# Sediment microbial communities contribute to shrimp intestine microbiota in cultural pond ecosystems

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# Abstract

Background Microorganisms in a shrimp cultural pond ecosystem (SCPE) play important roles for animal and environmental health. While the microbial diversity and assembly mechanism, and how communities of multiple habitats contribute to the SCPE metacommunity are poorly understood. Here, we analyzed phylogenetic structure of 792 samples of three habitats (water, shrimp intestine and sediment) from 88 shrimp cultural ponds across six regional sites in China. Results We show that the microbial communities of three habitats in the SCPE contained 15,197 operational taxonomic units (OTUs), and the microbial community of sediment had the highest OTUs (14,857). Despite the high microbial diversity, the SCPE has small core taxa (13 OTUs) among three habitats, and 30, 15, 53 core OTUs in water, shrimp intestine, and sediment, respectively. Especially, some opportunistic pathogens or functional microbes were core taxa in shrimp intestine or water and sediment. Of the SCPE metacommunity, the microbial diversity of the SCPE was mainly contributed by αLocalCommunities (66.0%), and followed by βInterHabitats (29.0%). The sediment microbial communities had a larger contribution (56.8%) to shrimp intestine microbiota compared to water communities (18.1%), which was also corroborated by the Sloan neutral community model analysis. Further, microbial assembly of three habitats appeared to be largely driven by stochastic processes (over 78%) in the SCPE. Conclusions Our results demonstrate that those core microbial taxa of three habitats were distinct with each other, that sediment communities mainly contributed to shrimp intestine communities, and that the microbial assembly was largely driven by stochastic processes. Our study enhances the mechanistic understanding of the microbial diversity and assembly in the SCPE for further analyzing metacommunities, and has important implications for microbial ecology and shrimp culture strategies.

# Background

With increasing demands for animal proteins due to rising populations, the global aquaculture production has increased by 500% since the late 1980s [1]. Aquaculture has become the third largest source of animal proteins accounting for 17% of the global protein consumption, and the annual output of aquaculture products in China is over 60% of the World [2]. Unfortunately, the frequent occurrence of diseases has threatened the development of the aquaculture industry. Shrimp are one of the most important aquatic products among fishery trading commodities worldwide [3]. Recently, the global production of shrimp is estimated to be reduced by 23% due to bacterial diseases, such as white feces syndrome (WFS), early mortality syndrome (EMS), acute hepatopancreatic necrosis disease (AHPND) and hepatopancreas necrosis syndrome (HPNS), leading to a loss of billions of dollars yearly [4–6].

Microorganisms play an indispensable role in ecosystems and provide a wide range of services [7–9]. In aquaculture ecosystems, microorganisms modulate ecosystem functions, including animal health, disease control, element cycling and water quality, and affect the productivity and sustainability of aquaculture [10–13]. For example, opportunistic pathogens, such as Vibrio species, are widely distributed in aquaculture systems [14, 15], while some functional microbes, such as Nitrosococcus and Nitrosopumilus species, may enhance nitrogen cycling [16]. More importantly, microbial communities of

multiple habitats (e.g., surrounding water, sediment, animal intestine) in aquaculture systems are all closely related to the occurrence of aquatic animal diseases [14, 17−20], challenging us to fully understand the structure, function and interaction in such complex aquaculture ecosystems. Therefore, it is necessary to understand the microbial ecology of aquaculture ecosystems for the sustainable outputs of aquaculture products.

The microbial communities of multiple habitats had high diversity [17, 21−23]and varied concurrently with some key environmental and geographic factors (e.g., host developmental stages, environmental factors, geographical distance) in aquaculture ecosystems [14, 16, 24, 25]. Despite recent advances in understanding microbial ecology of aquaculture ecosystems, their microbial assembly mechanisms remain unclear. In general, the mechanisms shaping the microbial diversity among species are considered as ecological processes [26]. Recently, our knowledge about ecological processes in shaping the microbial structure has been enriched substantially, and the importance of ecological stochasticity has also been emphasized [27]. For aquatic ecosystems (e.g., lakes), deterministic processes generally play a primary role in shaping the water or sediment microbial community structure [28, 29]. Of aquatic animal intestines, a dominant role of stochastic processes was observed for microbial assemblages, and deterministic processes became less important as hosts developed [18, 24, 30]. More importantly, microbial communities of multiple habitats (e.g., water, animal intestine, sediment habitats) constitute a metacommunity in aquatic ecosystems [31−34], and such close relationships were observed among them, which is important for aquatic animal productivity and health [15, 35]. However, how microbial communities of multiple habitats (water, animal intestine, sediment) in aquaculture ecosystems contribute to the metacommunity is poorly understood. Especially, it is essential to understand the interactions among microbial communities of animal intestine and surrounding environments for healthy aquaculture [35].

In this study, we aimed to understand the microbial assembly mechanisms for a metacommunity of three habitats (water, shrimp intestine and sediment) in the shrimp cultural pond ecosystem (SCPE) of Litopenaeus vannamei, and specifically focusing on three ecological questions: (i) Are there core microbial taxa among three habitats across the SCPE or in each habitat of the SCPE? (ii) What is the contribution of microbial communities from each habitat to the SCPE metacommunity? (iii) What ecological processes shape the microbial community structures in three habitats? To address those questions, we hypothesized that there would be core microbial taxa among communities of three habitats across the SCPE and in each habitat, and that sediment communities would have a decisive role to intestine microbiota of L. vannamei as it is one of benthic animals and has the characteristics of feeding from sediments. To test those hypotheses, we collected 792 samples from 88 cultural ponds in six regional sites of China (Additional file 1: Table S1), and analyzed microbial communities and the contribution of ecological processes to their assembly as well as the relationship among communities of three habitats in the SCPE based on 16S rRNA gene sequences. We found that those core microbial taxa of three habitats were distinct with each other, that sediment communities mainly contributed to shrimp intestine communities, and that the microbial assembly was largely driven by stochastic processes. Our study provides new insights into the understanding of microbial assembly mechanisms and the

developed framework will facilitate the metacommunity analysis in the SCPE, significantly advancing microbial ecology of aquaculture systems and shrimp culture strategies.

# Results

## Overview of microbial diversity of three habitats in the SCPE

To understand the microbial diversity in the SCPE, we sequenced 16S rRNA gene amplicons from 792 samples in 88 shrimp cultural ponds across six regional sites. A total of 38,662,478 high quality sequences (30,580 to 87,563 sequences for each sample) were obtained from all samples, and clustered into 15,197 operational taxonomic units (OTUs) with the highest number (i.e. 14,857) of OTUs in the sediment (Additional file 1: Tables S2 and S3). Such sequencing efforts were enough to capture a majority of microbial communities in all samples (Additional file 1: Table S3). The Shannon index was the highest in sediment (6.5 ± 0.3), and followed by water (4.4 ± 0.5) and shrimp intestine (3.2 ± 1.2), which significantly ($P$ < 0.001) differed among those three habitats, and Chao1 index showed similar results (Fig. 1a; Additional file 1: Table S3). To further evaluate the overall differences among three habitats, non-metric multidimensional scaling (NMDS) analysis showed that microbial communities were clustered by water, shrimp intestine and sediment habitats, which was further corroborated by analysis of similarity (ANOSIM), revealing that the microbial community structure significantly (r = 0.8115, $P$ < 0.001) differed between any two of compared habitats (Fig. 1b).

## Core OTUs among three habitats or of each habitat in the SCPE

To examine if core microbial taxa exist among three habitats in the SCPE, we defined that core microbial taxa should occur in ≥ 90% of all 792 samples tested (see Methods for details). About 0.08% (13 out of 15,197 OTUs) constituted core taxa among three habitats that accounted for the relative abundance of 7.98% of all sequences (Fig. 2a and 2b). The core OTUs belonged to Cyanobacteria (6 OTUs and a relative abundance of 5.63%), Actinobacteria (3 OTUs and 0.96%), Proteobacteria (3 OTUs and 0.98%), and Verrucomicrobia (1 OTU and 0.41%) (Additional file 1: Table S4).

Similarly, of each habitat, we defined that core microbial taxa should occur in ≥ 90% of 264 water, 264 shrimp intestine, or 264 sediment samples, respectively. The results showed that about 0.3% (30 out of 9,874 OTUs), 0.2% (15 out of 7,466 OTUs) and 0.4% (53 out of 14,857 OTUs) constituted core microbial taxa in water (Fig. 2c and 2d), shrimp intestine (Fig. 2e and 2f) and sediment (Fig. 2g and 2h) habitats, respectively, and they accounted for the relative abundance of 33.28%, 38.76% and 9.12% of all sequences obtained. The core OTUs in water belonged to Cyanobacteria (17.87%), Actinobacteria (8.50%), Proteobacteria (2.14%), Chlorobi (4.04%) and Verrucomicrobia (0.72%); the core OTUs in shrimp intestine belonged to Proteobacteria (29.38%), Cyanobacteria (1.52%), Actinobacteria (0.43%), Tenericutes (6.97%) and Verrucomicrobi (0.46%); the core OTUs in sediment belonged to 12 phyla, including Proteobacteria, Bacteroidetes, Cyanobacteria, Actinobacteria and Ignavibacteriae accounting for 4.64%, 1.98%, 0.88%, 0.68% and 0.51%, respectively (Additional file 1: Tables S5, S6, S7 and S8). Thus, the core OTU composition of water, shrimp intestine and sediment habitats were also distinct for each

habitat, suggesting that each habitat would select their core microbial taxa. Also, 15 core OTUs of shrimp intestine were also present in water and sediment habitats (table S3), suggesting possible sources (e.g., water and sediment) of shrimp intestine microbial communities. Interestingly, *Vibrio* OTU934, *Vibrio* OTU1793, *Photobacterium* OTU442 and *Candidatus* Bacilloplasma OTU384 were found to be core OTUs of shrimp intestine, and they are considered as animal opportunistic pathogens in aquatic ecosystems (Additional file 1: Table S6). Also, some functional microbes, such as *Synechococcus* OTU25011, *Synechococcus* OTU5786, *Synechococcus* OTU3905 and *Rhodobacter* OTU29858, or *Synechococcus* OTU25011, *Synechococcus* OTU5786, *Rhodobacter* OTU29858, *Truepera* OTU9796 and *Desulfomicrobium* OTU3310, were found to be core OTUs of water or sediment habitat, and they may enhance carbon and sulfur cycling in aquatic ecosystems (Additional file 1: Tables S5 and S7).

## Comparison of the microbial community composition in three habitats

To understand the composition of microbial communities from three habitats, we compared them at the OTU level using Venn analysis. The results showed that a total of 5,997 OTUs were commonly present in three habitats, and the number of OTUs was found to be in any two habitats: 6,094 (water, 81.6%) or 7,279 (sediment, 97.5%) out of 7,466 intestine OTUs, 6 094 (intestine, 77.2%) or 9,625 (sediment, 97.5%) out of 9,874 water OTUs, and 7,279 (intestine, 49.0%) or 9,625 (water, 64.8%) out of 14,857 sediment OTUs (Fig. 3). Most OTUs were in the sediment habitat, and a high percentage (~97.5%) of OTUs in shrimp intestine and water habitats were shared with the sediment habitat. For each pond and all six regional sites, similar trends were observed (Additional file 2: Figure S1).

We further compared the composition of microbial communities among three habitats at the phylum and genus levels, showing that all detected phyla and genera were always present in any of the three habitats (Additional file 2: Figures S2 and S3), but their relative abundances significantly ($P < 0.001$) differed (Additional file 1: Tables S9 and S10; Additional file 2: Figure S4a and S4b). Some opportunistic pathogens, such as *Vibrio*, *Photobacterium* and *Candidatus* Bacilloplasma, were detected in three habitats, and their relative abundances were significantly ($P < 0.001$) higher in the shrimp intestine than in the water or sediment habitat (Additional file 1: Tables S10 and S11; Additional file 2: Figure S4b and S4c).

## Sediment communities mainly contribute to shrimp intestine microbiota compared to water community in the SCPE metacommunity

In order to evaluate the contribution of microbial communities of three habitats to the SCPE metacommunity diversity, we used additive diversity partitioning of diversity across scales to determine whether the microbial diversity observed at the ecosystem level ($\gamma_{Ecosystem}$) was mainly from a high microbial dissimilarity among habitats ($\beta_{InterHabitats}$), a high dissimilarity among communities within each habitat ($\beta_{IntraHabitats}$) or from a high microbial diversity within each local community ($\bar{\alpha}_{LocalCommunities}$, i.e., water, shrimp intestine or sediment sample). The results showed that the contribution of $\alpha_{LocalCommunities}$ to the metacommunity diversity ($\gamma_{Ecosystem}$) was 66.0 ± 11.2%, outweighing

$\beta_{\text{InterHabitats}}$ (29.0 ± 11.0%) and $\bar{\beta}_{\text{IntraHabitats}}$ (5.0 ± 2.4%) in their contributions to $\alpha_{\text{Ecosystem}}$ (Fig. 4a). This high contribution of α-diversity and β-diversity to $\alpha_{\text{Ecosystem}}$ revealed that $\bar{\alpha}_{\text{LocalCommunities}}$ and $\beta_{\text{IntraHabitats}}$ were important for generating the microbial diversity in the SCPE.

To test if sediment communities have a decisive role to shrimp intestine microbial communities, we evaluated the contribution of different source communities to water, shrimp intestine and sediment habitats in the SCPE by SourceTracker analysis. For water microbial communities, the most dominant potential source was sediment (an average of 53.6%), followed by shrimp intestine (35.0%); for shrimp intestine communities, the most dominant potential source was also sediment (56.8%), followed by water (18.1%) (Fig. 4b). In contrast, for sediment communities, the source from water or shrimp intestine only attributed 17.9% or 15.4 %, respectively (Fig. 4b). These results indicated that each microbial community could be the source for the other two communities, and especially, sediment was found to be the most important source of water and shrimp intestine communities, and such similar trends were observed across all six regional sites (Additional file 2: Figure S5), indicating a possible general pattern in the SCPE.

The Sloan neutral community model was further applied to analyze the shared OTUs between the surrounding sediment or water and shrimp intestine. That is, neutral distribution (black points) accounted for 37.8% in sediment, and 30.8% in water microbial communities (Fig. 4c). In contrast, the proportion of under-represented (green points) and over-represented (red points) OTUs was 15.0% or 5.2%, and 44.7% or 45.7% in sediment or water, respectively (Fig. 4c). Thus, the proportion of shared and neutrally distributed OTUs between shrimp intestine and sediment was higher than these in water, suggesting that communities of shrimp intestine were tended to colonize from sediment rather than from water. More obvious similar trends were observed for the six locations across a regional scale (Additional file 2: Figure S6).

## The microbial assemblies of three habitats in the SCPE were largely controlled by stochastic processes

In order to understand microbial assembly mechanisms in three habitats of the SCPE, we used the null model-based approach to calculate stochastic ratios with taxonomic (Bray-Curtis, abundance-weighted and unweighted) and phylogenetic (weighted and unweighted UniFrac) metrics. The results showed that the average stochastic ratios based on taxonomic and phylogenetic metrics were higher than 78.4% in water, 80.8% in shrimp intestine and 80.3% in sediment habitats (Fig. 5a), suggesting that stochastic factors were more important than deterministic factors in influencing microbial community composition of three habitats in the SCPE.

Variation partitioning analysis (VPA) was performed to discern the relative importance of various factors (geographic distance and environmental factors) contributing to microbial communities of water and sediment habitats in the SCPE. Water environmental factors (pH, salinity, dissolved oxygen (DO), ammonia nitrogen ($NH_4^+$-N), nitrate nitrogen ($NO_3^-$-N), orthophosphate ($PO_4^{3-}$-P), concentrations of total

carbon (TC), total organic carbon (TOC), total phosphorus (TP), ratio of carbon to nitrogen (C/N) and ratio of nitrogen to phosphorus (N/P)) and sediment environmental factors (pH, TC, TOC, total nitrogen (TN), TP, C/N, ratio of carbon to phosphorus (C/P) and N/P) were selected by the BioEnv procedure, which provides the highest Pearson correlation with the microbial community structure. Overall, the combination of selected water or sediment environmental factors and geographic distance showed a significant correlation ($P < 0.001$) with the water or sediment microbial structure. These variables explained 29.5% or 24.9% of the observed variation in water or sediment habitats, respectively, leaving 70.5% or 75.1% of the variation unexplained (Fig. 5b and 5c). The water or sediment environmental factors explained 21.7% or 14.2% ($P < 0.001$), and geographic distance alone explained 2.5% or 9.0% ($P < 0.001$) variations with 5.3% or 1.7% interaction effect detected, respectively (Fig. 5b and 5c).

## Discussion

Microbial communities of multiple habitats constitute the metacommunity in aquatic ecosystems. However, a more comprehensive understanding of the microbial assembly mechanism and the relationships among communities of animal intestine and surrounding environments in aquatic ecosystems remains unclear. In the present study, we analyzed the microbial assembly of water, shrimp intestine and sediment habitats to understand how their microbial communities contribute to the SCPE metacommunity, and the results generally support our hypotheses.

Our first hypothesis is that there would be core taxa among microbial communities of three habitats across the SCPE and in each habitat. Core microbial taxa provide information on putatively important microorganisms for ecosystem functioning [36]. Previous studies showed that there were 9, 6, 2, 1 and 28 bacterial OTUs identified as core taxa in soil, human feces, air, freshwater and wastewater treatment plants, respectively [37], suggesting that various ecosystems may have different core microbial patterns, possibly due to their highly dissimilar community compositions as well as the number of samples examined. In this study, 13 bacterial OTUs were identified as core taxa among 792 samples of three habitats, and we also found that each habitat had their own core OTUs among 264 samples. The core OTU patterns of three habitats were markedly different, and 15 core OTUs in shrimp intestine could be from sediment and water habitats, which was supported by SourceTracker and Sloan neutral community model analyses. Such results are generally consistent with previous studies [15, 38, 39]. For example, the surroundings are indicated as sources of microbial species colonizing aquatic animal intestine, and vice versa [38, 39], and another study suggests that, to improve the host fitness, the colonization of microbes in shrimp intestine is selected from surrounding environments [15]. Interestingly, some core taxa detected in this study are derived from known shrimp opportunistic pathogens, including *Vibrio* OTU934, *Vibrio* OTU1793 and *Candidatus* Bacilloplasma OTU384, which were generally accompanied by disease outbreaks with the increased abundances in shrimp intestine [15, 18, 20]. Meanwhile, it is worthwhile to note that core taxa of water and sediment habitats in the SCPE associated with its known biological functions. For example, several core OTUs belonged to *Synechococcus* and *Rhodobacter* as well as some oxygenic photosynthetic microbes are known to enhance carbon cycling and capture energy form sunlight in aquatic ecosystems [40, 41]. A *Desulfomicrobium* OTU (OTU3310) and a *Truepera* OTU (OTU

9796) were identified as core taxa in a sediment habitat, reflecting their importance for sulfur cycling and organic matter degradation in aquatic ecosystems [42, 43]. Thus, core microbial taxa were among three habitats across the SCPE or in each habitat, and such core taxa in shrimp intestine may play key roles for shrimp and environment health in the SCPE.

Our second hypothesis is that sediment microbial communities would have a decisive role for shrimp intestine microbiota in the SCPE. Generally, multiple habitats constitute a metacommunity for the overall microbial diversity in aquatic ecosystems [31], and surrounding environments are the main sources of microbes colonizing aquatic animal intestines, and the host animal drives, in a large part, the selection of microorganisms [44, 45]. Aquatic animals are theoretically microorganism-free at birth, and all postnatally acquired intestine microorganisms should migrate from their surroundings [24]. As all activities carried out by aquatic animals (e.g., feeding, defecation) take place in water or/and sediment habitats, interactions between host and their environments may be much more direct than those between terrestrial animals and their environments, thus the assembly of aquatic animal intestine microbial communities is directly influenced by their environmental communities [36, 44]. Consistently, Cahill considered that the bacteria present in aquatic environments influenced the composition of animal intestine microbial communities [38]. Sullam *et al*. found that fish could acquire intestine bacteria through water cyclic transmission, where hosts obtained their bacterial communities from their environments [39]. Our results revealed that microbial communities of water, shrimp intestine and sediment habitats in the SCPE had close relationships as communities of three habitats are connected to each other by various biological and ecological processes, including nutrient share, dispersal and microbial interactions [35, 46, 47]. For instance, dispersal is a key factor influencing the metacommunity and its associated community structure [48]. In this study, we observed that a high percentage of dispersal rates from sediment to the other two habitats in the SCPE, and especially, compared with water communities, shrimp intestine communities were more closely related to sediment communities. The explanations can be largely due to shrimp lifestyles and sediment features. It is well-known that *L. vannamei* is a planktobenthos (mainly lives a benthic and sometimes floats in water), and their activities are more related to the sediment habitat. Also, *L. vannamei* has the characteristics of feeding from the sediment and ingestion of particulate matter into its intestine. Our results indicate that sediment communities mainly contribute to shrimp intestine microbiota, but such mechanisms need to be further investigated.

We also found that each habitat harbored distinct microbial communities, indicating that different taxa have obvious preferences in three habitats. For example, *Vibrio*, *Photobacterium*, *Candidatus* Bacilloplasma, *Shewanella*, *Spongiimonas*, *Rhodobacter* and *Aeromonas* were enriched in shrimp intestine. On one hand, *Vibrio*, *Photobacterium* and *Candidatus* Bacilloplasma are known as opportunistic pathogens. Previous studies indicated that these pathogens in shrimp intestine could be from their surrounding environments, and they are widespread in cultural pond ecosystems [14, 18, 49]. The crucial question is if the aquatic animal intestine offers a best-suited micro-environment for such rare taxa from the environment to become dominant in shrimp intestine. On the other hand, those opportunistic pathogens may also spread into their environments through the excretion of aquatic animals, making the

control of proliferation of opportunistic pathogens extremely challenging [13]. For example, they may be excreted by aquatic animals and spread into the environment as "seeds", when these pathogens in the environment are killed by disinfection [50]. As aquatic animals are very important for maintaining the microbial diversity in aquatic environments [51], more microbial ecological management strategies should be developed to restrain opportunistic pathogens in aquaculture.

Additionally, further understanding of underlying microbial assembly mechanisms was attempted, showing that the stochastic processes could play more important roles in influencing the microbial community structure than deterministic processes. A possible reason may explain such observations: ecological drift (e.g., stochastic processes of birth, death, colonization) becomes stronger due to high percentages of dispersal rates [52]. A recent study of activated sludge communities from wastewater treatment plants at a global scale indicated that microbial spatial turnover was largely driven by stochastic processes [37]. Similarly, in other natural and engineering ecosystems, such as temperate forest [53], grassland (under warming condition) [54], bioreactor [55]and groundwater system (perturbed by adding emulsified vegetable oil for uranium immobilization) [52], stochastic processes played larger roles than deterministic ones in explaining the microbial assembly. Our present study is largely consistently with those previous studies, suggesting that the microbial assembly was largely driven by stochastic processes in the SCPE.

# Conclusion

In summary, the importance of microbial communities in aquaculture ecosystems has been widely recognized. Here, we systematically evaluated the microbial community composition of water, shrimp intestine and sediment habitats in the SCPE as a metacommunity, and revealed their relationships and possible assembly mechanisms (Fig. 6). Specifically, we found that core microbial taxa were among three closely related habitats and in each habitat, and sediment communities dominated intestine microecosystem of L. vannamei, and microbial variations were largely controlled by stochastic processes in the SCPE. This study provides new insights into microbial assembly mechanisms and a framework for metacommunity analysis in the SCPE, which will have important implications for developing new strategies for shrimp healthy culture.

# Methods

### Sample collection and physicochemical analysis

A total of 792 water, shrimp intestine and sediment samples were collected from six regional sites in China (19.20º-39.29º N, 108.56º-117.93º E). The sampled 88 ponds had similar size, water depth, and stocking density (table S1). Site locations were recorded by global positioning system (GPS, Garmin Vista HCx, USA) and the geographical distances among sites ranged from 0.31 km to 2063.35 km. Each water sample (0.5 L) was taken from a depth of 0.5 m below the surface using a sterile bottle, and samples were immediately placed on ice before filtration through a 0.22 μm polyethersulfone membrane (Supor-

200, Pall, NY, USA) using a vacuum pump. The surface of shrimp was sterilized with 70% ethanol, then intestine was aseptically dissected from the musculature and placed into a 15 mL sterile centrifuge tube containing 10 mL PBS buffer. Each 5.0 g sediment sample was placed into a 15 mL centrifuge tube and washed with 10 mL PBS buffer for three times. All samples were stored at -80°C until DNA extraction.

Water temperature, pH, DO and salinity were measured on-site using a YSI handheld multi-parameter instrument (Model YSI 380, YSI Incorporated, USA), and meanwhile, sediment pH was measured on-site using a soil pH meter (ZD-05, Beijing Century Euron Co., Ltd., China). The TN, TP, dissolved inorganic nitrogen ($NH_4^+$-N, $NO_2^-$-N and $NO_3^-$-N), $PO_4^{3-}$-P of water samples, and TN, TP of sediment samples were measured using an auto discrete analyzer (Model CleverChem 380, DeChem-Tech, Germany). The TC and TOC of water and sediment samples were measured using a total organic carbon analyzer (Aurora 1030W, OI, USA). All physicochemical variables of water and sediment samples are described in the Additional file 1: Tables S12 and S13.

## DNA extraction, PCR amplification and sequencing of 16S rRNA gene amplicons

Genomic DNA from water, shrimp intestine and sediment samples were extracted by the Water DNA Isolation Kit (Omega Bio-tek, Doraville, GA, USA), PowerFecal DNA Isolation Kit (Mobio, Carlsbad, CA, USA) and PowerSoil DNA Isolation Kit (Mobio, Carlsbad, CA, USA), respectively. The 338F and 806R (5'-ACTCCTACGG GAGGCAGCAG-3'and 5'-GGACTACHVGGGTWTCTAAT-3') universal primer pair was used to amplify the V3-V4 regions of the bacterial/archaeal 16S rRNA gene. The PCR products from the samples were equally combined and then sequenced using the Illumina MiSeq platform (Illumina, San Diego, USA) by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

Paired-end sequences were merged using FLASH [56], and merged sequences were processed following the Quantitative Insights Into Microbial Ecology pipeline (QIIME version 1.9.0) [57]. In brief, the sequences with ambiguous bases or truncated at any site of more than three consecutive bases receiving a Phred quality score (Q) < 20 were removed. Chimeric sequences were discarded using the UCHIME algorithm [58]. Sequences with a distance-based identity of 97% or greater were grouped into OTU using UCLUST [59]. The most abundant sequence from each OTU was selected as representative and then was taxonomically assigned against the Silva SSU database 128 using the RDP Classifier algorithm (http://rdp.cme.msu.edu/), which enables each identified OTU to have a close relative. The core OTU was defined based on multiple reported measures: OTU with an occurrence frequency in more than 90% of all samples [60, 61]. Following the same criteria as described above, the core OTUs was identified for all 792 samples among three habitats or each habitat (e.g., 264 samples each in water, intestine and sediment habitats) in the SCPE.

## Relationships among microbial communities of water, intestine and sediment habitats

The relationships among microbial communities in water, shrimp intestine and sediment habitats of each pond and all sites were firstly analyzed using Venn analysis [62]. An additive partitioning framework was applied to separate the total microbial diversity at the ecosystem level ($\gamma_{Ecosystem}$) into contributions at

smaller scales from habitats to local communities [63]. More precisely, total ecosystem microbial diversity was expressed as the sum of inter-habitat difference in the community diversity, the mean intra-habitat difference and mean local community diversity with: $\beta_{Ecosystem} = \beta_{InterHabitats} + \bar{\beta}_{IntraHabitats} + \bar{\alpha}_{LocalCommunities}$. The ecosystem level ($\beta_{Ecosystem}$) may arise from a high microbial dissimilarity among habitats ($\beta_{InterHabitats}$), a high dissimilarity among communities within each habitat ($\beta_{IntraHabitats}$) or from a high diversity within each local community ($\bar{\alpha}_{LocalCommunities}$; i.e., each water, shrimp intestine or sediment sample). To further evaluate the relationships among microbial communities in water, shrimp intestine and sediment habitats, the different sources were used to estimate their contributions to microbial community composition of the shrimp cultural ponds using SourceTracker based on Bayesian algorithm [64], which was run through QIIME with default settings and with one habitat as the sink and the other two habitats as sources. The Sloan neutral community model [65] was used to analyze the OTUs that were shared between the shrimp intestine and surrounding water/sediment, in which the microbial community in water or sediment was the source of intestine microbiota. This model predicts that the probability of detecting an OTU in shrimp intestine due to dispersal is directly proportional to its abundance in the corresponding water/sediment community. OTUs were sorted into three categories depending on whether they occur more frequently (over-represented), less frequently (under-represented) or within (neutrally distributed) the 95% confidence interval of the neutral model predictions.

## Stochasticity of microbial assembly

We assessed microbial community-assembly stochasticity with a null-model-based index, stochasticity ratio, as described previously [52, 54, 66]. Since null-model approaches usually require adequate replicates, each sampled regional site in this study had more than 8 cultural ponds samples. We calculated stochasticity ratios using both taxonomic and phylogenetic metrics. When using dissimilarity index (abundance-weighted and unweighted Bray-Curtis), the stochasticity ratio was calculated based on typical null-model algorithms for taxonomic metrics [67, 68]. When using weighted and unweighted UniFrac, the stochasticity ratio was calculated based on typical null-model algorithms for phylogenetic metrics [68, 69]. Samples within each regional site were considered sharing the same regional species pool in null model algorithms.

## Statistical analysis

A ternary plot was applied to reveal the distribution of the dominant genera (> 0.1%) among water, shrimp intestine and sediment habitats using the package "ggtern" in R 3.3.2 [70]. Welch's *t*-test was used to compare the microbial diversity indices among water, shrimp intestine and sediment habitats. NMDS and ANOSIM were performed to evaluate the overall differences in microbial communities of water, shrimp intestine and sediment using weighted UniFrac distance [14]. Then, the differentially abundant taxa among three habitats were identified using one-way analysis of variance [34]. BioEnv and canonical correspondence analysis were used to identify the environmental factors importance to the microbial

community structure, which was used to construct the environmental factor matrix for VPA in R 3.3.2 with the vegan package [70].

# Additional Files

Additional file 1: Supplementary Tables S1-S13 (XLSX 196 kb)

Additional file 2: Supplementary Figures S1-S6 (DOCX 3,598kb)

# Abbreviations

AHPND: Acute hepatopancreatic necrosis disease; ANOSIM: Analysis of similarity; C/N: Ratio of carbon to nitrogen; C/P: Ratio of carbon to phosphorus; DO: Dissolved oxygen; EMS: Early mortality syndrome; GPS: Global positioning system; HPNS: Hepatopancreas necrosis syndrome; NMDS: Non-metric multidimensional scaling; N/P: Ratio of nitrogen to phosphorus; OTU: Operational taxonomic unit; SCPE: Shrimp cultural pond ecosystem; TC: Concentrations of total carbon; TN: Total nitrogen; TOC: Total organic carbon; TP: Total phosphorus; VPA: Variation partitioning analysis; WFS: White feces syndrome.

# Declarations

### Ethics approval and consent to participate

Not applicable

### Consent for publication

Not applicable

### Availability of data and materials

The 16S rRNA gene sequencing data used in this study are available in the NCBI Short Read Archive (https://www.ncbi.nlm.nih.gov/sra) under Bioproject PRJNA545396.

### Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

All authors contributed experimental assistance and intellectual input to this study. The original concept was conceived by ZJH, JGH, ZLH and DWH; Experimental strategies and sampling design were developed by ZJH, DWH, ZLH and JGH; Sample collections, DNA extraction, DNA sequencing and data analyses were performed by DWH, ZJH, RJZ, SZZ, CGX, DDW, XSD, LFY, HW, ZXD, SPW, DLN, CLX, QYY, JZZ, ZLH and JGH. The manuscript was written by ZJH, DWH, JZZ, ZLH and JGH. All authors read and approved the final manuscript.

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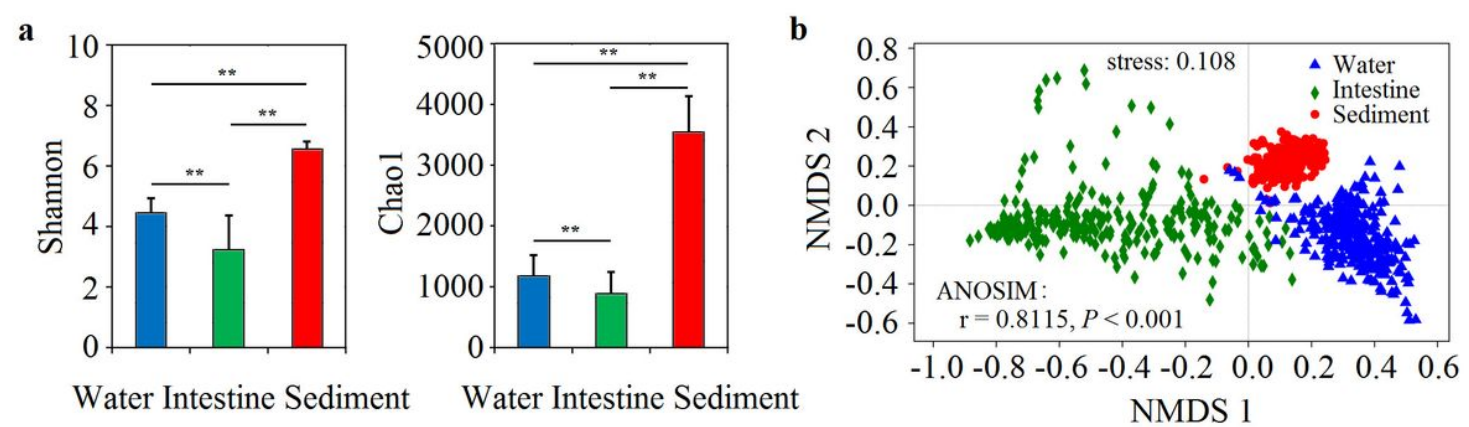
# Figures



Figure 1

Microbial diversity among water, shrimp intestine and sediment habitats in the SCPE. a Statistical significance of the α-diversity indices among water, shrimp intestine, and sediment habitats were based on the Welch's t-test (**: $P < 0.01$). b β-diversity of water, shrimp intestine, and sediment microbial communities in the SCPE analyzed by NMDS and ANOSIM based on weighted UniFrac distance.
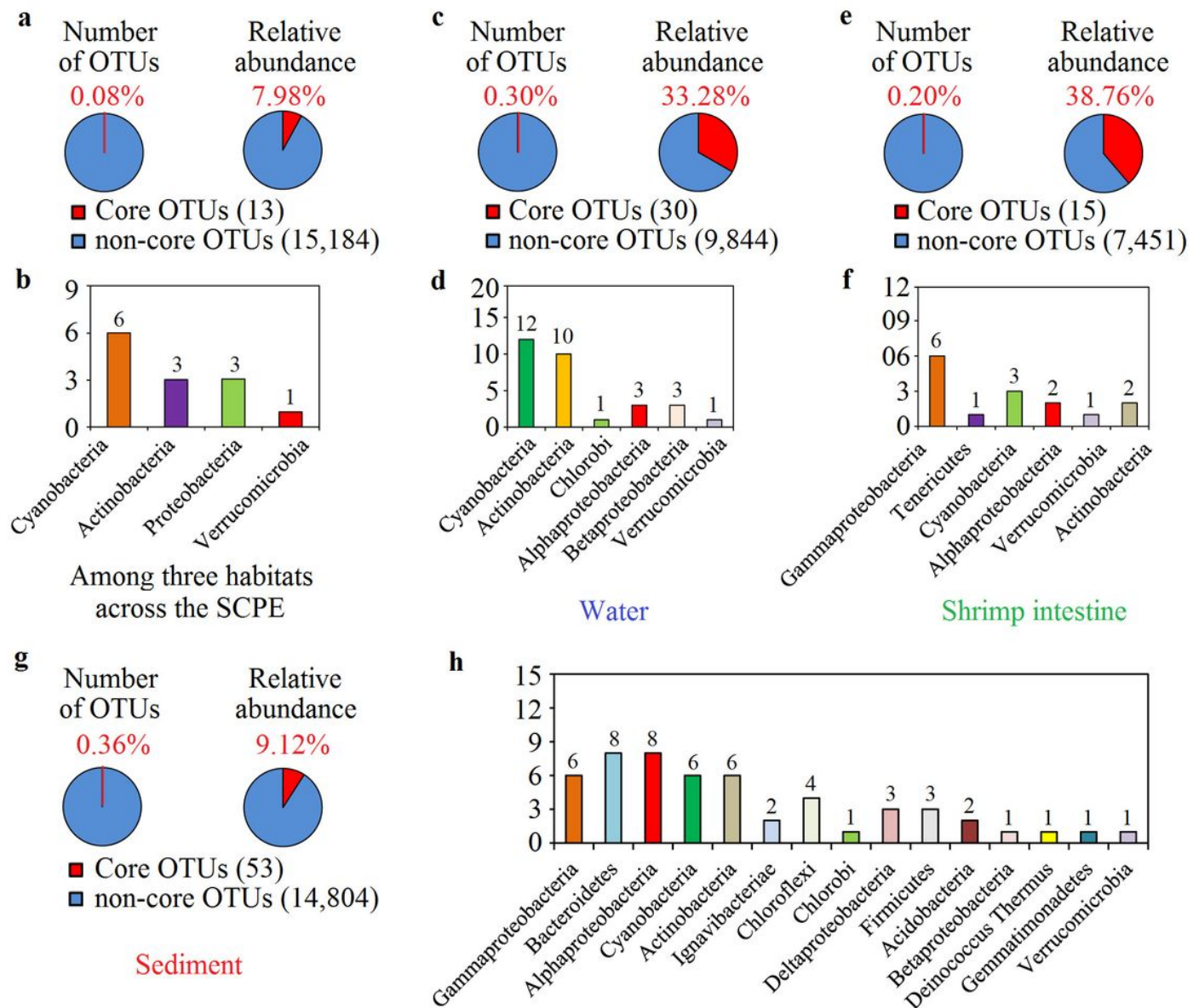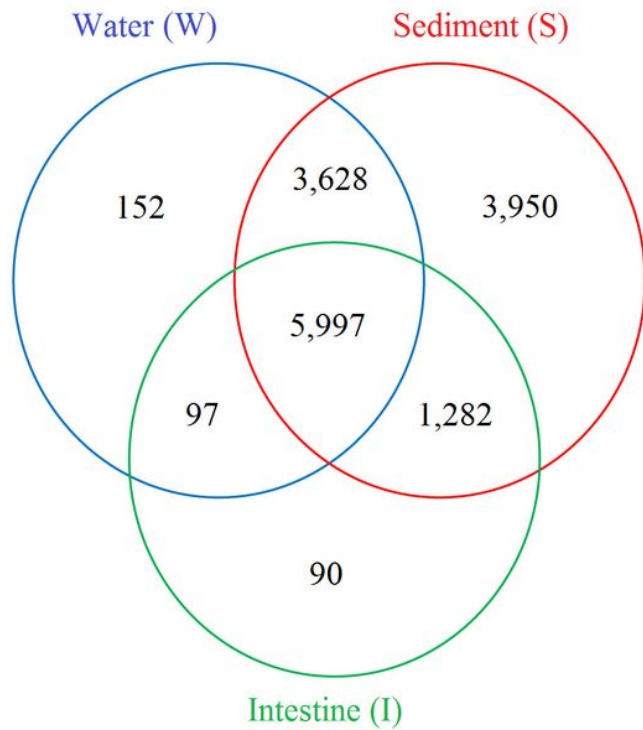
**Figure 2**

The abundance and composition of core microbial OTUs among three habitats or in each habitat. a The percentages and relative abundances of core OTUs and other remaining OTUs. b The taxonomic composition of core OTUs among three habitats across the SCPE at the phylum level. c and d The percentages and relative abundances of core OTUs and other remaining OTUs in water. e and f The percentages and relative abundances of core OTUs and other remaining OTUs in shrimp intestine. g and h The percentages and relative abundances of core OTUs and other remaining OTUs in sediment.

OTUs in Water = 9,874
OTUs in Intestine = 7,466
OTUs in Sediment = 14,857

OTUs shared in W and I = 6,094
OTUs shared in W and S = 9,625
OTUs shared in I and S = 7,279

OTUs shared in W and S/OTUs in W = 97.5%
OTUs shared in W and I/OTUs in W = 77.2%

OTUs shared in I and W/OTUs in I = 81.6%
OTUs shared in I and S/OTUs in I = 97.5%

OTUs shared in S and I/OTUs in S = 49.0%
OTUs shared in S and W/OTUs in S = 64.8%

**Figure 3**

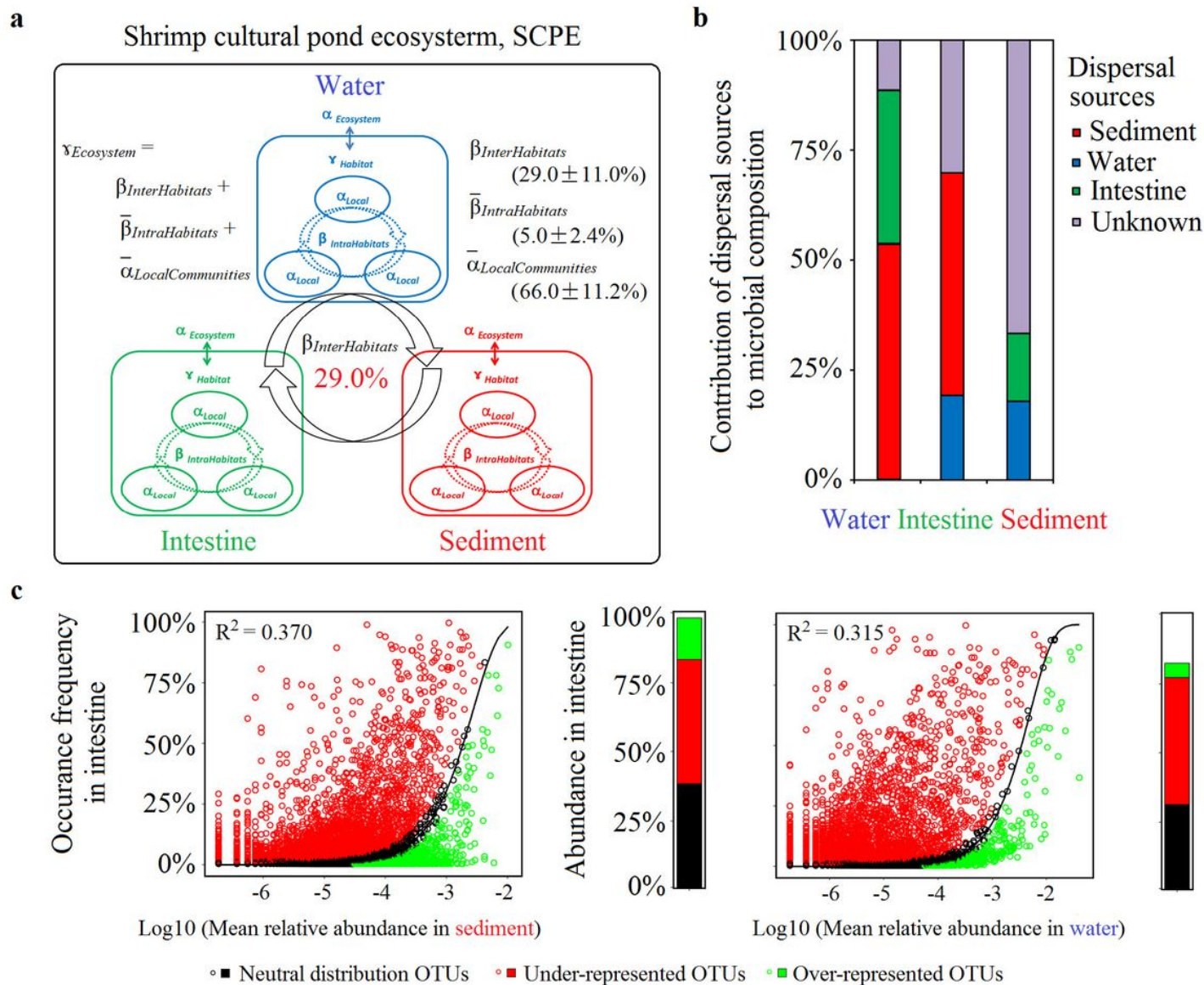Venn analysis of microbial compositions in water, shrimp intestine and sediment habitats based on detected OTUs.

**Figure 4**

The contribution of communities in water, shrimp intestine and sediment habitats to the SCPE metacommunity. a Multiscale hierarchical partitioning of microbial community diversity. b SourceTracker analysis of contributions of water, shrimp intestine, and sediment source communities to each other's microbial communities. c The neutral model applied to shrimp intestine microbial communities with their corresponding surrounding water/sediment communities as the sources. Stacked bar chart depicts the relative abundance of sequences in the neutrally distributed (black), under-represented (red) and over-represented (green) OTUs in shrimp intestine. The model was applied independently for the OTUs that were shared between the shrimp intestine and surrounding water/sediment for all ponds.
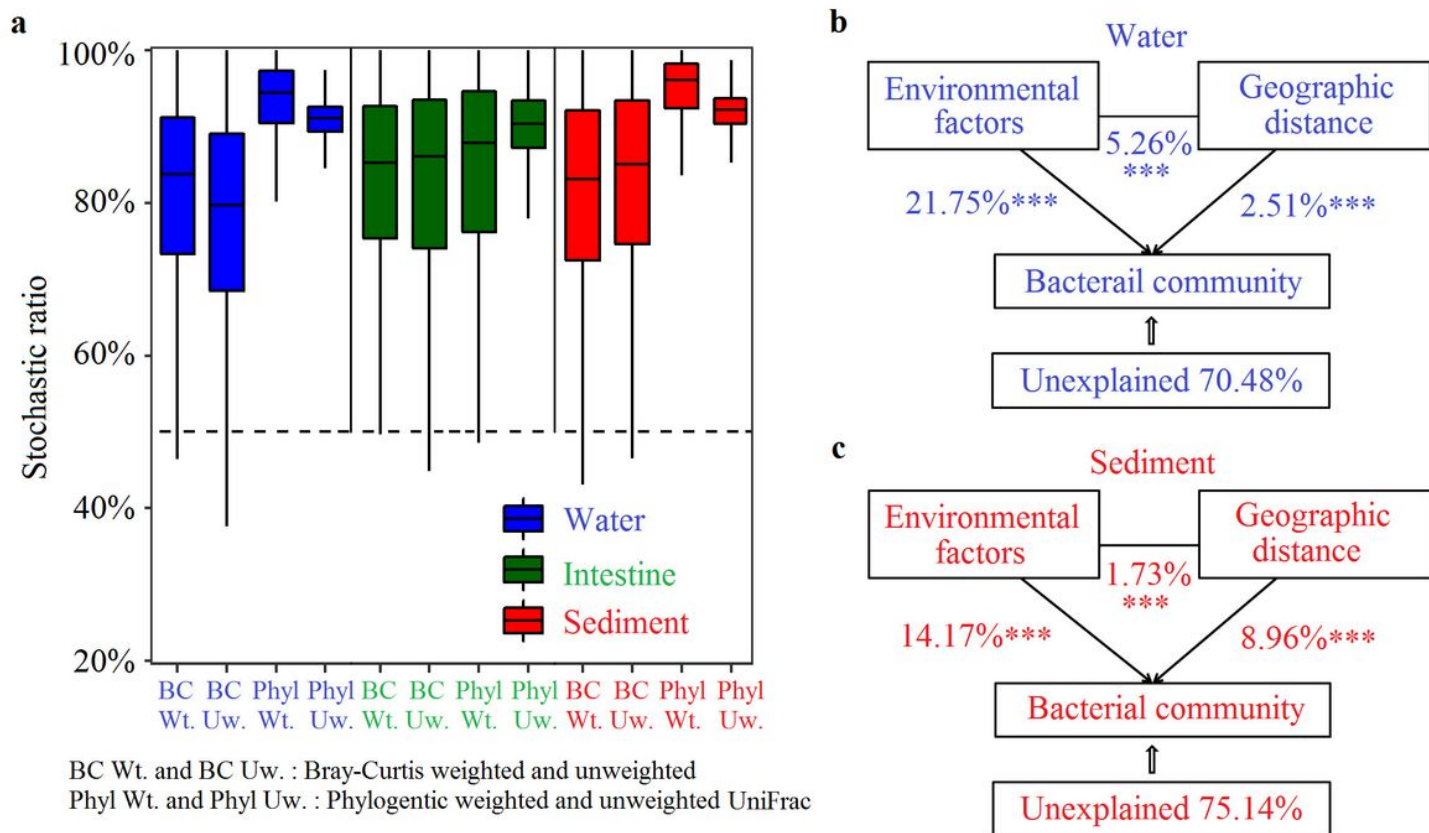
**Figure 5**

Microbial assembly mechanisms of water, shrimp intestine and sediment habitats in the SCPE. a Ecological stochasticity of microbial assembly estimated by stochasticity ratio, which was calculated for each pair of samples based on taxonomic diversity (Bray-Curtis, BC) and phylogenetic diversity (Phyl., UniFrac) weighted with abundance (Wt) or not (Uw). Boxes and whiskers indicate quartiles. b c VPA of geographic distance and environmental factors on the microbial community structure of water and sediment samples. Numbers under double lines indicate the interaction effect of water or sediment environmental factors and geographic distance on the microbial community structure. ***: P < 0.001.
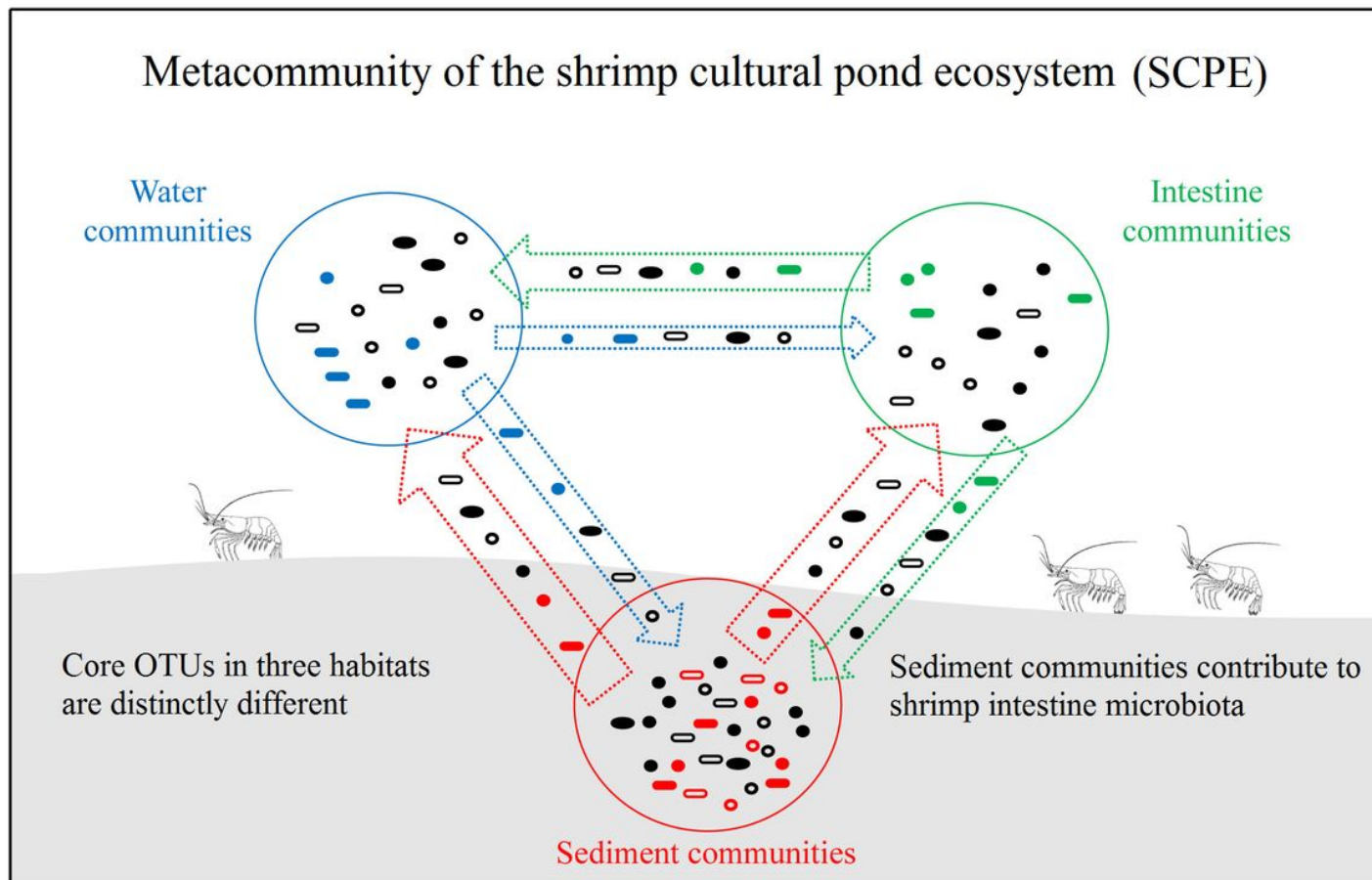
**Figure 6**

A schematic presentation of microbial characteristics and relationships among communities of water, shrimp intestine and sediment habitats in the SCPE.

## Supplementary Files

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- Additionalfile2.docx
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