1.2	7.8 ± 0.2	8.2 ± 0.0
DO (mg L^{-1})	4.2 ± 1.7^{a}	3.8 ± 1.2^{a}	5.2 ± 2.8^{ab}	8.4 ± 0.7^{b}
Hardness ($CaCO_3$, mg L^{-1})	93.2 ± 4.2^{c}	34.1 ± 1.1^{a}	82.7 ± 6.0^{b}	83.9 ± 4.5^{b}
Ammonia (mg L^{-1})	2.41 ± 1.31^{b}	0.22 ± 0.01^{a}	4.82 ± 0.74^{c}	2.84 ± 1.61^{bc}
Nitrite (mg L^{-1})	0.109 ± 0.077^{ab}	0.004 ± 0.004^{a}	0.184 ± 0.106^{b}	0.269 ± 0.110^{b}
Nitrate (mg L^{-1})	0.38 ± 0.28	0.05 ± 0.04	0.41 ± 0.40	0.04 ± 0.02
Reactive phosphate (mg L^{-1})	0.03 ± 0.02	0.07 ± 0.00	0.25 ± 0.30	0.29 ± 0.27
TN (mg L^{-1})	4.64 ± 0.57	2.67 ± 0.63	5.95 ± 2.72	6.51 ± 3.82
TP (mg L^{-1})	0.31 ± 0.10^{a}	0.32 ± 0.09^{a}	0.86 ± 0.36^{b}	0.85 ± 0.26^{b}
COD_{Mn} (mg L^{-1})	12.5 ± 2.4	11.3 ± 2.2	13.2 ± 1.5	22.8 ± 15.7
BOD_5 (mg L^{-1})	9.9 ± 2.6^{ab}	4.8 ± 0.8^{a}	10.5 ± 4.7^{ab}	14.4 ± 6.0^{b}

BCW, FMW, MSW and PFW represented the water samples from black carp ponds, pearl mussel ponds, largemouth bass ponds and yellow catfish ponds respectively. DO, dissolved oxygen; TN, total nitrogen; TP, total phosphorus; COD_{Mn}, chemical oxygen demand; BOD_5, biochemical oxygen demand. The superscripts present the results of Duncan's test, and the values with different superscripts in the same row are significantly different ($P < 0.05$).

sediment samples of 12 fish polyculture or mussel–fish integrated culture ponds. The number of OTUs was more, and bacterial diversity was higher, in the sediment than in the water column. These results are consistent with the previous reports that microbial richness and diversity were higher in the sediment than in the water (Zeng *et al.* 2010; Garcia-Moyano, Gonzalez-Toril, Aguilera & Amils 2012).

In the present study, a total of 213 common OTUs were identified from the water column, while 1035 common OTUs were identified from the sediment. The bacterial OTUs in the water column were completely different from those in the sediment. The variation in the number of special OTUs among the ponds with different aquaculture modes was greater in the water column (26–173) than in the sediment (79–131). More common OTUs and smaller variation in the number of special OTUs suggest that bacterial community was more stable in the sediment than in the water column. The number of special OTUs was least in the water column of the ponds stocked with largemouth bass, while the number of special OTUs was most in the water column of the ponds stocked with pearl mussel. Meanwhile, bacterial diversity in the water column was lower in the largemouth bass ponds than in the pearl mussel ponds. This result suggests that the number of special OTUs might be an indicator for evaluating bacterial diversity in aquaculture ponds.

In previous studies, bacterial composition in water column is similar between the ponds stocked with Pacific white shrimp *Litopenaeus vannamei* and ponds stocked with grass carp (Zhang, Fu, Deng, Liang, Zheng, Sun, Zhu, Peng, Wang, Shen & Li 2013; Zhou *et al.* 2013; Tang *et al.* 2014; Zhang *et al.* 2016). In the present study, bacterial composition in the water column was similar at the phylum level but different at the genus level among the ponds with different aquaculture modes. *Proteobacteria*, *Actinobacteria* and *Bacteroidetes* dominated the bacterial communities in the water column of the ponds stocked with pearl mussel and fish. These results are consistent with the reports that *Proteobacteria*, *Actinobacteria* and *Bacteroidetes* generally dominate the bacterial communities in aquaculture ponds (Liu *et al.* 2013; Zhang *et al.* 2013; Zhou *et al.* 2013; Tang *et al.* 2014). *Flavobacterium* was abundant in the water column of the ponds stocked with pearl mussel, largemouth bass and yellow catfish. This result implies the risk of diseases in these ponds as *Flavobacterium* is a opportunistic pathogenic genus (Wiklund, Madsen, Bruun & Dalsgaard 2000). The percentage of *CL500-29_marine_grou*p in the water column was higher, while the nitrite was lower, in the pearl mussel ponds than in the yellow catfish ponds. This result is inconsistent with the conclusion that *CL500-29_marine_grou*p is positively correlated to the nitrate or nitrite (Liu, Fu, Yang, Zhao, He & Zhang 2015). The



percentage of *Zymomonas* in the water column was higher in the largemouth bass ponds than in the pearl mussel ponds and yellow catfish ponds. The higher percentage of *Zymomonas* in the largemouth bass ponds might be attributable to denitrification as *Zymomonas mobilis* can utilize gaseous nitrogen (Kremer, LaSarre, Posto & McKinlay 2015).

Results of the present study indicate that bacterial composition in the sediment was similar among the ponds with different aquaculture modes at the phylum level, but varied at the genus level. The percentage of *Proteobacteria*, *Chloroflexi* and *Bacteroidetes* in the sediment of the pearl mussel ponds differed from those of the black carp, largemouth bass and yellow catfish ponds. The variation in *Proteobacteria* among the ponds with different aquaculture modes was attributed to the change in percentage of *Nitrospinaceae_uncultured*, *Sva0485_norank*, *Rhodobacteraceae_unclassified*, *Nitrosomonadaceae_uncultured* and *Geobacter*. *Nitrospira* and *Nitrospinaceae_uncultured* were regarded as nitrite oxidizers (Lücker & Daims 2014; Daims, Lebedeva, Pjevac, Han, Herbold, Albertsen, Jehmlich, Palatinszky, Vierheilig, Bulaev, Kirkegaard, von Bergen, Rattei, Bendinger,

Nielsen & Wagner 2015). The percentage of *Nitrospira* and *Nitrospinaceae_uncultured* in bacterial composition was relatively high in the sediment of the pearl mussel ponds, suggesting the high activity in transforming nitrite to nitrate in these ponds. *Nitrosomonadaceae_uncultured* was regarded as ammonia-oxidizing bacteria (Prosser, Head & Stein 2014). The percentage of *Nitrosomonadaceae_uncultured* in bacterial composition in the sediment and ammonia in the water column were higher in the black carp, largemouth bass and yellow catfish ponds than in the pearl mussel ponds. These results suggest that ammonia oxidation by *Nitrosomonadaceae_uncultured* could not significantly reduce ammonia accumulation in these fish ponds.

The relationship between the bacterial composition and abiotic environments remains unclear. Li, Li, Yu, Qin and Wang (2013) reported that bacterial composition in the sediment of intertidal regions for mariculture is correlated with reactive phosphate, salinity, ammonia and chlorophyll *a*. In the present study, the abiotic parameters varied among the ponds with different aquaculture modes. The ammonia, nitrite, TN and TP in the water column were higher in the largemouth bass and yellow catfish ponds than in the pearl mussel ponds due to high dietary nitrogen input in these fish ponds (Wang *et al.* 2009; Ye, Tan, Chen & Luo 2009; Tang *et al.* 2015; Tidwell, Webster & Coyle 1996). Hardness is generally lower in the pearl mussel ponds as the pearl mussel absorbs calcium for shell growth and pearl formation (Wang *et al.* 2009). Results of RDA indicate that bacterial composition was significantly correlated with hardness, ammonia and TN in the water column in the fish polyculture and mussel–fish integrated culture ponds. Considering hardness, ammonia and TN is significantly affected by the aquaculture mode, these results reveal that aquaculture mode should be a factor regulating the structure of bacterial community in the ponds.

In conclusion, 3701 and 11 150 OTUs were identified from the water column and sediment in 12 freshwater fish polyculture and mussel–fish integrated culture ponds, respectively, and bacterial diversity and stability were higher in the sediment than in the water column. Bacterial composition in the water column and sediment between the ponds with different aquaculture modes were similar at the phylum level, but varied at the genus level. The dominated bacteria were *Proteobacteria*, *Actinobacteria* and *Bacteroidetes* in the water column, and *Proteobacteria*, *Chloroflexi*, *Bacteroidetes*, *Acidobacteria* and *Nitrospirae* in the sediment. Bacterial composition in either water column or sediment of the ponds correlated with hardness, ammonia and TN in the water column.

Conflict of Interest

The authors declare no conflict of interest.

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