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Nutrients, heavy metals and microbial communities co-driven distribution of antibiotic resistance genes in adjacent environment of mariculture☆

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ABSTRACT

With the rapid development of aquaculture, the large amounts of pollutants were discharged into the aquatic environment, where the detected antibiotic resistance genes (ARGs) have drawn increasing attention due to their potential threats to ecological environment and human health. Thus, the impact of mariculture on ARGs was assessed and the underlying mechanism of their propagation was explained. Sediments from eight sampling sites were collected along a mariculture drainage ditch, and the sediment in Yellow River Delta National Park was used as a non-mariculture control. Microbial ARGs qPCR array and illumina sequencing of 16S rRNA gene were applied to examine the changing patterns of ARGs and bacterial communities. Results showed that 18 ARGs (3 fluoroquinolone, 1 aminoglycoside, 3 macrolide-lincosamide-streptogramin B, 2 tetracycline, and 9 beta-lactam resistance genes) were influenced by mariculture, and ARGs abundance and diversity were significantly increased in mariculture sediments ($p < 0.05$). A remarkable shift in bacterial community structure and composition was also observed. The abundance of most of ARGs were significantly decreased in the estuary samples, implying that seawater had a significant dilution effect on the ARGs emission from the mariculture sites. Partial redundancy analysis showed that nutrients, heavy metals, and bacteria communities might directly and indirectly contribute to ARGs propagation, suggesting that the profile and dissemination of ARGs were driven by the combined effects of multiple factors in mariculture-impacted sites.

1. Introduction

Recently, growing attention has been paid to the worldwide emergence and spread of antibiotic resistance genes (ARGs) owing to their unfavorable consequences of human health in modern medicines (Levy and Marshall, 2004). The increase of antibiotic resistance in pathogens bequeathed by the ARGs can complicate the treatment of diseases (Cosgrove et al., 2004) and has resulted in more than two million infections and 14,000 deaths each year in the United States (WHO, 2001). It was reported that the expansion of aquaculture has accelerated the prophylactic and therapeutic usage of antimicrobials, among which some of them were crucial in clinical therapeutics (Cabello et al., 2013). In general, the large number of incomplete metabolites and intact antibiotics exist in aquaculture sites, which has promoted the development of antibiotic resistance (Liang et al., 2013; Sapkota et al., 2008). Aquaculture pond is considered as one kind of the reservoir of ARGs (Seyfried et al., 2010; Xiong et al., 2015). Diverse ARGs have been detected in water and sediment of aquaculture and effluent-receiving aquatic environments (Buschmann et al., 2012; Di Cesare et al., 2013; Harnisz et al., 2015; Nonaka et al., 2007).

The awareness of antibiotic misuse harmful to human health has stimulated the surveillance on antibiotic administration (Laganà et al., 2011; Scarano et al., 2014). Denmark has banned the use of antibiotics as growth promoters (Aarestrup et al., 2010). In the

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United States, the antibiotics used for human medicine will also cease for animal growth promotion after 2016, and veterinary oversight will be required for therapeutic uses (White House, 2015). However, antibiotics are not the only selective pressure of ARGs in aquaculture areas. Recent studies showed that ARGs were detected even in the aquaculture environments where antibiotics were never used. For example, antibiotic-resistant bacteria had been isolated from the fish, feed and environmental samples in an aquaculture ecosystem with no known antibiotics history (Huang et al., 2015). Tetracycline, sulphonamide and trimethoprim resistance genes persisted in a fish farm of the Baltic Sea, in which antibiotics were stopped to use 6 years ago (Muziasari et al., 2014; Tamminen et al., 2011b). Several mechanisms affecting the dynamics of ARGs were found in different environments. Horizontal gene transfer (HGT) mediated by mobile genetic elements such as integrons and plasmids has been demonstrated as the major driving force in the dissemination of ARGs (Chen et al., 2013; Luo et al., 2014; Szczepanowski et al., 2008). Additionally, co-selection of antibiotic and heavy metal resistances resulted from heavy metals, particularly Cd, Hg, Cu, and Zn was frequently in water and soil (Seiler and Berendonk, 2012). Moreover, recent studies have proved that bacterial community variation has a more contribution to resistome than HGT in soil, sludge and drinking water (Forsberg et al., 2014; Jia et al., 2015; Su et al., 2015). Thus, more exhaustive mechanisms of ARGs dynamics in environments influenced by aquaculture should be revealed for reducing the risks of ARGs.

To date, most studies on ARGs in aquaculture environment focused on a few human pathogens, such as *Aeromonas* (Deng et al., 2014; Han et al., 2012), *Vibrio* (Aedo et al., 2014; Reboucas et al., 2011) and *Enterococci* (Di Cesare et al., 2013) by using culture-dependent methods. Some researches analyzed ARGs in aquaculture environment by culture-independent methods, however, most of them merely targeted at a few specific ARGs according to their research purposes (Harnisz et al., 2015; Muziasari et al., 2014). Until now, there is no research obtained the comprehensive ARGs profile and how aquaculture activities drove the distribution of ARGs in aquaculture environment. Furthermore, the bacterial community composition in aquaculture environment has not been entirely explored. The objectives of this study were to investigate the response of ARGs and bacterial community to mariculture activities and the factors which involved in the ARGs shift in mariculture sediments. To the best of our knowledge, this paper makes the first pioneer to investigate ARGs variation in aquaculture environment using the microbial qPCR array technique.

2. Materials and methods

2.1. Sample collection and DNA extraction

Surface sediment samples (~500 g) were collected at 8 stations along a mariculture drainage ditch (N 37°34′44.0″, E 118°56′24.9″) in June (wet season) and October (dry season) 2014 in Dongying, China (Fig. 1). Different seasons are of different natural conditions such as temperature, rainfall and salinity, which can change the physical and chemical properties and microbial community of the research sites (Patel et al., 2014; Suh et al., 2015; Kaevska et al., 2016). Shrimp and sea cucumber were reared in the mariculture ponds around the drainage ditch. In front of the mariculture facilities, there are some houses regularly connected to sewage systems which were not linked with the drainage ditch. The sediments on

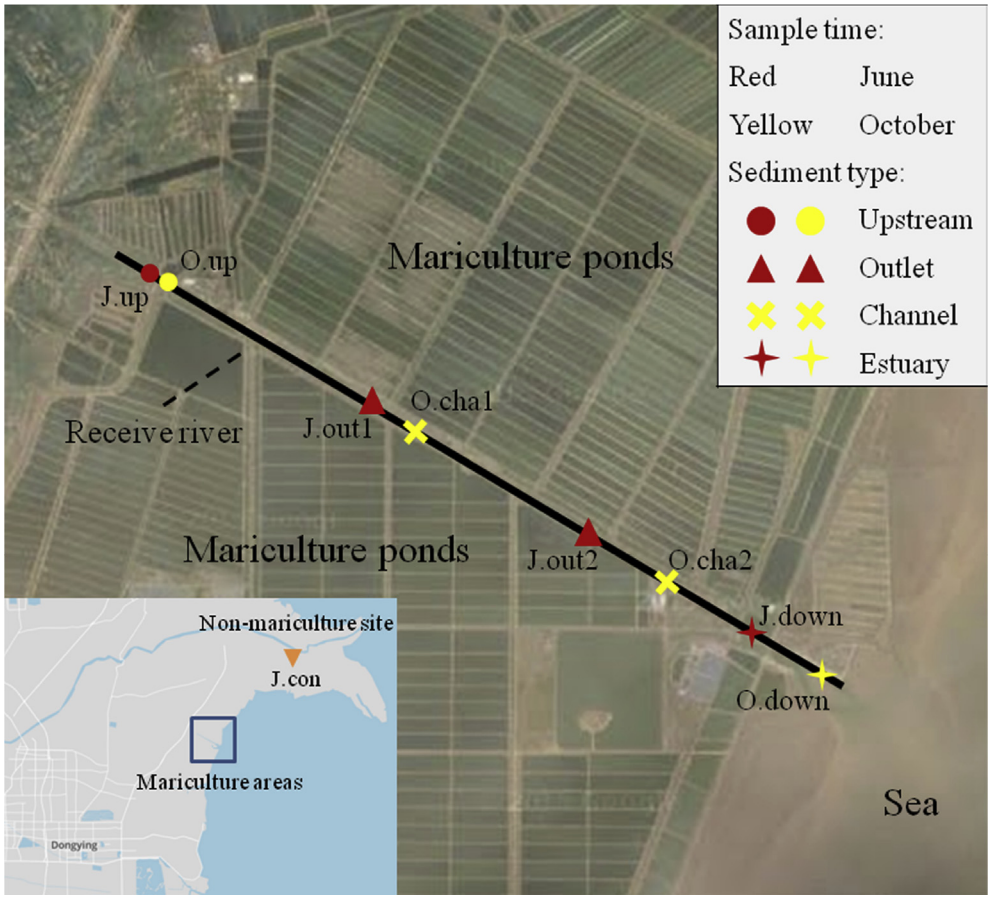

Fig. 1. Map of the sampling sites.

the upstream and downstream of the mariculture discharge area were sampled in June and October. The sediments located at mariculture pond outlets and drainage ditch channel were sampled in June and October, respectively. A surface sediment in Yellow River Delta National Park collected in July 2014 used as a non-mariculture control. It should be pointed out that all sampling was completed during the ebb tide period. The sediments were aseptically collected in triplicates from each site with sterile containers, immediately placed on ice, transported to the laboratory and stored at −20 °C for further analysis. The water quality parameters of the sampling sites were listed in Table S1 (Supporting Information).

All DNA were extracted using the FastDNA SPIN Kit for Soil (MP Biomedicals, CA), and the concentration and purity of them were measured by NanoPhotometer® Classic Launched (IMPLEN, GRE). DNA extraction of each sample was performed in triplicates, and mixed to eliminate heterogeneity in each sample and potential bias during the DNA extraction.

2.2. Sediment characterization

TOC was achieved by using Elementar Vario EL III with 0.25 g sample after removing inorganic carbon with 10% HCl (Wu et al., 2014). TN, NH_4^+-N and available phosphates analyses were performed as described by Islam et al. (Islam et al., 2004). Cu, Pb, Zn, Hg and Cr were analyzed using an inductively coupled plasma-mass spectrometry (ICP-MS, Optima, 2000 DV, Perkin Elmer, USA) (Yuan et al., 2004).

2.3. 16S rRNA gene amplication, sequencing, and data processing

The V4 region of 16S rRNA gene in each sample was amplified, quantified, pooled, purified and sequenced by Illumina Miseq platform at Novogene, Tianjin, China (Yan et al., 2015). Paired-end reads were appointed to the samples based on their unique barcodes and truncated by cutting off the barcode and primer sequences, and assembled into raw tags by using FLASH (Magoc and Salzberg, 2011). Quality filtering of raw tags was conducted on QIIME v1.8.0 (Caporaso et al., 2010) according to the specific filtering conditions (Bokulich et al., 2013) for achieving the high-quality clean tags. Sequences with ≥97% similarity were assigned to the same operational taxonomic unit (OTU). Representative sequence of each OTU was screened by the default method, which was assigned to a taxonomy using RDP classifier (Wang et al., 2007) based on Greengenes Database (DeSantis et al., 2006).

OTUs abundance was normalized using a standard of the number of sequences according to the sample with the least sequences. Subsequent analyses of alpha and beta diversities were performed with the normalized data. Alpha diversities including Observed-species, Chao1, Shannon, Simpson, ACE and Good-coverage, and beta diversities on Bary-Curtis, weighted unifrac and unweighted unifrac distances were calculated by using QIIME software (Version 1.8.0). Principal component analysis (PCA) and principal coordinate analysis (PCoA) were analyzed by FactoMineR and WGCNA packages in R software. Unweighted pair-group method with arithmetic means (UPGMA) clustering was executed to illustrate the weighted and unweighted unifrac matrix using average linkage and run by QIIME software (Version 1.8.0).

2.4. Microbial ARGs quantitative PCR array, quantification of 16S rRNA and class I integrons, and data analysis

To figure out the diversity and abundance of ARGs in samples, a microbial ARGs quantitative PCR (qPCR) array (cat. no. 330261 BAID-1901ZRA, QIAGEN, USA) was performed using the Agilent Mx3005p qPCR system (Agilent Technologies, USA) according to the manufacturer's instructions. This array contains 87 set of primers targeting at antibiotic resistance genes. The expression products of these ARGs resist to aminoglycoside, β-lactam, erythromycin, fluoroquinolone, macrolide-lincosamide-streptogramin B, tetracycline, vancomycin, and multidrug resistance classifications (Table S2). Pan-bacteria primer detecting a broad range of bacterial species was included to evaluate the number of bacteria in samples, and the positive PCR control was also included to test the presence of PCR inhibitors and the efficiency of the PCR. The PCR mixture (2550 μL) contained 1275 μL Microbial qPCR Mastermix, 500 ng microbial genomic DNA and microbial DNA-free water. Then it was equal divided into the 96-well specification for the microbial ARGs qPCR array that contained a mix of two pre-dispensed, gene specific primers and one fluorescent hydrolysis probe. The thermal cycle reaction was performed with an initial denaturation at 95 °C for 10 min, followed by a 40 cycles of denaturation at 95 °C for 15 s and annealing at 60 °C for 2 min. For each sample amplification was conducted in triplicates, and a non-template control was also included. The Microbial ARGs quantitative PCR array (cat. no. 330261 BAID-1901ZRA, QIAGEN, USA) performs with the primer efficiencies between 80 and 120%, the correlation coefficients (R) > 0.995, and the limit of quantification (LLOQ) < 100 gene copies for 97% of the ARGs. The baseline and threshold fluorescence values were manually adjusted to the same levels across all qPCR runs, and a threshold cycle (Ct) value of 37 was used as the detection limit. The normalized copy number (Eq. (1)) of ARGs and pan-bacteria were calculated referring to a previous study (Looft et al., 2012).

$$\text{Normalized copy number} = 10^{((37-\text{Ct})/(10/3))} \quad (1)$$

The absolute copy number of 16S rRNA and class I integrase (*intI1*) gene were quantified by Agilent Mx3005p qPCR system (Agilent Technologies, USA) using a SYBR Green approach. The qPCR mixture (20 μL) consisted of 10 μL 2 × SYBR® Premix DimerEraser (TaKaRa, Dalian, China), 0.2 μM of each primer (Table S3), 1 μL DNA template and nuclease-free water. The qPCR reactions were performed using the following protocol: 95 °C for 30 s followed by 40 cycles of denaturation at 95 °C for 5 s, annealing at the given temperatures (Table S3) for 30 s and extension at 72 °C for 30 s, finally with a melting curve analysis auto-generated by the program. Each reaction was run in triplicates, meanwhile sterile water was used as a negative control. Standard curves for copy number calculation of these genes were established according to the previous studies (Zhang and Fang, 2006; Zhang et al., 2009) and listed in Table S4. A significantly high correlation was obtained between the Ct value of pan-bacteria primer and the absolute copy number of 16S rRNA gene ($p < 0.01$). Both of them represent the number of bacteria in samples. Thus, the normalized copy number of ARGs generated by the microbial ARGs qPCR array could be transformed to absolute copy number of ARGs by dividing absolute 16S rRNA gene copy number (Ouyang et al., 2015) (Eq. (2)).

$$\begin{aligned}\text{Absolute copy number of ARG} &= \text{Absolute copy number of 16S rRNA genes} \\ &\quad * \text{Normalized copy number of ARG/Normalized copy number of pan} - \text{bacteria}\end{aligned} \quad (2)$$

The average number of 16S rRNA gene per bacteria cell was predicted at 4.1 based on the Ribosomal RNA Operon Copy Number Database (rrnDB version 4.3.3) (Stoddard et al., 2015). Bacterial cell number of each sample was then determined by dividing 16S rRNA gene copy number by this value, and the relative abundance of ARGs per bacterial cell was calculated.

2.5. Statistical analyses

Pearson correlation analysis was performed to explain the correlations between the abundance of ARGs and the concentration of chemical parameters. To evaluate the contribution of nutrients (TN, NH_4^+-N, available phosphates and TOC), heavy metals (Cu, Cr, Pb, Hg and Zn) and bacterial communities (the OTU profiles) to the ARGs propagation, PCoAs based on Bray-Curtis distance of these three groups were calculated first. Partial redundancy analysis (pRDA) was further conducted to determine the contribution of bacterial communities, nutrients, and heavy metals to the shift of ARGs using the first two PCs of the three groups PCoA. The correlation between bacterial community and ARGs profile was examined by Mantel test. Adonis test was conducted to determine the significance of difference in ARGs profile or bacterial community structure among samples. RDA and pRDA were performed in R3.2.3 with vegan package. Heatmap was performed using Heml v1.0 software. Other statistical analyses were conducted using PAST v3.08 (Hammer et al., 2001).

3. Results and discussions

3.1. Mariculture sediments characteristics

The chemical characteristics of the mariculture sediments were summarized in Table S5. The TN and TOC contents in the sediment samples were 2.00–4.03 and 13.81–16.46 g/kg, respectively. Similar result was also found in the Dongjiang River estuary (1.7–3.1 g/kg of TN and 18.2–21.6 g/kg of TOC) (Su et al., 2014). But the TOC concentrations in this study were higher than that in the sediments of neighboring Laizhou Bay (Wu et al., 2014). The contents of ammonia nitrogen and available phosphates were 81.6–220 and 9.91–44.1 mg/kg, respectively. Sediments in October had significantly higher ammonia nitrogen than June ($p < 0.05$). And no significant difference in ammonia nitrogen and available phosphates was found between mariculture and control samples.

All five heavy metals (Cr, Cu, Hg, Pb, and Zn) were detected in both the mariculture and control samples (Table S5). The concentration ranges of heavy metals were as follows: Cr, 45.0–88.0 mg/kg; Cu, 18.0–32.0 mg/kg; Hg, 26.0–66.0 mg/kg; Pb, 0.00–24.0 mg/kg; Zn, 36.0–107 mg/kg. In a study of mariculture sediments around the Pearl River Delta region, Liang et al. (2016) found that the mean concentrations of Cu, Zn, Cr, and Pb in all mariculture sediment samples were 109, 273, 99 and 33 mg/kg, compared with 63, 209, 56 and 23 mg/kg for the reference sediment samples, respectively. The concentration of most of heavy metals in the mariculture sediments from Dongying were much lower than those reported in Pearl River Delta. However, a range of 26.0–66.0 mg/kg Hg was found in this study, which was dozens of times higher than that in Chinese safety guideline (1.00 mg/kg, GB18668-2002). No significant difference in heavy metal concentrations was found between mariculture and control samples, suggesting that the heavy metals in mariculture area were independent from mariculture activities but their persistence could bring some potential risks.

3.2. Characterization of bacterial community

Few previous studies have investigated the change of microbial communities in aquaculture environments, while the vast majority of researches focused on the interior of the aquaculture ponds (Xiong et al., 2015) and the functional microorganisms associated with the growth of cultured food animals (Blancheton et al., 2013; Zhang et al., 2016). The studies of the bacterial communities around the ponds which were influenced by aquaculture were still limited. This study investigated the shift of bacterial communities in mariculture sediments by illumina sequencing based on 16S rRNA gene. A total of 557,815 tags with average 59,962 high quality tags per sample were obtained (the range of 50,875 to 64,378 tags, Table S7). These sequences were clustered into 6918 OTUs at 3% dissimilarity level (the range of 3351 to 4179 per sample, Table S6).

The dominant phyla in all samples were Proteobacteria (64.2%), Bacteroidetes (12.2%), Firmicutes (9.2%), Acidobacteria (2.3%), and

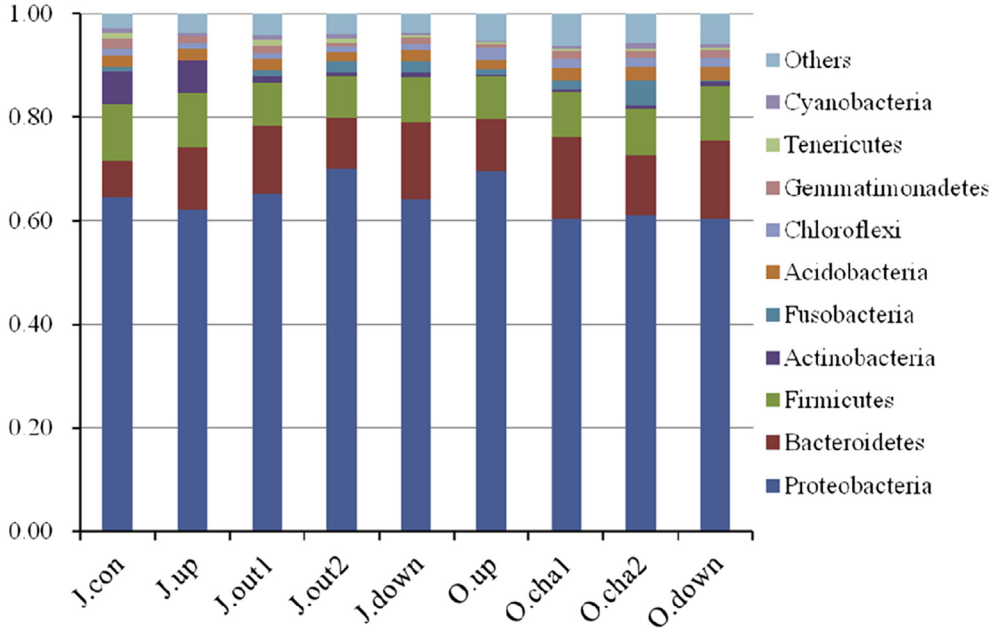

Fig. 2. Structure of microbial communities at phylum level.

Actinobacteria (2.1%), accounting for over 90% of the total bacterial 16S rRNA gene sequences (Fig. 2). Proteobacteria was the largest group in samples, which only represented a slight shift among all samples at phylum level. Further analysis found that at class level, Alphaproteobacteria decreased from 12.4% in J.con and 11.8% in J.up to 4.6–6.7% in other samples, and Betaproteobacteria declined from 7.1% in J.con to 0.8–4.3% in others. However, Deltaproteobacteria increased from 7.8% in J.con to 12.3–15.4% in other samples. Gammaproteobacteria represented 26.4% in O.up compared to 34.4–46.5% in others (Fig. S1). As the second abundant phylum, Bacteroidetes increased from 6.9% in J.con to 9.6–16.0% in other samples, which was attributed to the contribution of the classes Bacteroidales and Flavobacteriia (Fig. S1). The Firmicutes was primarily composed of class Clostridia, which occupied 10.3–10.9% in J.con, J.up and O.down but 8.0–8.9% in others (Fig. S1). The Actinobacteria was enriched in J.con (6.3%) and J.up (6.4%) compared to others (0.4–1.2%). Actinobacteria as the dominant class mainly contributed to the variation of the Actinobacteria phylum (Fig. S1). Generally, mariculture activities could lead to the increase of Deltaproteobacteria, Gammaproteobacteria and Bacteroidetes, and the decrease of Alphaproteobacteria, Betaproteobacteria, Firmicutes and Actinobacteria. Typical genera associated with opportunistic human pathogens were also observed. It should be noted that the genera of *Aermonas*, *Escherichia*, *Pseudomonas*, *Staphylococcus*, and *Vibrio* were present in all sediment samples with their relative abundance up to 0.1% (Table S7).

The overall bacterial communities were significantly altered by mariculture (Adonis test, $p < 0.05$). Based on the Bray-Curtis distance, PCoA showed that the mariculture-impacted samples were clustered together and separated from the other samples (Fig. 3A). The first two PCs explained a total of 60.6% variance of bacterial communities. The J.con and J.up samples were separated form others along PC1, which explained 39.5% of the variation. The O.up sample was separated from others along PC2, which explained 21.1% of the variation. These results suggested that mariculture activities significantly changed the bacterial communities at

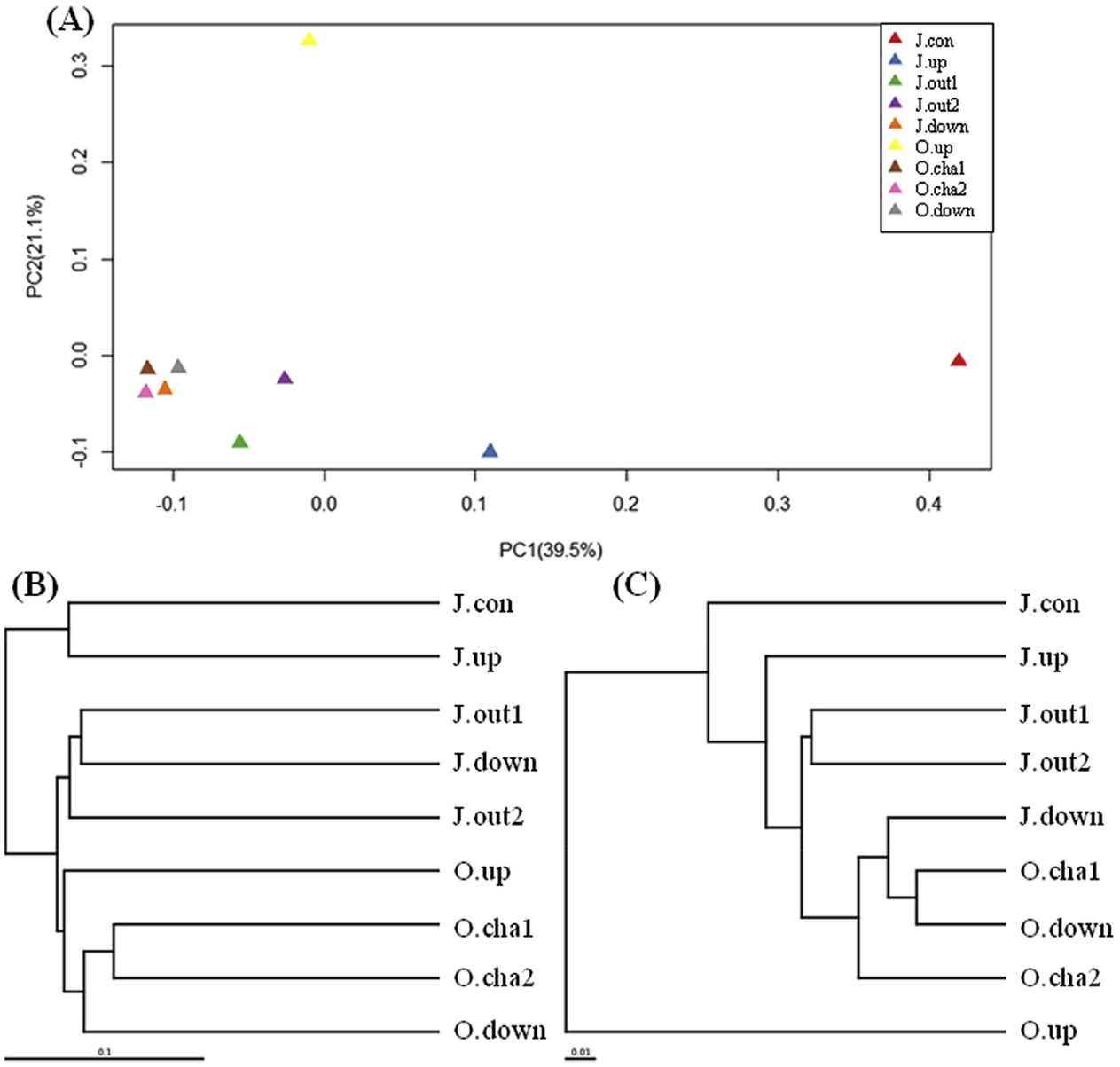

Fig. 3. (A) PCoA based on Bray-Curtis distance showing the overall distribution pattern of bacterial communities in all samples. (B) UPGMA clustering based on unweighted unifrac distance and (C) weighted unifrac distance.

downstream sediments of the ponds owing to the discharge of wastewaters. These divergences in bacterial communities were further demonstrated by PCoA (Fig. S2) and UPGMA clustering analysis based on the weighted and unweighted unifrac distances. UPGMA clustering analysis based on unweighted unifrac distance showed that samples were clustered into two groups according to the sampling time (Fig. 3B). Unweighted unifrac distance only considered the composition of the species without their relative abundance, indicating that seasonal variation was the main driving force of the change of bacterial community composition in mariculture sites. Similar results were also found in drinking water (Henne et al., 2013), coastal water (Patel et al., 2014), soil (Lipson, 2007) and mariculture ponds (Pereira et al., 2011). The above results demonstrated the need for an in-depth understanding of bacterial community change through the year in order to determine appropriate breeding management strategies. Furthermore, weighted unifrac distance considers not only the composition of species but also their relative abundance. UPGMA clustering analysis based on weighted unifrac distance showed that the samples of the sewage outfall and the river channel were clustered, respectively, and separated with upstream samples (Fig. 3C), which meaning that mariculture could significantly altered the microbial community structures of surrounding environments and had a strong effect on the sites close to mariculture wastewater drain outlets. Intensive aquaculture was well known to contribute to the eutrophication and oxygen depletion by introducing uneaten fish feed and fish feces into the aquatic environment (Kawahara et al., 2009). In fish farms, antibiotics were often mixed with fish feed to treat and prevent different fish illnesses (Cabello et al., 2013). The combined effect of high nutrient levels, oxygen depletion and antibiotic usage could change the microbiota of the sediments (Tamminen et al., 2011a). At the same time, marine could significantly reduce the impacts of mariculture activities, resulting that the microbial community structure was quite similar with that of the channel and downstream samples. The above results demonstrated that seasonal variation was the major driving force in microbial composition, and seawater dilution had a great effect on microbial relative abundance.

3.3. Variation of antibiotic resistance genes

Significant increase of the ARGs diversity was detected in mariculture drainage ditch sediments compared with that of control (Fig. 4A, $p < 0.05$), implying that more ARGs persisted in the place where there were strong anthropogenic pressures. Owing to anthropogenic activities, the emergence and dissemination of ARGs

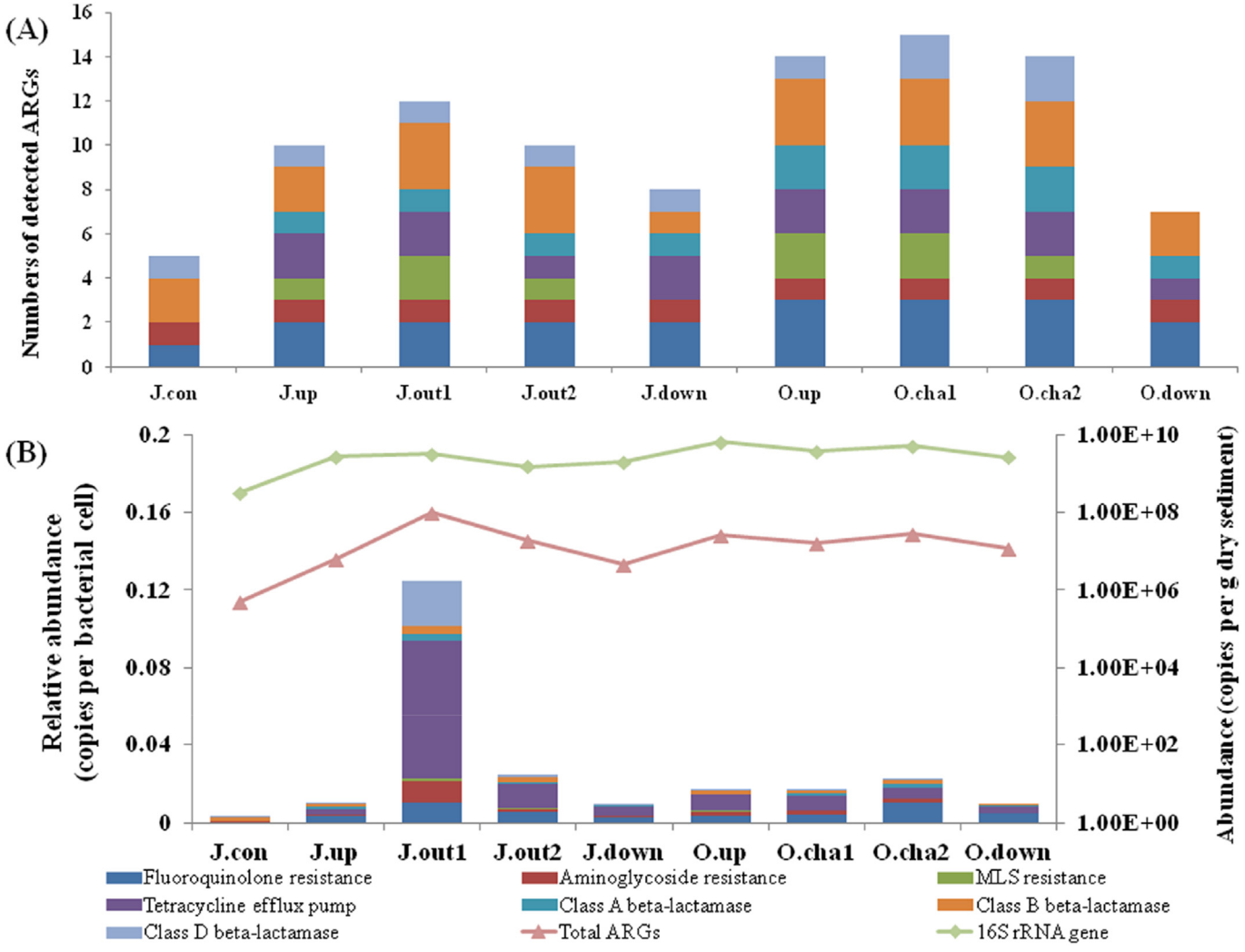

Fig. 4. (A) Number of ARGs detected in samples. (B) The relative abundance of ARGs presented as copies of ARGs per bacterial cell, and total copy number ARGs and 16S rRNA genes presented as copies per g dry sediment in samples.

have been found in urban river (Ouyang et al., 2015), agricultural soils (Woegerbauer et al., 2015) and feedlots environments (He et al., 2014). The number of detectable ARGs could reach 10–12 and 14–15 among upstream, outlet and channel samples in June and October, respectively. The number of detected ARGs significantly reduced in downstream samples compared with other mariculture samples at the same sampling time (Fig. 4A, $p < 0.05$), which could due to the highly disturbed by marine. The concentration of ARGs in downstream samples (see below) were also declined, some of which were even below the detection limit.

To assess the abundance of ARGs in the total bacterial community, the relative abundance of ARGs was calculated with the estimated bacterial cell number. The relative abundance of ARGs gradually increased in mariculture drainage ditch sediments, at least three times higher compared with that in control sediments (Fig. 4B). In mariculture area, the relative abundance of ARGs at outlet exploded compared with that at the upstream, and then it reduced at the downstream to the similar level with that at the upstream (Fig. 4B). Fluoroquinolone resistance genes and tetracycline resistance genes related to active efflux of antibiotic were the most abundant ARGs in the mariculture-impacted sediments. Some studies have quantified the abundance of ARGs in aquaculture surrounding environment by the culture-independent or culture-dependent methods. For instance, Tamminen et al. (2011b) found that no tetracycline, sulphonamide and trimethoprim resistance genes were detected in the surrounding environment of Baltic Sea aquaculture farms. In Drweca River, no significant difference in the abundance of oxytetracycline and doxycycline resistance bacteria and *tet* genes between upstream and downstream river water of a fish farm (Harnisz et al., 2015). Conflicting results between the above and present studies may be due to the different research methods. A small number of specific ARGs were investigated qualitatively and quantitatively in the above studies, which were not applicable to obtain a comprehensive result. However, in the present study, a high-throughput qPCR array was applied to evaluate the profile of ARGs, which could reflect the authentic overall change when some specific ARGs were relative stable.

Heatmap based on Euclidean distance presenting the normalized copy number of ARGs showed that the control sample alone was clustered to a group (Fig. 5). Thus, it is clear that the diversity and abundance of ARGs in the environment could be significantly changed by mariculture. In addition, the abundance of most of the ARGs was significantly decreased in J.down and O.down samples which highly disturbed by marine diffusion, suggesting that seawater had a significant dilution effect on the ARGs released from mariculture activities (Lu et al., 2015). However, it is worth noticing that the abundance of *ACC(6)-bl-cr* gene in J.down and O.down did not change significantly compared with that in other samples, and

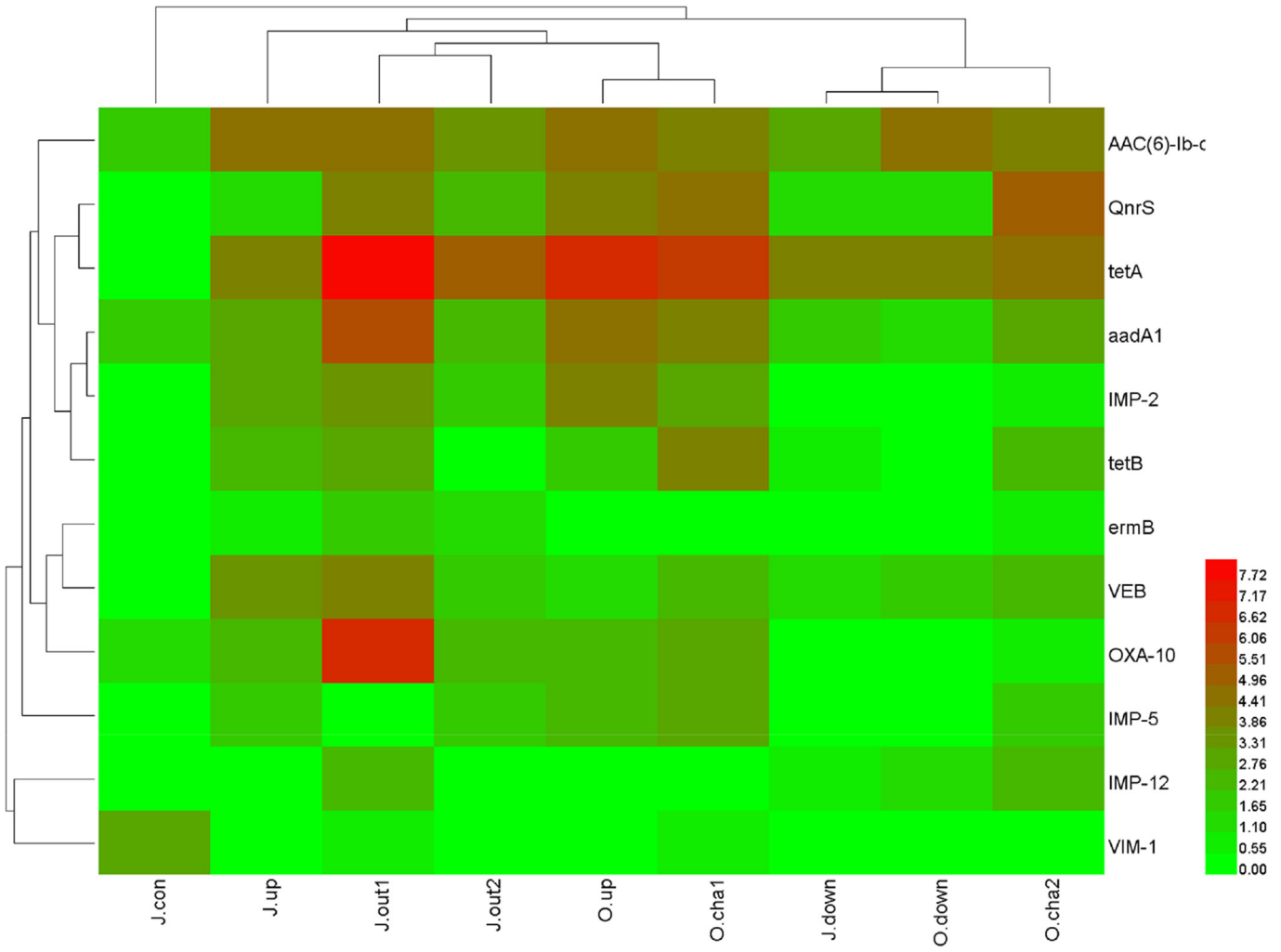

Fig. 5. ARGs distribution profiles in all samples. Plotted values were the natural logarithm transformed the normalized copy number of each ARG. Columns and rows were clustered based on Euclidean distance.

the abundance of *IMP-12* gene even had little improvement. These implied that seawater dilution could not completely eliminate the effect of mariculture on ARGs proliferation and dissemination. Bacteria in marine sediments could play an important role in the global exchange of antibiotic resistance. The introduction of ARGs in marine environment, together with the changes related to human activities and natural conditions, might be relevant for the future evolution and dissemination (Martínez, 2008). The high nucleotide identity of ARGs detected between mariculture sediment bacteria in China and human pathogens in clinical setting implied that HGT have occurred recently among these microorganism (Yang et al., 2013).

3.4. Relationships among nutrients, heavy metals, bacterial communities and ARGs

ARGs were generally located on mobile genetic elements such as plasmids, transposons and integrons which were the vehicles for ARGs dissemination via HGT. Among these mobile genetic elements, integrons were particularly adapted to transfer and disseminate antibiotic resistance. They can capture and integrate one or more gene cassettes, and convert them into functionally expressed genes. In this way, integrons quickly acquire diverse resistance genes (Gillings et al., 2015; Mazel, 2006). However, the increase of *intI1* gene copy number in mariculture sediments was not detected. Additionally, both the absolute and relative abundance of *intI1* gene and ARGs were not significantly correlated (Table S8). This result suggested that integrons was not the main mechanism of ARGs propagation in mariculture areas. Further researches on the relationship between other HGT vehicles and the ARGs in mariculture are needed. In addition to HGT, the co-selection of heavy metals was also confirmed to be the important mechanism for ARGs transmission in animal manures (Ji et al., 2012), swine farms (Zhu et al., 2013), wastewater treatment plants (Gao et al., 2015; Mao et al., 2015), landfill leachates (Wu et al., 2015) and isolates from various environments (Gullberg et al., 2014; Martins et al., 2014). Pearson correlation analysis showed that the concentrations of Cu and Cr were significantly positive correlated with the abundance of some ARGs (*ACC(6)-Ib-cr*, *IMP-12*, *IMP-5*, and *QnrS*). The strong correlations between the particular ARGs and heavy metals suggested that heavy metals contamination may be considered as a potential factor that contributed to the dissemination of ARGs in mariculture environment. Furthermore, the pearson correlation analysis of the normalized copy number of ARGs among all samples showed the significant correlation between the *tetA*, *aadA1*, and *OXA-10* genes (Fig. S3). The detection frequencies of these three genes in all samples were 100%. *tetA* gene was the most abundant gene in all sediments; the normalized copy number of *aadA1* and *OXA-10* genes were 2.89–47.84 and 1.14–101.83, respectively. These results implied that a large number of such genes in mariculture wastewaters and co-selection of these genes were driven by mariculture activities. The relationship of microbial communities and ARGs profiles was measured to estimate the contribution of microbial communities to ARGs variations. Mantel test showed that no significant correlation was found between bacterial communities and ARGs profiles based on Bray-Curtis distance ($r = 0.53$, $p > 0.05$, permutations = 9999). These above results implied that the HGT, the co-selection of heavy metals and the change of microbial communities were not the dominant mechanism for the ARGs propagation in the mariculture environment.

To comprehensively evaluate the contribution of nutrients, heavy metals and bacterial communities to the ARGs propagation, PCoAs based on Bray-Curtis distance of these three groups were calculated first. The partial redundancy analysis (pRDA) was performed on ARGs profiles and the first two PCs of each group. A total of 77.5% variance of ARGs could be explained by selected variables in mariculture samples. Nutrients explained the largest variation (19.6%), followed by the interaction between nutrients and bacterial communities which accounted for 15.2% of the variation. Bacterial communities, heavy metals and the interaction of them explained similar variation (10.7%, 12.5%, and 9.5%, respectively), which were higher than those explained by the interaction between nutrients and heavy metals (5.6%) and the interaction between all variables (4.4%) (Fig. 6). A study on Dongjiang River basin discovered that nutrients, heavy metals and antibiotics explained 21% of the cumulative percentage variance of the ARGs data, and some ARGs showed certain correlations with TS, TOC, TN, Cu and Zn (Su et al., 2014). In the soils from the Mezquital Vally, long-term wastewater irrigation led to an increase in the abundance of ARGs and the concentration of S and P. Further analysis found that the concentrations of S and P were positively correlated with antibiotic resistance levels (Jechalke et al., 2015). Bactierial communities were found to drive antibiotic resistome in drinking water chlorination (Jia et al., 2015) and sewage sludge composting (Su et al., 2015). In this study, no dominant factor for ARGs dissemination was observed in the research sites, maybe because the concentrations of the chemical contaminants were relatively low and they were not powerful human disturbance such as the chlorination or composting. Therefore, the combined effects of nutrients, heavy metals and microbial communities drove the distribution of ARGs in mariculture sediments.

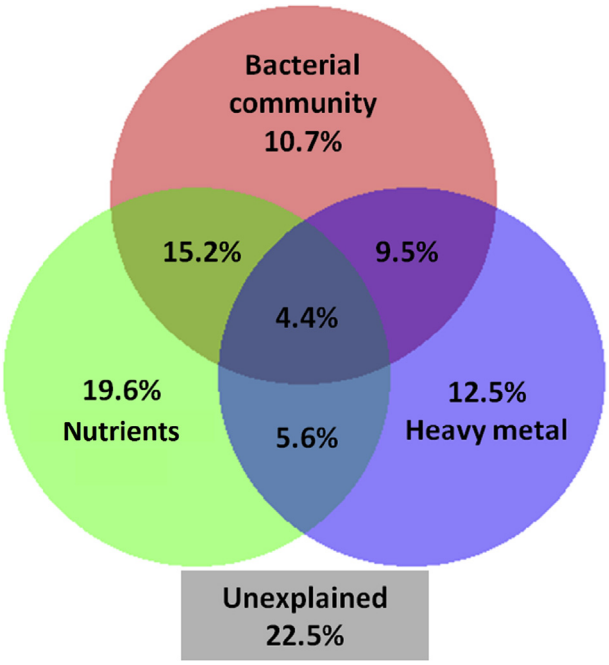

Fig. 6. Partial redundancy analysis differentiated the effects of nutrients, heavy metals and bacterial communities on the shift of overall ARGs in mariculture sediments.

4. Conclusions

In conclusion, the findings in this paper indicated that mariculture could alter bacterial communities and increase the abundance of ARGs in adjacent sediments. The change of nutrients, heavy metals and microbial communities could jointly contribute to the distribution of ARGs in sediments impacted by mariculture. Since marine diffusion decreased the abundance of most of ARGs, mariculture activities are less likely to cause serious ARGs

contamination in marine environment under the current tested conditions. However, changes in environmental conditions or long-term exposure to nearby mariculture activities could probably lead to the propagation of ARGs eventually. To reduce the potential risks, specific management strategies should be carried out to control the antibiotic resistance dissemination by mariculture.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2016.10.075.

References

Aarestrup, F.M., Jensen, V.F., Emborg, H.D., Jacobsen, E., Wegener, H.C., 2010. Changes in the use of antimicrobials and the effects on productivity of swine farms in Denmark. Am. J. Vet. Res. 71, 726–733.
Aedo, S., Ivanova, L., Tomova, A., Cabello, F.C., 2014. Plasmid-related quinolone resistance determinants in epidemic *Vibrio parahaemolyticus*, uropathogenic *Escherichia coli*, and marine bacteria from an aquaculture area in Chile. Microb. Ecol. 68, 324–328.
Blancheton, J.P., Attramadal, K.J.K., Michaud, L., D'Orbcastel, E.R., Vadstein, O., 2013. Insight into bacterial population in aquaculture systems and its implication. Aquacul. Eng. 53, 30–39.
Bokulich, N.A., Subramanian, S., Faith, J.J., Gevers, D., Gordon, J.I., Knight, R., Mills, D.A., Caporaso, J.G., 2013. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. Nat. Methods 10, 57–59.
Buschmann, A.H., Tomova, A., Lopez, A., Maldonado, M.A., Henriquez, L.A., Ivanova, L., Moy, F., Godfrey, H.P., Cabello, F.C., 2012. Salmon aquaculture and antimicrobial resistance in the marine environment. PLoS One 7, e42724.
Cabello, F.C., Godfrey, H.P., Tomova, A., Ivanova, L., Doelz, H., Millanao, A., Bushmann, A.H., 2013. Antimicrobial use in aquaculture re-examined: its relevance to antimicrobial resistance and to animal and human health. Environ. Microbiol. 15, 1917–1942.
Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336.
Chen, B., Yang, Y., Liang, X., Yu, K., Zhang, T., Li, X., 2013. Metagenomic profiles of antibiotic resistance genes (ARGs) between human impacted estuary and deep ocean sediments. Environ. Sci. Technol. 47, 12753–12760.
Cosgrove, S.E., Carroll, K.C., Perl, T.M., 2004. *Staphylococcus aureus* with reduced susceptibility to vancomycin. Clin. Infect. Dis. 39, 539–545.
Deng, Y.T., Wu, Y.L., Tan, A.P., Huang, Y.P., Jiang, L., Xue, H.J., Wang, H.J., Wang, W.L., Luo, L., Zhao, F., 2014. Analysis of antimicrobial resistance genes in *Aeromonas* spp. isolated from cultured freshwater animals in China. Microb. Drug Resist 20, 350–356.
DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P., Andersen, G.L., 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl. Environ. Microb. 72, 5069–5072.
Di Cesare, A., Luna, G.M., Vignaroli, C., Pasquaroli, S., Tota, S., Paroncini, P., Biavasco, F., 2013. Aquaculture can promote the presence and spread of antibiotic-resistant *Enterococci* in marine sediments. PLoS One 8, e62838.
Forsberg, K.J., Patel, S., Gibson, M.K., Lauber, C.L., Knight, R., Fierer, N., Dantas, G., 2014. Bacterial phylogeny structures soil resistomes across habitats. Nature 509, 612–616.
Gao, P., He, S., Huang, S., Li, K., Liu, Z., Xue, G., Sun, W., 2015. Impacts of coexisting antibiotics, antibacterial residues, and heavy metals on the occurrence of erythromycin resistance genes in urban wastewater. Appl. Mcirobiol. Biot. 99, 3971–3980.
Gillings, M.R., Gaze, W.H., Pruden, A., Smalla, K., Tiedje, J.M., Zhu, Y.G., 2015. Using the class 1 integron-integrase gene as a proxy for anthropogenic pollution. ISME J. 9, 1269–1279.
Gullberg, E., Albrecht, L.M., Karlsson, C., Sandegren, L., Andersson, D.I., 2014. Selection of a multidrug resistance plasmid by sublethal levels of antibiotics and heavy metals. Mbio 5 e01918–14.
Hammer, Ø., Harper, D.A., Ryan, P.D., 2001. PAST: paleontological statistics software package for education and data analysis. Palaeontol. Electron. 4, 1–9.
Han, J.E., Kim, J.H., Choresca Jr., C.H., Shin, S.P., Jun, J.W., Chai, J.Y., Park, S.C., 2012. Prevalence of tet gene and complete genome sequencing of tet gene-encoded plasmid (pAHH01) isolated from *Aeromonas* species in South Korea. J. Appl. Microbiol. 112, 631–638.
Harnisz, M., Korzeniewska, E., Golas, I., 2015. The impact of a freshwater fish farm on the community of tetracycline-resistant bacteria and the structure of tetracycline resistance genes in river water. Chemosphere 128, 134–141.
He, L.Y., Liu, Y.S., Su, H.C., Zhao, J.L., Liu, S.S., Chen, J., Liu, W.R., Ying, G.G., 2014. Dissemination of antibiotic resistance genes in representative broiler feedlots environments: identification of indicator ARGs and correlations with environmental variables. Environ. Sci. Technol. 48, 13120–13129.
Henne, K., Kahlisch, L., Hoefle, M.G., Brettar, I., 2013. Seasonal dynamics of bacterial community structure and composition in cold and hot drinking water derived from surface water reservoirs. Water Res. 47, 5614–5630.
House, White, 2015. National Action Plan for Combating Antibioticresistant Bacteria. The White House, Washington, DC.
Huang, Y., Zhang, L., Tiu, L., Wang, H.H., 2015. Characterization of antibiotic resistance in commensal bacteria from an aquaculture ecosystem. Front. Microbiol. 6, 914.
Islam, M.S., Sarker, M.J., Yamamoto, T., Wahab, M.A., Tanaka, M., 2004. Water and sediment quality, partial mass budget and effluent N loading in coastal brackishwater shrimp farms in Bangladesh. Mar. Pollut. Bull. 48, 471–485.
Jechalke, S., Broszat, M., Lang, F., Siebe, C., Smalla, K., Grohmann, E., 2015. Effects of 100 years wastewater irrigation on resistance genes, class 1 integrons and incp-1 plasmids in mexican soil. Front. Microbiol. 6, 1–8.
Ji, X., Shen, Q., Liu, F., Ma, J., Xu, G., Wang, Y., Wu, M., 2012. Antibiotic resistance gene abundances associated with antibiotics and heavy metals in animal manures and agricultural soils adjacent to feedlots in Shanghai. China. J. Hazard. Mater 235, 178–185.
Jia, S., Shi, P., Hu, Q., Li, B., Zhang, T., Zhang, X.X., 2015. Bacterial community shift drives antibiotic resistance promotion during srinking water chlorination. Environ. Sci. Technol. 49, 12271–12279.
Kaevska, M., Videnska, P., Sedlar, K., Slana, I., 2016. Seasonal changes in microbial community composition in river water studied using 454-pyrosequencing. Springerplus 5, 1–8.
Kawahara, N., Shigematsu, K., Miyadai, T., Kondo, R., 2009. Comparison of bacterial communities in fish farm sediments along an organic enrichment gradient. Aquaculture 287, 107–113.
Laganà, P., Caruso, G., Minutoli, E., Zaccone, R., Santi, D., 2011. Susceptibility to antibiotics of *Vibrio* spp. and *Photobacterium damsela* ssp. *piscicida* strains isolated from Italian aquaculture farms. New Microbiol. 34, 53–63.
Levy, S.B., Marshall, B., 2004. Antibacterial resistance worldwide: causes, challenges and responses. Nat. Med. 10, 122–129.
Liang, X., Shi, Z., Huang, X., 2013. Occurrence of antibiotics in typical aquaculture of the Pearl River estuary. Ecol. Environ. Sci. 22, 304–310.
Liang, P., Wu, S.C., Zhang, J., Cao, Y., Yu, S., Wong, M.H., 2016. The effects of mariculture on heavy metal distribution in sediments and cultured fish around the pearl river delta region, South China. Chemosphere 148, 171–177.
Lipson, D.A., 2007. Relationships between temperature responses and bacterial community structure along seasonal and altitudinal gradients. FEMS Microbiol. Ecol. 59, 418–427.
Looft, T., Johnson, T.A., Allen, H.K., Bayles, D.O., Alt, D.P., Stedtfeld, R.D., Sul, W.J., Stedtfeld, T.M., Chai, B., Cole, J.R., Hashsham, S.A., Tiedje, J.M., Stanton, T.B., 2012. In-feed antibiotic effects on the swine intestinal microbiome. Proc. Natl. Acad. Sci. U. S. A. 109, 1691–1696.
Lu, Z., Na, G., Gao, H., Wang, L., Bao, C., Yao, Z., 2015. Fate of sulfonamide resistance genes in estuary environment and effect of anthropogenic activities. Sci. Total. Environ. 527, 429–438.
Luo, Y., Wang, Q., Lu, Q., Mu, Q., Mao, D., 2014. An ionic liquid facilitates the proliferation of antibiotic resistance genes mediated by class 1 integrons. Environ. Sci. Technol. Lett. 1, 266–270.
Magoc, T., Salzberg, S.L., 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27, 2957–2963.
Mao, D., Yu, S., Rysz, M., Luo, Y., Yang, F., Li, F., Hou, J., Mu, Q., Alvarez, P.J., 2015. Prevalence and proliferation of antibiotic resistance genes in two municipal wastewater treatment plants. Water Res. 85, 458–466.
Martínez, J.L., 2008. Antibiotics and antibiotic resistance genes in natural environments. Science 321, 365–367.
Martins, V.V., Barboza, Z.M.O., Pitondo-Silva, A., Stehling, E.G., 2014. Aquatic environments polluted with antibiotics and heavy metals: a human health hazard. Environ. Sci. Pollut. R. 21, 5873–5878.
Mazel, D., 2006. Integrons: agents of bacterial evolution. Nat. Rev. Microbiol. 4, 608–620.
Muziasari, W.I., Managaki, S., Parnanen, K., Karkman, A., Lyra, C., Tamminen, M., Suzuki, S., Virta, M., 2014. Sulphonamide and trimethoprim resistance genes persist in sediments at Baltic Sea aquaculture farms but are not detected in the surrounding environment. PLoS One 9, e92072.
Nonaka, L., Ikeno, K., Suzuki, S., 2007. Distribution of tetracycline resistance gene, *tet(M)*, in Gram-positive and Gram-negative bacteria isolated from sediment and seawater at a coastal aquaculture site in Japan. Microbes Environ. 22, 355–364.
Ouyang, W.Y., Huang, F.Y., Zhao, Y., Li, H., Su, J.Q., 2015. Increased levels of antibiotic resistance in urban stream of Jiulongjiang River, China. Appl. Microbiol. Biot. 99, 5697–5707.
Patel, V., Munot, H., Shouche, Y.S., Madamwar, D., 2014. Response of bacterial community structure to seasonal fluctuation and anthropogenic pollution on coastal water of Alang-Sosiya ship breaking yard, Bhavnagar, India. Bioresour. Technol. 161, 362–370.

Pereira, C., Salvador, S., Arrojado, C., Silva, Y., Santos, A.L., Cunha, A., Gomes, N.C., Almeida, A., 2011. Evaluating seasonal dynamics of bacterial communities in marine fish aquaculture: a preliminary study before applying phage therapy. J. Environ. Monit. 13, 1053–1058.

Reboucas, R.H., De Sousa, O.V., Lima, A.S., Vasconcelos, F.R., De Carvalho, P.B., Vieira, R.H., 2011. Antimicrobial resistance profile of *Vibrio* species isolated from marine shrimp farming environments (*Litopenaeus vannamei*) at Ceara. Braz. Environ. Res. 111, 21–24.

Sapkota, A., Sapkota, A.R., Kucharski, M., Burke, J., McKenzie, S., Walker, P., Lawrence, R., 2008. Aquaculture practices and potential human health risks: current knowledge and future priorities. Environ. Int. 34, 1215–1226.

Scarano, C., Spanu, C., Ziino, G., Pedonese, F., Dalmasso, A., Spanu, V., Virdis, S., De Santis, E.P.L., 2014. Antibiotic resistance of *Vibrio* species isolated from *Sparus aurata* reared in Italian mariculture. New Microbiol. 37, 329–337.

Seiler, C., Berendonk, T.U., 2012. Heavy metal driven co-selection of antibiotic resistance in soil and water bodies impacted by agriculture and aquaculture. Front. Miciobiol. 3, 399.

Seyfried, E.E., Newton, R.J., Rubert, K.F., Pedersen, J.A., McMahon, K.D., 2010. Occurrence of tetracycline resistance genes in aquaculture facilities with varying use of oxytetracycline. Microb. Ecol. 59, 799–807.

Stoddard, S.F., Smith, B.J., Hein, R., Roller, B.R.K., Schmidt, T.M., 2015. rrnDB: improved tools for interpreting rRNA gene abundance in bacteria and archaea and a new foundation for future development. Nucleic. acids. Res. 43, D593–D598.

Su, H.C., Pan, C.G., Ying, G.G., Zhao, J.L., Zhou, L.J., Liu, Y.S., Tao, R., Zhang, R.Q., He, L.Y., 2014. Contamination profiles of antibiotic resistance genes in the sediments at a catchment scale. Sci. Total Environ. 490, 708–714.

Su, J.Q., Wei, B., Ouyang, W.Y., Huang, F.Y., Zhao, Y., Xu, H.J., Zhu, Y.G., 2015. Antibiotic resistome and its association with bacterial communities during sewage sludge Composting. Environ. Sci. Technol. 49, 7356–7363.

Suh, S.S., Park, M., Hwang, J., Kil, E.J., Jung, S.W., Lee, S., Lee, T.K., 2015. Seasonal dynamics of marine microbial community in the South Sea of Korea. PLoS One 10, e0131633.

Szczepanowski, R., Bekel, T., Goesmann, A., Krause, L., Kroemeke, H., Kaiser, O., Eichler, W., Pühler, A., Schlüter, A., 2008. Insight into the plasmid metagenome of wastewater treatment plant bacteria showing reduced susceptibility to antimicrobial drugs analysed by the 454-pyrosequencing technology. J. Biotechnol. 136, 54–64.

Tamminen, M., Karkman, A., Corander, J., Paulin, L., Virta, M., 2011a. Differences in bacterial community composition in Baltic Sea sediment in response to fish farming. Aquaculture 313, 15–23.

Tamminen, M., Karkman, A., Lohmus, A., Muziasari, W.I., Takasu, H., Wada, S., Suzuki, S., Virta, M., 2011b. Tetracycline resistance genes persist at aquaculture farms in the absence of selection pressure. Environ. Sci. Technol. 45, 386–391.

Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microb. 73, 5261–5267.

WHO, 2001. Global Strategy for Containment of Antimicrobial Resistance (accessed 16.03.15). http://www.who.int/csr/resources/publications/drugresist/WHO_CDS_CSR_DRS_2001_2_EN/en/index.html.

Woegerbauer, M., Zeinzinger, J., Gottsberger, R.A., Pascher, K., Hufnagl, P., Indra, A., Fuchs, R., Hofrichter, J., Kopacka, I., Korschineck, I., Schleicher, C., Schwarz, M., Steinwider, J., Springer, B., Allerberger, F., Nielsen, K.M., Fuchs, K., 2015. Antibiotic resistance marker genes as environmental pollutants in GMO-pristine agricultural soils in Austria. Environ. Pollut. 206, 342–351.

Wu, B., Song, J., Li, X., 2014. Evaluation of potential relationships between benthic community structure and toxic metals in Laizhou Bay. Mar. Pollut. Bull. 87, 247–256.

Wu, D., Huang, Z., Yang, K., Graham, D., Xie, B., 2015. Relationships between antibiotics and antibiotic resistance gene levels in municipal solid waste leachates in Shanghai, China. Environ. Sci. Technol. 49, 4122–4128.

Xiong, W., Sun, Y., Zhang, T., Ding, X., Li, Y., Wang, M., Zeng, Z., 2015. Antibiotics, antibiotic resistance genes, and bacterial community composition in fresh water aquaculture environment in China. Microb. Ecol. 70, 425–432.

Yan, Q., Bi, Y., Deng, Y., He, Z., Wu, L., Van Nostrand, J.D., Shi, Z., Li, J., Wang, X., Hu, Z., Yu, Y., Zhou, J., 2015. Impacts of the Three Gorges Dam on microbial structure and potential function. Sci. Rep. 5, 8605.

Yang, J., Wang, C., Chang, S., Li, L., Geng, J., Hu, S., Feng, J., 2013. Marine sediment bacteria harbor antibiotic resistance genes highly similar to those found in human pathogens. Microb. Ecol. 65, 975–981.

Yuan, C.G., Shi, J.B., He, B., Liu, J.F., Liang, L.N., Jiang, G.B., 2004. Speciation of heavy metals in marine sediments from the East China Sea by ICP-MS with sequential extraction. Environ. Int. 30, 769–783.

Zhang, T., Fang, H.H.P., 2006. Applications of real-time polymerase chain reaction for quantification of microorganisms in environmental samples. Appl. Microbiol. Biot. 70, 281–289.

Zhang, X.X., Zhang, T., Zhang, M., Fang, H.H.P., Cheng, S.P., 2009. Characterization and quantification of class 1 integrons and associated gene cassettes in sewage treatment plants. Appl. Microbiol. Biot. 82, 1169–1177.

Zhang, H., Sun, Z., Liu, B., Xuan, Y., Jiang, M., Pan, Y., Zhang, Y., Gong, Y., Lu, X., Yu, D., Kumar, D., Hu, X., Cao, G., Xue, R., Gong, C., 2016. Dynamic changes of microbial communities in *Litopenaeus vannamei* cultures and the effects of environmental factors. Aquaculture 455, 97–108.

Zhu, Y.G., Johnson, T.A., Su, J.Q., Qiao, M., Guo, G.X., Stedtfeld, R.D., Hashsham, S.A., Tiedje, J.M., 2013. Diverse and abundant antibiotic resistance genes in Chinese swine farms. Proc. Natl. Acad. Sci. U. S. A. 110, 3435–3440.