

Influence of environmental variables on methane related microbial activities in a tropical bio-secured zero-exchange shrimp culture system

Elaine A. Sabu^{a,b}, Maria Judith Gonsalves^{a*}, Delcy Nazareth^a, Rayadurga Anantha Sreepada^a

^aBiological Oceanography Division, CSIR-National Institute of Oceanography,
Dona Paula, Goa – 403 004, India

^bSchool of Earth, Ocean and Atmospheric Sciences, Goa University,
Taleigao Plateau, Goa – 403 206, India

*Corresponding author: M.J.B.D. Gonsalves, Senior Principal Scientist,
Biological Oceanography Division, CSIR-National Institute of Oceanography,
Dona Paula, Goa – 403004, India. Tel: +91–832–2450624; Fax: +91–832–2450606;
E-mail: mjudith@nio.org

Abstract

Recently, aquaculture has emerged as the fastest growing food-producing sector accounting for nearly 50% of the world's food fish and is an activity of economic importance worldwide. Aquaculture systems are considered as one of the potential contributors to atmospheric methane (CH₄)—the second most important greenhouse gas (GHG). The present study was undertaken to assess the influence of environmental variables on the abundance of culturable methanotrophs (MOB), methane oxidation activity (MOA) and methane production activity (MPA) during a grow-out cycle in zero-exchange shrimp pond growing whiteleg shrimp, *Litopenaeus vannamei*. During the 96 days of culture (DoC), the abundance of MOB in the pond water and sediment, ranged between 3.04×10^3 to 1.8×10^4 CFU mL⁻¹ and 3.36×10^5 to 3.17×10^6 CFU g⁻¹, respectively. The mean potential rates of MOA and MPA in pond sediments were 392.71 ± 53.06 $\mu\text{mole g}^{-1}\text{d}^{-1}$ and 0.60 ± 0.30 $\mu\text{mole g}^{-1}\text{d}^{-1}$, respectively. The abundance of MOB and methane related activities were strongly influenced by the dissolved nitrogen species and total organic carbon ($p < 0.05$). In addition to providing the baseline data on microbial mediated methane activities, the study highlights the importance of methane oxidation/production processes in commercial earthen shrimp aquaculture system and the influence of environmental variables in regulating these processes. The results of this study would aid in developing environmental friendly management processes to reduce the efflux of GHG methane from shrimp aquaculture systems which is a thriving industry in the food sector.

Keywords: Shrimp aquaculture; *Litopenaeus vannamei*; Methane oxidation; Methane production

1. Introduction

Shrimp aquaculture is currently undergoing tremendous expansion due to its economic importance across the world, to meet the protein requirement of burgeoning world population (Klinger and Naylor, 2012; Yuan et al., 2019; Gamboa-Delgado et al., 2020). With a total production of 4.97 million tonnes (~53% of the total shrimps and prawns production), the whiteleg shrimp, *Litopenaeus vannamei* was the highest harvested crustacean species in the world during 2018 (FAO, 2020). By virtue of its attributes such as high density tolerance, physiological adaptability to environmental variables like salinity and temperature, relatively faster growth during short culture periods and omnivore feeding habit, have made *L. vannamei* the most dominant shrimp species for large-scale commercial aquaculture (Gamboa-Delgado et al., 2020). Rapid large-scale transformation from traditional aquaculture to the high-density and high-yield culture systems have the potential to cause hypernutrification and eutrophication in the surrounding coastal ecosystems. Currently many coastal areas worldwide are experiencing pollution from several anthropogenic sources (Hou et al., 2016) and the effluents from aquaculture systems may deteriorate the coastal ecosystem health. Hence, an understanding of the variations of different environmental parameters and by-products formed during shrimp farming will contribute to the improvement of the ecological and environmental sustainability of shrimp farming.

In contrast to other natural water bodies, aquaculture ponds have potential in contributing to carbon emissions by virtue of addition of management inputs to enhance aquatic production (Boyd et al., 2007, 2010). With the progress of the culture, organically rich residues (uneaten feed, faeces and detrital aggregates) that accumulate on the pond bottom provide abundant sources of labile organic matter (carbon and nitrogen) to microbes, thereby stimulating the microbial decomposition (Green et al., 2012; Chen et al., 2016; Yang et al., 2017b). This leads to concomitant decrease in pH (Fazli et al., 2013) and dissolved oxygen levels thus triggering the development of anaerobic conditions. The formation of anaerobic conditions in the pond sediment favors the production of green house gases (GHGs) like methane (CH₄) and its subsequent ebullition into the atmosphere (Krüger et al., 2001; Bastviken et al., 2004; Yang et al., 2017b; Yuan et al., 2019, 2021). Methane being the second most important GHG has a global warming potency of 34 times higher than CO₂ (Yang et al., 2019b) and increased emissions of CH₄ during shrimp culture, especially during the middle and late phase in the brackish marsh ecosystem converted shrimp ponds (Yang et al., 2017a). This has a severe impact on the global climate change hence the methane emission from aquaculture systems are a matter of concern and needs to be monitored. However, the mechanisms involved in CH₄ cycling in aquaculture ponds are not fully understood (Martinez-Cruz et al., 2017). The methane cycle consists of distinct microbial taxa that are involved in the production and oxidation of methane thereby

controlling the methane emission from the ponds into the atmosphere (Fazli et al., 2013). In the aquaculture ponds, the presence of substrates and anaerobic niches favor the production of methane by methanogenesis. This CH₄ gas gets released into the atmosphere from the sediment and water column (Dutta et al., 2013; Neue et al., 1997). During the carbon mineralization more than 50% is reported to get converted into methane through methanogenesis in the aquaculture ponds (Brigolin et al., 2009). Methane thus released, can be oxidised under aerobic and anaerobic conditions by various methanotrophic microbial groups (Kalyuzhnaya et al., 2019). These methanotrophs act as bio-filters thus playing an important role in mitigating the emission of methane. Bastviken et al. (2011) reported that about 80% of CH₄ is oxidised into CO₂ by the methanotrophic bacteria (Kumaraswamy et al., 2000). Hence, the sediment-water interface plays a significant role in the biogeochemical cycling of CH₄ in aquaculture ponds (Xiong et al., 2017). The balancing between the sources and sinks of methane by methanogens and methanotrophs respectively, determine the net CH₄ flux from the aquaculture ponds (Grinham et al., 2018; Sabu et al., 2021).

Studies have reported that the methane emissions from various aquaculture practices are influenced by factors such as high water salinity, substrate (Yang et al., 2019c) and dissolved oxygen (Hu et al., 2016). Yang et al. (2020) reported that methane is emitted from the aquaculture ponds majorly through ebullition. Erazo and Bowman (2021) studied the impact of aquaculture effluent released into the mangrove ecosystem which could act as a source for methane emission rather than a sink (Maher et al., 2016). The above studies deals majorly on the methane emission rates from the aquaculture systems. However the present study focuses on the interaction of microbial communities involved in the production and oxidation of CH₄. These activities significantly govern the net CH₄ emission. Hence, considering the global footprint of shrimp aquaculture, study on methane related physiological microbial groups and its activity in relation to the environmental variables during the 96 days of culture of *L. vannamei* in zero-exchange shrimp culture system was undertaken. The findings of the study would help in upgrading and improvising the farm management practices for mitigating the emissions of GHG methane from shrimp aquaculture systems.

2. Materials and methods

2.1. Sampling site and farm management

A commercial shrimp farm located near Alvekodi village near Kumta town (Karnataka State, India), south-western coastal India (Lat. 14.42° N; Long. 74.40° E) was chosen for the present study. The farm is tide-fed from the adjoining mangrove creek located around 3 km from the coast. The investigations for the present study were carried out in a biosecured zero-exchange shrimp pond (water spread area, 0.84 ha; mean water depth, 1.2 ± 0.10 m). The farm management practices

involved the stocking of certified shrimp seeds, stringent feed management, periodic water quality assessment, probiotics application etc. The pond bottom was prepared (sun drying, fertilization and liming) 30 days prior to the stocking of post larvae. During the pond preparation prior to larval stocking 800 kg of agrilime was applied and during the production cycle agrilime, dolomite, calcium hydroxide and calmag were added at different quantities on regular basis to buffer the pH and alkalinity fluctuations. The culture pond was filled with dechlorinated and conditioned seawater from reservoir followed by the application of inorganic fertilizer (4 ppm of urea:single super phosphate in a ratio of 1:1). The farm-prepared liquid mixture containing rice/wheat bran, jaggery, yeast, cow dung and protein sources (groundnut oil cake, old feed, buttermilk) were added (100 to 150 L) during the initial 15 days of culture (DoC) for enhancing the natural pond productivity. This fermented liquid mixture is expected to act as a probiotic and also help in regulating pH fluctuations and in maintaining the optimum C:N ratio.

2.2. Stocking and feed management

Post-larvae (PL₁₈) of *Litopenaeus vannamei* which tested negative to white spot syndrome virus by polymerase chain reaction test procured from a nearby shrimp hatchery were stocked after two weeks of pond preparation. Before stocking into the pond, the PL₁₈ were observed for visible signs of morbidity and the stocking was done in the early morning hours at a density of 11 PL₁₈ m⁻². During the culture, shrimps were fed with formulated pellet feed (CP-Aquaculture, India, proximate composition, 38–40% protein; 5% lipids and 3% fibre) split into three rations a day. A progressive decrease in feeding rate from 10% to 2% with the increase of shrimp body weight was followed. The feeding rates were adjusted based on the monitoring of survival and shrimp biomass.

2.3. Culture conditions

The shrimp production cycle lasted for a duration of 96 days with zero-water exchange. During the production to compensate the water loss through evaporation and seepage, fresh dechlorinated water was added to maintain the minimum water depth (~ 1.2 m). The frequent monitoring of pH helped in buffering the wide fluctuations with the application of hydrated lime and pH fixers. Paddle-wheel aerators were operated with an aeration schedule of 8, 12 and 16 h d⁻¹ for 0–50; 51–80 and 81–96 DoC, respectively for maintaining optimum dissolved oxygen (DO) levels in the pond. Commercially available liquid and powdered probiotics were administered directly to the pond water as well as with the feed. The consistent application of liquid probiotics ‘Super PS’ (mixture of *Rhodobacter* spp. and *Rhodococcus* spp., ~ 10⁹ CFU mL⁻¹) restricted the formation of hydrogen sulfide (H₂S), increased the DO levels, supported the non-pathogenic bacterial growth and helped

decomposition of the accumulated organic matter (carbohydrates, proteins and lipids). The commercial feed-based probiotics containing *Bacillus subtilis* (10^8 CFU mL⁻¹) and vitamin-mineral feed additive were also added for enhancing the growth and survival rates of shrimp.

2.4. Environmental parameters and analysis

The study involved nine sampling campaigns, 0 DoC (one day prior to transfer of post larvae) and thereafter on 12, 25, 41, 55, 68, 78, 88 and 96 DoC. Water and sediment samples were collected from three pre-determined points within the shrimp pond. The three pre-determined points in the pond were close to pond outlet; middle of the pond and proximate to feeding tray in the pond and the selection of these sampling locations were based on the positioning of paddle-wheel aerators. A Niskin water sampler was used to collect pond water (30 cm above the bottom) and a handheld acrylic core (50 mm diameter) was used to collect the sediment from the top 0–5 cm of the pond bed. All the samplings were carried out at a fixed time of the day. Samples were transported aseptically in an ice box to the laboratory for processing and analysis. Water temperature, pH and salinity were measured *in situ* with the help of calibrated thermometer, portable pH meter (Cole-Parmer-99361-12) and a handheld refractometer (Atago, Japan), respectively. The DO concentrations in the pond water samples were estimated by Winkler's method (Parsons et al., 1984). The biochemical oxygen demand (BOD₅) was estimated according to the 5-day BOD test as per the method suggested by Grasshoff et al. (2009). Content of total suspended solids (TSS) was estimated by filtration of a known volume of water sample on pre-weighed Whatman GF/F glass fibre filter paper (47 mm diameter; nominal pore size: 0.7 µm). The filter paper was oven dried to attain constant weight and the results were computed (Grasshoff et al., 2009). The concentrations of dissolved nutrients in the pond were determined using the salicylate method for ammonium (NH₄⁺), diazotization method using nitrite (NO₂⁻), cadmium column reduction followed by diazotization method for nitrate (NO₃⁻) and ascorbic acid method for orthophosphate (PO₄³⁻) (Grasshoff et al., 2009). Water samples for chlorophyll *a* (Chl *a*) analysis were filtered using GF/F, extracted overnight in 90% acetone and analyzed spectrophotometrically (Parsons et al., 1984). The acetone extracts of Chl *a* were treated with 10% HCl and analyzed for phaeophytin (Phaeo) pigments using spectrophotometer (Parsons et al., 1984). All the environmental variables were analyzed in triplicate.

The concentrations of total carbohydrates (CHO) in the pond sediment were estimated using phenol-sulfuric acid method as described by Kochert (1978), with glucose as the standard. The total protein (PRT) content was analyzed using Lowry's Folin-Ciocalteu method with bovine serum albumin as a standard (Lowry et al., 1951). Bligh and Dyer (1959) method using chloroform and methanol extraction was used to determine the total lipid (LIP) concentrations in the sediment with

stearic acid as standard. Total organic carbon (TOC) in the pond sediment were analyzed by wet oxidation method (El Wakeel and Riley, 1957) with glucose as standard. The total nitrogen (TN) content in the pond sediments was estimated by Kjeldahl digestion (Bremner, 1965) and total phosphorus (TP) (Parsons et al., 1984).

2.5. Bacteriological groups

The total heterotrophic bacteria in water (THBw) and sediment (THBs) were enumerated by spread plate technique on nutrient agar plates (Rodina, 1972) and the total anaerobic bacteria (TAB) were enumerated using anaerobic agar medium (HiMedia, India) (Rodina, 1972). Abundance of methane-oxidizing bacteria (MOB) in pond water and sediments were enumerated on nitrate mineral salts medium (NMS) (Whittenbury et al., 1970). The enumeration of methanotrophs were performed at ambient room temperature (26 ± 2 °C) on NMS agar plates which were incubated in anaerobic bags with a headspace of roughly 80% air and flushed with methane contributing 20% (Kalyuzhnaya et al., 2019). The colonies were counted after 4-5 days of incubation. The source for each bacterial group is indicated by 'w' and 's' for water and sediment, respectively.

2.6. Methane related activities

The methane production activity (MPA) and potential methane oxidation activity (MOA) were determined for the pond sediments for the entire production cycle. For detecting the rates of MOA, slurries (in triplicate) were prepared using 1 g (wet weight) of pond sediment and 9 mL of pond water (filtered and autoclaved) in a gas-tight vial (20 mL capacity) and vials, were purged with 0.1 mL of methane gas. To inhibit the methanogenic activity 100 μ L of 2-bromoethanesulphonate (Sigma-Aldrich, USA) was added. The gas-tight vials were incubated at ambient room temperatures (26 ± 2 °C). The methane concentrations on days, 0 (D_0) and 4 (D_4) of incubation were estimated following the headspace method (Gonsalves et al., 2011) using gas chromatography (GC) equipped with a flame ionization detector (Shimadzu GC-2010). Nitrogen was used as a carrier gas (9 mL min^{-1}) and the oven temperature was maintained isothermally at 60 °C. The GC was calibrated using a reference mixture of methane standards (concentration range, 1–5 ppm; Alchemie Gases & Chemicals Pvt. Ltd., Mumbai, India). The methane oxidation activity for the sediments were computed using Bunsen solubility coefficient according to Bastviken et al. (2002) and Tishchenko et al. (2005). The values obtained were expressed as $\mu\text{mole g}^{-1} \text{d}^{-1}$.

For estimation of MPA in sediments, slurries were prepared similarly as defined for MOA, except that the vials were purged with 1 mL of acetylene gas to inhibit methane oxidation. At the beginning of incubation, the vials were flushed with N_2 gas to maintain anaerobic conditions (Pack et

al., 2011). Methane concentrations were quantified based on the differences of values on D₀ and D₄ (Gonsalves et al., 2011). The rates were calculated as mentioned above.

2.7. Statistical analyses

Before subjecting the data for statistical analysis, data on environmental variables were square root transformed, and bacterial abundances were $\log(x+1)$ transformed. The significant interrelationships between these variables were deciphered using Spearman correlation analysis using the software Statistica version 12.0 (Zar, 1996). The correlation coefficient showed the significant relationships between environmental variables and bacterial groups which influence the methane related activities (MPA and MOA). Three levels of significance ($p < 0.05$, $p < 0.01$ and $p < 0.001$) were reported. The network diagrams for visualizing the significant relationships ($p < 0.05$) were built using the software Cytoscape version 3.7.0 (Shannon et al., 2003). Principal component analysis was performed for delineating a set of environmental parameters controlling the abundance of bacterial groups using PRIMER 6 software (Primer-E Ltd., Plymouth, UK) after data normalization.

3. Results

3.1. Environmental parameters

All the measured water quality parameters during the culture period (Fig. S1) fell within the optimal range for the growth and survival of *L. vannamei*. During the culture period, the temperature varied from normal range of 25 °C to 32 °C (29.4 ± 1.85 °C) and nearly alkaline pH values (7.91 ± 0.08) were maintained. A wide variation in alkalinity values (80 to 140 mg L⁻¹; 106.56 ± 15.89 mg L⁻¹) recorded during the production cycle were largely due to the frequent application of lime to buffer pH fluctuation. Salinity increased from an initial value of 32 (0 DoC) to 44 by the end of the culture period (96 DoC) and *L. vannamei* is known to adapt to wide variations in salinity levels (Ponce-Palafox et al., 2019). Concentration of total suspended solids (TSS) peaked during the middle of the culture period and declined thereafter and remained low until the end of the culture period. Optimum concentrations of DO (5.50 ± 1.14 mg L⁻¹) were maintained during the entire culture duration with the help of paddle-wheel aerators and commercial oxygen releasing compounds. With the progress of culture, the concentrations of Chl *a* (4.18 ± 3.80 mg L⁻¹) increased from the 0 DoC (0.69 ± 0.05 mg L⁻¹) towards the end of the culture period (11.08 ± 4.42 mg L⁻¹). Levels of nutrients showed a decreasing trend after the 41 DoC. The concentration of NH₄⁺ (19.48 ± 0.14 μM) peaked during the middle culture period (41 DoC). On the other hand, the mean concentrations of NO₂⁻ (13.66 ± 0.49 μM) and NO₃⁻ (122.76 ± 27.97 μM) showed a similar trend peaking on 12 DoC and declined

thereafter progressively with the DoC. The concentration of PO_4^{3-} increased towards the end of the culture period ($19.14 \pm 1.56 \mu\text{M}$). The mean concentrations of TN and TP in the pond sediments during the culture period (dry weight) were 2.39 ± 0.17 and $0.28 \pm 0.03 \text{ mg kg}^{-1}$ respectively and varied within a narrow range. An increase in the concentrations of nutrients (NH_4^+ , NO_2^- and NO_3^-) and sediment TN and TP on 12 DoC was discernible. The average TOC in the pond sediment was $4.32 \pm 1.63\%$. During the production cycle, the mean content of CHO for pond sediment was $276.71 \pm 87.02 \mu\text{g g}^{-1}$. The mean LIP concentration was $2206.94 \pm 405.14 \mu\text{g g}^{-1}$ with a sudden decline on 55 DoC and the mean concentration of PRT for pond was $311.46 \pm 83.41 \mu\text{g g}^{-1}$ with a gradual decline towards the 96 DoC. The survival rate, mean biomass and the food conversion ratio (FCR) recorded during the culture were 78%, $20.83 \pm 0.5 \text{ g shrimp}^{-1}$ and 1.66, respectively.

3.2. Bacteriological abundance

The abundance of THBw increased by an order of magnitude on 12 DoC ($5.5 \times 10^6 \text{ CFU mL}^{-1}$) from an initial count of $1.5 \times 10^5 \text{ CFU mL}^{-1}$ and dropped to $3.3 \times 10^4 \text{ CFU mL}^{-1}$ post 41 DoC (Fig. 1). The highest abundance of THBs was during the 41 DoC ($1.0 \times 10^{10} \text{ CFU g}^{-1}$) and decreased gradually to $3.3 \times 10^7 \text{ CFU g}^{-1}$ at the time of crop harvest (Fig. 2). Both in water and sediment, the abundance of TAB showed a decreasing trend from the initial to the final harvesting time. The culturable TAB in water and sediment were more abundant during the initial stages of culture: $2 \times 10^6 \text{ CFU mL}^{-1}$ (water) and $8.8 \times 10^4 \text{ CFU g}^{-1}$ (sediment), respectively and decreased with the increase in shrimp biomass to $1.1 \times 10^4 \text{ CFU mL}^{-1}$ and $7.4 \times 10^3 \text{ CFU g}^{-1}$ on 96 DoC. The counts of TAB peaked (0 – 30 DoC) during the initial stages of culture and progressively decreased after 30 DoC (Fig. 1 & 2). The abundance of THBw showed the highest count when compared to the other bacterial groups. The abundance of THBs was two-orders higher than those of TAB. The MOB in the sediment ranged from 3.36×10^5 (0 DoC) to $11.2 \times 10^5 \text{ CFU g}^{-1}$ (96 DoC). Comparatively, the MOB abundance was almost two-orders lower in the water: 5.2×10^3 (0 DoC) to $6.8 \times 10^3 \text{ CFU mL}^{-1}$ (96 DoC). However, the general trend of MOB population in water (Fig. 1) and sediment (Fig. 2) was almost similar.

3.3. Methane related activities

The levels ($\mu\text{mole g}^{-1} \text{ d}^{-1}$) of potential MOA and MPA in the shrimp pond sediments during the production cycle is depicted in Fig. 3. The lowest rates of MOA ($15.23 \pm 6.55 \mu\text{mole g}^{-1} \text{ d}^{-1}$) was observed on 25 DoC and the maximum of $599.61 \pm 167.18 \mu\text{mole g}^{-1} \text{ d}^{-1}$ was on 55 DoC. The rate of MOA did not show any specific trend during the entire production cycle. The rate of MPA in the pond sediments peaked on 41 DoC ($3.45 \pm 0.97 \mu\text{mole g}^{-1} \text{ d}^{-1}$), post 41 DoC, the rate of MPA showed

a declining trend till the harvest. The lowest MPA ($0.102 \pm 0.05 \mu\text{mole g}^{-1} \text{d}^{-1}$) was recorded on 88 DoC.

3.4. Interrelationship between environmental variables and bacterial abundance

The results of Spearman correlation analysis revealed the significant interrelationships ($p < 0.05$) between the environmental parameters and the bacterial groups which influence the rates of MPA and MOA in the pond ecosystem (Fig. 4). The network of interactions between the bacterial groups (water and sediment) and methane related activities (sediment) and various environmental variables as revealed by Cytoscape network in shrimp pond is shown in Fig. 4. The abundance of heterotrophs and anaerobes in both water and sediments showed significant positive correlation with dissolved nitrogen species, while negative correlation was obtained for methanotrophs in water (Fig. 4). Similarly, TOC negatively correlated with THB and TAB in water and sediment. MOBw positively correlated with TOC ($p < 0.01$, $r = 0.556$) and negatively correlated with NH_4^+ ($p < 0.01$, $r = -0.569$), NO_2^- ($p < 0.001$, $r = -0.686$), NO_3^- ($p < 0.01$, $r = -0.519$), THBw ($p < 0.001$, $r = -0.600$) and TABw ($p < 0.01$, $r = -0.582$) in the water. In the sediment, the CHO ($p < 0.05$, $r = 0.460$) and PRT ($p < 0.05$, $r = 0.403$) showed positive correlation with the MOB_s, while negative correlated with TABw ($p < 0.05$, $r = -0.782$). The methane production correlated with ammonia ($p < 0.001$, $r = 0.785$), TOC ($p < 0.05$, $r = -0.402$) and TAB_s ($p < 0.05$, $r = 0.420$).

The results of principal component analysis (PCA) for assessing the magnitude of influence of environmental variables on the bacterial groups and the methane related microbial activities are summarized in the Table 1. Based on the eigen values ($\geq \pm 0.2$), the first five principal components (PC) were retained and they explained a cumulative variance of 87.30%. The PC1 explained as much as 40% of the total variance with positive loadings by NO_2^- , NO_3^- and the bacterial groups such as THBw, TABw and negative loadings by salinity, temperature, Chl *a*, Phaeo and TOC (Table 1). PC2 explained 18.5% of the variability and included negative loadings by pH, TSS, NH_4^+ , CHO, MPA, MOB_s and phytoplankton abundance (Table 1). There was a significant positive loading for potential MOA on this axis. PC3 explained 12.35% of the variance with positive loadings of DO, BOD, TP, PRT, MOB_s while the axis had LIP, TABw and THB_s with negative loadings. PC4 and PC5 explained 10.55% and 5.9% of the total variability, respectively. This is indicated by the positive loading of MOB_s on PC4. PC5 was positively loaded with TN and PRT (Table 1).

4. Discussion

Aquaculture has emerged as the fastest-growing food-producing sector to meet the increasing demand for aquatic products (Muralidhar et al., 2017; FAO, 2020). The emission of potential GHGs mainly CH_4 and N_2O (Yang et al., 2017a, 2017b) formed as end products from the degradation of

rich organic matter (Bridgham et al., 2013) during the aquaculture production has been documented (Queiroz et al., 2019). Considering CH₄ as the second most important GHG, few studies (Yang et al., 2017a; Queiroz et al., 2019; Raul et al., 2020; Erazo and Bowman, 2021) on the emission of CH₄ in aquaculture systems have been investigated (IPCC, 2018). Yang et al. (2017a) reported a mean CH₄ flux of $123 \pm 48 \text{ mg CH}_4 \text{ m}^{-2} \text{ h}^{-1}$ from shrimp ponds, whereas Queiroz et al. (2019) reported a CH₄ flux of $0.8 \text{ mg CH}_4 \text{ m}^{-2} \text{ h}^{-1}$ from a mangrove site receiving shrimp pond effluents. Besides, coastal marine shrimp ponds have significantly higher methane emissions compared to that from freshwater aquaculture ponds (Hu et al. 2016) that could be attributable to the sub-optimal water quality (Hasan and Soto, 2017).

The pond water quality gets influenced by the microbial processes, especially towards the end of the production cycle (Moriarty, 1997; Funge-Smith and Briggs, 1998). In order to obtain sustainable yields, shrimp farmers strive to maintain the key water quality parameters within the desired levels by adopting best pond management practices including the application of probiotics (Shariff et al., 2001; Nakayama et al., 2009). In a healthy shrimp farming system, the indigenous microbial flora aid in the remineralization of organic nutrients and also tend to influence the methane related activities (Sahu et al., 2008). Various complex biogeochemical processes (Holmer et al., 2005; Yang et al., 2019b) including microbial methane related activities which form an integral part of aquaculture pond ecosystems has seldom been taken into account while explaining the variability in global methane budgets (Yang et al., 2020). The present study was undertaken in order to gain comprehensive understanding of pond ecosystem functioning and magnitude of influence of environmental variables that are intrinsically linked to the methane related microbial activities. Such an information is crucial for achieving environmentally friendly standards for pond management and for the sustainability of shrimp culture farms.

Environmental variables play an important role in determining the shrimp productivity and health (Ray et al., 2011). These environmental variables influenced the microbial biogeochemical processes which determine the pond dynamics (Sabu et al., 2021). Accumulation of dissolved nutrients (NH₄⁺, NO₂⁻, NO₃⁻ and PO₄³⁻) and organic matter (uneaten feed, faeces and metabolites) with the progress of the culture period tend to affect the water quality and shrimp productivity in the zero-exchange shrimp systems (Ray et al., 2010). It has been documented that as low as ~15% of the formulated feed (amounting to 24–37% of nitrogen and 11–20% of phosphorus) is assimilated as shrimp biomass and the remainder is discharged into the pond water and sediment (Funge-Smith and Briggs, 1998; Christopher et al., 2003). This accumulation of organic matter contributes to CH₄ formation (Boyd et al., 2010; Boyd and Tucker, 2014). The paddle-wheel aerators used in augmenting the DO levels are also known to contribute to the circulation and facilitate water column

mixing (Tendencia et al., 2015). Furthermore, maintenance of oxic conditions through aeration in enhancing the microbial activity (Fernandes et al., 2010) such as the aerobic methane oxidation (Yuan et al., 2019; Yang et al., 2019a) in shrimp ponds have been reported.

Bacterial and phytoplankton communities in the pond ecosystem recycle the nutrients (Hargreaves 1998; Fellows et al., 2006). In the present study, THBw showed highly significant correlation with dissolved nitrogen species (NH_4^+ , NO_2^- , NO_3^-) and an inverse relationship with Chl *a* ($p < 0.001$, $r = -0.771$) and phytoplankton abundance ($p < 0.05$, $r = -0.435$) implying the occurrence of effective recycling and assimilation of nutrients (Lemmonier et al., 2010). Significant negative correlation of phytoplankton abundance with NO_2^- ($p < 0.01$, $r = -0.503$) and NO_3^- ($p < 0.01$, $r = -0.537$), implies that the uptake of dissolved inorganic nitrogen by phytoplankton might constitute the primary nitrogen removal pathway aiding in the growth and abundance of phytoplankton populations (Hargreaves, 1998). An increase in dissolved nutrients during the initial days supported the gradual establishment of the phytoplankton community in shrimp pond ecosystem which has also been reported from our previous study (Fernandes et al., 2019).

Heterotrophic ammonia assimilation helps in building cellular protein (Ray et al., 2010), this was corroborated with the positive correlation obtained for THB with NH_4^+ ($p < 0.001$, $r = 0.705$), as well as in the efficient uptake of inorganic nutrients by THB which was more than the phytoplankton (Løvdaal et al., 2008; Liu et al., 2014; Sabu et al., 2021). The concentrations of all dissolved nutrients were within the safe limits for the culture of *L. vannamei* throughout the production cycle (Chien, 1992), except for a peak of NH_4^+ on 41 DoC. The initial increase of NH_4^+ could mainly be due to the breakdown of abundant supply of labile carbon and nitrogen (protein from uneaten feed, reduction of nitrogen species and shrimp excretion) (Brune et al., 2004) for the microbial assimilation (Burford and Glibert, 1999; Yang et al., 2018). Negative correlation between abundance of MOBw and NH_4^+ ($p < 0.01$, $r = -0.568$) and NO_2^- ($p < 0.001$, $r = -0.686$) explains the competitive inhibitory effect of nitrogenous compounds on methane monooxygenase (MMO) enzyme which regulates methane oxidation (Bodelier and Laanbroek, 2004; Nyerges et al., 2010, Stein and Klotz, 2011). Nyerges and Stein (2009) reported that the ability of NH_4^+ to act as cosubstrate and competitive inhibitor of MMO enzyme varies amongst species of methanotrophic bacteria. Besides, ammonia and nitrite are known to strongly influence methanotrophic activity and composition of methanotrophic communities (Nyerges et al., 2010).

Apart from nitrogen, the high concentration of dissolved phosphate is of major concern for the sustainable shrimp aquaculture (Boyd et al., 2007; Sabu et al., 2021). An increase in the concentration of dissolved PO_4^{3-} towards the end of the culture period, was perhaps due to the excessive remineralization processes (Burford et al., 2003) which could have stimulated the

phytoplankton growth. In the marine ecosystems, phosphonates are one of the major forms of organic-P compounds which are also constituents of phytoplankton, particulate organic matter (Karl et al., 2008; Kolowitz et al., 2001; Benitez-Nelson et al., 2004; Kittredge et al., 1969). The formation of methylphosphonate and its subsequent utilization result in the aerobic formation of CH₄ in the coastal marine ecosystems (Karl et al., 2008; Sosa et al., 2019). During the concurrent metabolism of methylphosphonate and phosphate, the uptake of the latter is more favored and this results in the systematic reduction of CH₄ yield from the utilization of methylphosphonate (Karl et al., 2008). This process of CH₄ production from the methylphosphonates could be inhibited by the increasing concentration of dissolved phosphate in the pond ecosystem (Carini et al., 2014) and hence in the present study an inverse relationship of PO₄³⁻ with MPA was observed.

Significant relationships between pond water quality parameters and heterotrophic bacterial population suggest the active role of microbes in processing the organic matter and the resultant nutrients in the pond (Das et al., 2013; Sabu et al., 2021). MOB_w and MOA positively correlated ($p < 0.05$) with phytoplankton (Fig. 4). This could be due to the presence of methane formed from the degradation of organic matter or from the methylated compounds (dimethylsulfoniopropionate) which are produced as metabolic by-product of microalgae (Damm et al., 2008). This methane undergoes oxidation by methanotrophs resulting in the release of CO₂ as an end product. The CO₂ thus produced serves as substrate for phytoplankton and in turn leads to an increase in the DO levels (Yang et al., 2019b). In addition, the increased Chl *a* concentration towards the end of the culture period concomitant with the higher phytoplankton abundance reflects the increased photosynthetic rates as observed by Fernandes et al. (2019). This effectively helps in the uptake of atmospheric CO₂ and fixes into organic matter (Maberly, 1996; Gu et al., 2011; Wang et al., 2015). This organic biomass eventually adds to the TOC in the sediment thus explaining the positive correlation of phytoplankton with TOC ($p < 0.001$, $r = 0.816$). TOC is negatively correlated with THB in water ($p < 0.001$, $r = -0.679$) and sediments ($p < 0.001$, $r = -0.673$) (Fig. 4). Thus implying that heterotrophic bacterial communities are actively involved in the organic matter mineralization and nutrient biota-interactions (Freitas and Godinho-Orlandi, 1991; Bhakta et al., 2006). In the present study, the abundance of THB in the sediment (Fig. 2) was four times higher than that recorded in pond water (Fig. 1). Abraham et al. (2004) reported a heterotrophic count of 1.02×10^7 CFU mL⁻¹ in the semi-intensive shrimp culture system on the day of harvest. In the present study, the highest abundance of THB in water of 2.03×10^6 CFU mL⁻¹ was on 0 DoC and of 1.04×10^{10} CFU g⁻¹ in sediment on 41 DoC. This variation in the abundance of THB might reflect the obvious differences in stocking density, species, and pond management practices (Ampofo and Clerk, 2003). Aquaculture pond bottoms are recipients of a large amount of nitrogen, phosphorus and organic matter (Boyd, 1990;

1992) and these substances tend to accumulate in the bottom sediment. Accumulation of high organic matter in the sediment layer is likely to result in anaerobic processes in the pond bottom which lead to the formation of reducing conditions and the release of potentially toxic compounds such as sulfide and methane (Boyd, 1992). In the pond water, abundance of TAB was an order higher than those recorded in the sediment. This could be due to the mechanical aeration maintained in the pond leading to the resuspension of the bottom sediments (Seo and Boyd, 2001; McGraw et al., 2001) which serve as microniches for the anaerobes in the pond water. However, the mineralization of organic matter results in the regeneration of nutrients at the sediment-water interface which act as an important source of ammonia and a sink for dissolved oxygen (Hargreaves, 1998). Similar to heterotrophs, the TABw and TABs showed significant negative relationship ($p < 0.001$) with dissolved nutrients and TOC (TABw: $r = -0.791$; TABs: $r = -0.839$) (Fig. 4). In oxygen deficient conditions, other electron acceptors are used to mediate the decomposition of organic matter. In both pond water and sediment, the TAB significantly correlated with THB. The significant correlation of THB, TAB with TOC reflects the decomposition of organic matter along with subsequent emission of GHGs (Yang et al., 2017b). Content of sediment TOC recorded during the present study (1.68–6.18%) was relatively higher than those reported previously from shrimp culture ponds (scampi culture ponds, 1.24–1.3%; polyculture ponds, 1.33–1.39%) (Adhikari et al., 2012).

In the present study, the concentrations of TOC (organic matter from uneaten feed, dead plankton, organic fertilizers) influenced the abundance of MOBw ($p < 0.01$, $r = 0.556$). The counts of MOB in the sediments were two to three orders magnitude higher than pond water throughout the culture period. The abundance of MOBs correlated with TSS ($p < 0.01$, $r = 0.504$) and sedimentary CHO ($p < 0.05$, $r = 0.46$) and PRT ($p < 0.5$, $r = 0.403$) which represent the labile components of the organic matter. Methanotrophs and their activity has been reported (Gonsalves et al., 2011; Kamaleson et al., 2019) to be influenced by the labile organic matter such as CHO and PRT. The organic matter serves s substrates for methanogenesis which is done by anaerobes. The high abundance of TAB in the initial phase till 41 DoC might have contributed to the higher rates of MPA towards the middle of the production cycle (41 DoC) followed by a decline. This decline in MPA may be due to the gradual increase in the size of post larvae, frequency of aeration, feeding rates and the luxuriant growth of microalgae with the progress of the culture. However, the attainment of highest MOA on 55 DoC could be attributed to the higher aerobic microbial activities as a consequence of increased aeration frequency and probiotic application (Yang et al., 2017a, Yang et al., 2019a).

During the entire production cycle, the highest level of MPA in sediments (3.45 ± 0.97 mole $\text{g}^{-1}\text{d}^{-1}$) was recorded on 41 DoC. The Spearman correlation analysis between methane related

microbial activities and environmental variables showed significant positive relationships of MPA with TSS ($p < 0.001$, $r = 0.846$), NH_4^+ ($p < 0.001$, $r = 0.785$), TABs ($p < 0.001$, $r = 0.628$), and inverse relationships with PO_4^{3-} ($p < 0.05$, $r = -0.454$), Chl *a* ($p < 0.05$, $r = -0.514$) and TOC ($p < 0.05$, $r = -0.438$). This positive correlation of MPA with ammonia (Fig. 4) could be due to increase in the nutrient availability (up to 41 DoC) in the pond which influenced the methanogenic activity (Purvaja and Ramesh, 2001) (Fig. S1).

Methanotrophs also help in keeping the concentration of CH_4 at lower levels (Yang et al., 2019c) by oxidizing CH_4 to CO_2 for energy generation and growth (Trotsenko and Murrell, 2008). Although the potential MOA ranged from $15.23 \pm 6.55 \mu\text{mole g}^{-1}\text{d}^{-1}$ to $599.61 \pm 167.18 \mu\text{mole g}^{-1}\text{d}^{-1}$, the frequent aeration can lead to rapid oxidation of methane in the overlying water column (Yang et al., 2017a) which in turn could lower the levels of methane in the water column. Bastviken et al. (2008) reported that 80% of methane produced in the deep wetland sediments could get oxidized in the water column during transport. Besides, the chances of bioturbation triggered ebullition (bubble flux from sediment) and diffusion of methane into the atmosphere are possible (Bastviken et al., 2004; Tranvik et al., 2009). However, in the pond sediments, a complex array of environmental variables seemed to influence the methane oxidation potential in the biosecured system as the MOA showed negligible correlations with most of the physicochemical variables.

In the present study, the overall MPA was comparatively lower than the MOA in the pond sediments. This is influenced by increased abundance of methanotrophic microbial population than the methanogenic archaeal groups in water and sediment. Also, the pond management practices such as the increased frequency of aeration, addition of probiotics and oxygen releasing compounds provide favorable conditions for methanotrophic microbial communities to flourish over the methanogenic archaeal groups. This enhanced activity of methanotrophs can utilize most of the CH_4 (80%) produced via methanogenesis which leads to a higher MOA in comparison to MPA (Kumaraswamy et al., 2000; Brigolin et al., 2009; Bastviken et al., 2011). The suppression of methanogenic activity restricting the CH_4 emission in the presence of higher levels of the dissolved oxygen in the aquaculture ponds has been well documented (Raul et al., 2020; Krüger et al., 2001). All the above factors enhance the efficiency for MOA over MPA. The concentration of CH_4 in sediment is directly related to the competition between methanogens and sulfate reducers and denitrifiers; (Marinho et al., 2012). Results of PCA showed that abundance of MOB and methane related activities interacted with salinity, temperature, dissolved nitrogen species, phytoplankton pigments, TOC and other bacterial groups in the shrimp pond ecosystem. The Cytoscape network diagram (Fig. 4) elucidated the complex interactions between various physicochemical and biological parameters in relation to CH_4 cycle. The results obtained from the present study is

suggestive that concentrations of GHGs like CH₄ is influenced by different environmental parameters to varying extent and can also be impacted by aquaculture pond management practices.

5. Conclusion

The fate of methane emission from an aquaculture system is governed by the abundance and the levels of activity of methane related bacterial communities. These communities are influenced by the environmental variables which in turn vary in accordance with the pond management practices. Considering a lacunae in the understanding of complex interactions between the above parameters, the present study was aimed to study the role of these microbes in the pond water and sediment and thus aid in developing mitigatory measures for aquaculture wastewater treatment. The *in situ* bacterial communities in conjunction with pond management practices (feed, aeration, maintenance of optimum pH levels, probiotics and oxygen releasing compounds etc.) result in the build-up of dissolved nutrients and organic matter, thereby contributing to the methane production in the pond ecosystem. The methane oxidation activity by the microbial community during the aquaculture production could be a factor controlling the concentration of methane being effluxed from the system. Future application of ecofriendly methanotrophs will be expected to minimize the emission of methane. Besides, different bacterial groups with their varied activities contributed to the bioremediation of aquaculture pollutants. The results of the present study provide a baseline information on microbial processes and insights into the environmental factors which may aid in the development of shrimp farming industry.

Declaration of Competing Interests

The authors have declared no competing interest exists.

Acknowledgments

The authors acknowledge the Director, CSIR-National Institute of Oceanography (CSIR-NIO) for the facilities. EAS acknowledges the University Grants Commission (UGC), India for providing the Junior and Senior Research Fellowship. Authors extend their thanks to the anonymous reviewers for their constructive criticisms on the manuscript. The authors would like to record the cooperation of the shrimp farm staff for fieldwork. This is CSIR-NIO contribution No.xxxx.

References

- Abraham, T.J., Ghosh, S., Nagesh, T.S., Sasmal, D., (2004). Distribution of bacteria involved in nitrogen and sulphur cycles in shrimp culture systems of West Bengal, India. *Aquaculture*.239, 275–288. <https://doi.org/10.1016/j.aquaculture.2004.06.023>
- Adhikari, S., Lal, R., & Sahu, B.C. (2012). Carbon sequestration in the bottom sediments of aquaculture ponds of Orissa, India. *Ecological Engineering*, 47, 198–202. <https://doi.org/10.1016/j.ecoleng.2012.06.007>
- Ampofo, A. and Clerk, G.C. (2003). Bacterial flora of fish feeds and organic fertilizers for fish culture ponds in Ghana. *Aquaculture. Research*, 34(8), 677–680. <https://doi.org/10.1046/j.1365-2109.2003.00844.x>
- Bastviken, D., Cole, J. J., Pace, M. L., & Van de Bogert, M. C. (2008). Fates of methane from different lake habitats: Connecting whole-lake budgets and CH₄ emissions. *Journal of Geophysical Research*, 113, G02024. <https://doi.org/10.1029/2007JG000608>
- Bastviken, D., Cole, J., Pace, M., & Tranvik, L. (2004). Methane emissions from lakes: Dependence of lake characteristics, two regional assessments, and a global estimate. *Global Biogeochemical Cycles*, 18(4). <https://doi.org/10.1029/2004GB002238>
- Bastviken, D., Ejlertsson, J., & Tranvik, L. (2002). Measurement of methane oxidation in lakes: a comparison of methods. *Environmental Science & Technology*, 36(15), 3354–3361. <https://doi.org/10.1021/es010311p>
- Bastviken, D., Tranvik, L. J., Downing, J. A., Crill, P. M., & Enrich-Prast, A. (2011). Freshwater methane emissions offset the continental carbon sink. *Science*, 331(6013), 50-50. <https://doi.org/10.1126/science.1196808>
- Benitez-Nelson, C. R., O'Neill, L., Kolowith, L. C., Pellechia, P., & Thunell, R. (2004). Phosphonates and particulate organic phosphorus cycling in an anoxic marine basin. *Limnology and Oceanography*, 49(5), 1593-1604. <https://doi.org/10.4319/lo.2004.49.5.1593>
- Bhakta, J. N., Bandyopadhyay, P. K., & Jana, B. B. (2006). Effect of different doses of mixed fertilizer on some biogeochemical cycling bacterial population in carp culture pond. *Turkish Journal of Fisheries and Aquatic Sciences*, 6(2), 165–171.
- Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37(8), 911–917. <https://doi.org/10.1139/o59-099>
- Bodelier, P. L., & Laanbroek, H. J. (2004). Nitrogen as a regulatory factor of methane oxidation in soils and sediments. *FEMS Microbiology Ecology*, 47(3), 265–277. [https://doi.org/10.1016/S0168-6496\(03\)00304-0](https://doi.org/10.1016/S0168-6496(03)00304-0)
- Boyd, C. E. (2010). Aquaculture ponds hold carbon. *Global Aquaculture Advocate*, 13(2), 23–24.
- Boyd, C. E., & Tucker, C. S. (2014). Handbook for aquaculture water quality. *Handbook for Aquaculture Water Quality*, 439.

- Boyd, C. E., Tucker, C. S., Mc-Nevin, A., Bostick, K., & Clay, J. (2007). Indicators of resource use efficiency and environmental performance in fish and crustacean aquaculture. *Reviews in Fisheries Science*, 15, 327–360. <https://doi.org/10.1080/10641260701624177>
- Boyd, C. E., Wood, C. W., Chaney, P. L., & Queiroz, J. F. (2010). Role of aquaculture pond sediments in sequestration of annual global carbon emissions. *Environmental Pollution*, 158(8), 2537-2540. <https://doi.org/10.1016/j.envpol.2010.04.025>.
- Boyd, C.E., (1990). Water quality in ponds for aquaculture, p 473. Alabama Agriculture Experiment Station, Auburn University, Alabama, pp 370-/380.
- Boyd, C.E., (1992). Shrimp pond bottom soil and sediment management. In J.A. Wyban, editor. Proceedings of the special session on shrimp farming. World Aquacult. Soc. Baton Rouge, Louisiana, USA. Pp. 168–181.
- Bremner, J. M. (1965). Total nitrogen. In: Methods of soil analysis, Part 2. pp 1149-1178. Ed. by C. A. Black. Madison: American Society of Agronomy
- Bridgham, S. D., Cadillo-Quiroz, H., Keller, J. K., & Zhuang, Q. (2013). Methane emissions from wetlands: biogeochemical, microbial, and modeling perspectives from local to global scales. *Global Change Biology*, 19(5), 1325–1346. <https://doi.org/10.1111/gcb.12131>
- Brigolin, D., Pastres, R., Nickell, T. D., Cromey, C. J., Aguilera, D. R., & Regnier, P. (2009). Modelling the impact of aquaculture on early diagenetic processes in sea loch sediments. *Marine Ecology Progress Series*, 388, 63-80. <https://doi.org/10.3354/meps08072>
- Brune, D.E., Kirk, K., Eversole, A.G., (2004). Autotrophic intensification of pond aquaculture; shrimp production in a partitioned aquaculture system. Proceedings of the Fifth International Conference on Recirculating Aquaculture, Roanoke, Virginia, pp. 201–210.
- Burford, M.A. and Glibert, P.M., (1999). Short-term N cycling in early and late growth-phase shrimp ponds. *Aquaculture Research* 30, 215–227. <https://doi.org/10.1046/j.1365-2109.1999.00314.x>
- Burford, M.A., (2003). Nutrient and microbial dynamics in high intensity, zero-exchange shrimp ponds in Belize. *Aquaculture* 219, 393–411. [https://doi.org/10.1016/S0044-8486\(02\)00575-6](https://doi.org/10.1016/S0044-8486(02)00575-6)
- Carini, P., White, A. E., Campbell, E. O., & Giovannoni, S. J. (2014). Methane production by phosphate-starved SAR11 chemoheterotrophic marine bacteria. *Nature Communications*, 5(1), 1–7. <https://doi.org/10.1038/ncomms5346>
- Chen, Y., Dong, S., Wang, F., Gao, Q., & Tian, X. (2016). Carbon dioxide and methane fluxes from feeding and no-feeding mariculture ponds. *Environmental Pollution*, 212, 489–497. <https://doi.org/10.1016/j.envpol.2016.02.039>
- Chien, Y. H. (1992). Water quality requirements and management for marine shrimp culture. In *Proceedings of the special session on shrimp farming* (pp. 144-156). World Aquaculture Society Baton Rouge, LA, USA.

- Christopher, J., Preston, N., Thompson, P. J., & Burford, M. (2003). Nitrogen budget and effluent nitrogen components at an intensive shrimp farm. *Aquaculture (Amsterdam, Netherlands)*, 218(1–4), 397–411. [https://doi.org/10.1016/S0044-8486\(03\)00014-0](https://doi.org/10.1016/S0044-8486(03)00014-0)
- Damm, E., Kiene, R. P., Schwarz, J., Falck, E., & Dieckmann, G. (2008). Methane cycling in Arctic shelf water and its relationship with phytoplankton biomass and DMSP. *Marine Chemistry*, 109(1-2), 45-59. <https://doi.org/10.1016/j.marchem.2007.12.003>
- Das, M., Ayyappan, S. Dash, B. and Muduli, H.K. (2013). Heterotrophic bacterial populations and dehydrogenase activity in fish ponds under different fertilization practices. *Indian Journal of Fisheries*, 60(3), 87–90.
- Dutta, M. K., Chowdhury, C., Jana, T. K., & Mukhopadhyay, S. K. (2013). Dynamics and exchange fluxes of methane in the estuarine mangrove environment of the Sundarbans, NE coast of India. *Atmospheric Environment*, 77, 631–639. <https://doi.org/10.1016/j.atmosenv.2013.05.050>
- El Wakeel, S. K., & Riley, J. P. (1957). The determination of organic carbon in marine muds. *ICES Journal of Marine Science*, 22(2), 180–183. <https://doi.org/10.1093/icesjms/22.2.180>
- Erazo, N. G., & Bowman, J. S. (2021). Sensitivity of the mangrove-estuarine microbial community to aquaculture effluent. *Isience*, 24(3), 102204. <https://doi.org/10.1016/j.isci.2021.102204>
- FAO (2020) The state of world fisheries and aquaculture 2020 Sustainability in action. Rome. <https://doi.org/10.4060/ca9229en>
- Fazli, P., Man, H. C., Shah, U. K. M., & Idris, A. (2013). Characteristics of methanogens and methanotrophs in rice fields: a review. *Asia-Pacific Journal of Molecular Biology and Biotechnology*, 21(1), 3–17.
- Fellows CS, Valett HM, Dahm CN, Mulholland PJ, Thomas SA (2006). Coupling nutrient uptake and energy flow in headwater streams. *Ecosystems*, 9, 788–804. <https://doi.org/10.1007/s10021-006-0005-5>
- Fernandes SO, Kulkarni SS, Shirodkar RR, Karekar SV, Kumar RP, Sreepada RA, LokaBharathi PA (2010). Water quality and bacteriology in an aquaculture facility equipped with a new aeration system. *Environment Monitoring Assessment* 64:81–92. <https://doi.org/10.1007/s10661-009-0876-y>
- Fernandes V, Sabu EA, Shivaramu MS, Gonsalves MJB, Sreepada RA (2019). Dynamics and succession of plankton communities with changing nutrient levels in tropical culture ponds of whiteleg shrimp. *Aquaculture Environment Interactions* 11:639–655. <https://doi.org/10.3354/aei00341>
- Freitas, E. A.C and Godinho-Orlandi, M.J.L. (1991). Distribution of bacteria in sediment of an Oxbow tropical lake. *Hydrobiologia*, 211(1), 33–41. <https://doi.org/10.1007/BF00008614>
- Funge-Smith, S. J., & Briggs, M. R. (1998). Nutrient budgets in intensive shrimp ponds: implications for sustainability. *Aquaculture*, 164(1-4), 117–133. [https://doi.org/10.1016/S0044-8486\(98\)00181-1](https://doi.org/10.1016/S0044-8486(98)00181-1)

- Gamboa-Delgado, J., Nieto-López, M. G., Maldonado-Muñiz, M., Villarreal-Cavazos, D., Tapia-Salazar, M., & Cruz-Suárez, L. E. (2020). Comparing the assimilation of dietary nitrogen supplied by animal-, plant-and microbial-derived ingredients in Pacific white shrimp *Litopenaeus vannamei*: A stable isotope study. *Aquaculture Reports*, 17, 100294. <https://doi.org/10.1016/j.aqrep.2020.100294>
- Gonsalves, M. J., Fernandes, C. E., Fernandes, S. O., Kirchman, D. L., & Bharathi, P. L. (2011). Effects of composition of labile organic matter on biogenic production of methane in the coastal sediments of the Arabian Sea. *Environmental Monitoring and Assessment*, 182(1-4), 385–395. <https://doi.org/10.1007/s10661-011-1883-3>
- Grasshoff, K., Kremling, K., & Ehrhardt, M. (Eds.). (2009). *Methods of seawater analysis*. John Wiley & Sons.
- Green, D. S., Boots, B., & Crowe, T. P. (2012). Effects of non-indigenous oysters on microbial diversity and ecosystem functioning. *PLoS One*, 7(10). <https://doi.org/10.1371/journal.pone.0048410>
- Grinham, A., Dunbabin, M., & Albert, S. (2018). Importance of sediment organic matter to methane ebullition in a sub-tropical freshwater reservoir. *Science of the Total Environment*, 621, 1199-1207. <https://doi.org/10.1016/j.scitotenv.2017.10.108>
- Gu, B.H., Schelske, C.L., Coveney, M.F., (2011). Low carbon dioxide partial pressure in a productive subtropical lake. *Aquatic Sciences*, 73, 317–330. <https://doi.org/10.1007/s00027-010-0179-y>
- Hargreaves JA (1998). Nitrogen biogeochemistry of aquaculture ponds. *Aquaculture*, 166,181–212. [https://doi.org/10.1016/S0044-8486\(98\)00298-1](https://doi.org/10.1016/S0044-8486(98)00298-1)
- Hasan, M. R. & Soto, S. (2017). Improving feed conversion ratio and its impact on reducing greenhouse gas emissions in aquaculture. FAO Non-Serial Publication. Rome, FAO. 33 pp. <http://www.fao.org/3/a-i7688e.pdf>
- Holmer, M., Wildish, D., & Hargrave, B. (2005). Organic enrichment from marine finfish aquaculture and effects on sediment biogeochemical processes. In *Environmental effects of marine finfish aquaculture* (pp. 181-206). Springer, Berlin, Heidelberg. <https://doi.org/10.1007/b136010>
- Hou, J., Zhang, G., Sun, M., Ye, W., & Song, D. (2016). Methane distribution, sources, and sinks in an aquaculture bay (Sanggou Bay, China). *Aquaculture Environment Interactions*, 8, 481–495. <https://doi.org/10.3354/aei00189>
- IPCC. Special report on global warming of 1.5°C (Press Release). Incheon, Republic of Korea; 2018. Retrieved August 11, 2020 Available:https://www.ipcc.ch/site/assets/uploads/2018/11/pr_181008_P48_spm_en.pdf
- Kalyuzhnaya, M. G., Gomez, O. A., & Murrell, J. C. (2019). The methane-oxidizing bacteria (methanotrophs). *Taxonomy, Genomics and Ecophysiology of Hydrocarbon-Degrading Microbes*, 245-278. https://doi.org/10.1007/978-3-030-14796-9_10

- Kamaleson, A. S., Gonsalves, M. J., & Nazareth, D. R. (2019). Interactions of sulfur and methane-oxidizing bacteria in tropical estuarine sediments. *Environmental Monitoring and Assessment*, 191(8), 1-18. <https://doi.org/10.1007/s10661-019-7616-8>
- Karl, D. M., Beversdorf, L., Björkman, K. M., Church, M. J., Martinez, A., & Delong, E. F. (2008). Aerobic production of methane in the sea. *Nature Geoscience*, 1(7), 473–478. <https://doi.org/10.1128/AEM.00289-19>
- Kittredge, J. S., & Roberts, E. (1969). A carbon-phosphorus bond in nature. *Science*, 164(3875), 37-42.
- Klinger, D., & Naylor, R. (2012). Searching for solutions in aquaculture: charting a sustainable course. *Annual Review of Environment and Resources*, 37, 247–276. <https://doi.org/10.1146/annurev-environ-021111-161531>
- Kochert, G. (1978). Carbohydrate determination by the phenol-sulfuric acid method. *Handbook of phycological methods, Phycological and biochemical methods.*, 95.
- Kolowith, L. C., IngaKolowith, L. C., Ingall, E. D., and Benner, R. (2001). Composition and cycling of marine organic phosphorus. *Limnol. Oceanogr.* 46, 309–320. <https://doi.org/10.4319/lo.2001.46.2.0309>
- Krüger, M., Frenzel, P., & Conrad, R. (2001). Microbial processes influencing methane emission from rice fields. *Global Change Biology*, 7(1), 49-63. <https://doi.org/10.1046/j.1365-2486.2001.00395.x>
- Kumaraswamy, S., Rath, A. K., Ramakrishnan, B., & Sethunathan, N. (2000). Wetland rice soils as sources and sinks of methane: a review and prospects for research. *Biology and Fertility of Soils*, 31(6), 449-461. <https://doi.org/10.1007/s003740000214>
- Lemonnier, H., Courties, C., Mugnier, C., Torréton, J. P., & Herbland, A. (2010). Nutrient and microbial dynamics in eutrophying shrimp ponds affected or unaffected by vibriosis. *Marine Pollution Bulletin*, 60(3), 402-411. <https://doi.org/10.1016/j.marpolbul.2009.10.012>
- Liu, L., Yang, J., Lv, H., & Yu, Z. (2014). Synchronous dynamics and correlations between bacteria and phytoplankton in a subtropical drinking water reservoir. *FEMS Microbiology Ecology*, 90(1), 126–138. <https://doi.org/10.1111/1574-6941.12378>
- Løvdal, T., Eichner, C., Grossart, H. P., Carbonnel, V., Chou, L., Martin-Jézéquel, V., & Thingstad, T. F. (2008). Competition for inorganic and organic forms of nitrogen and phosphorus between phytoplankton and bacteria during an *Emiliania huxleyi* spring bloom. *Biogeosciences*, 5(2), 371-383. <https://doi.org/10.5194/bg-5-371-2008>
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 193, 265–275.
- Maberly, S. C. (1996). Diel, episodic and seasonal changes in pH and concentrations of inorganic carbon in a productive lake. *Freshwater Biology*, 35(3), 579–598. <https://doi.org/10.1111/j.1365-2427.1996.tb01770.x>

- Maher, D. T., Sippo, J. Z., Tait, D. R., Holloway, C., & Santos, I. R. (2016). Pristine mangrove creek waters are a sink of nitrous oxide. *Scientific Reports*, 6(1), 1-8. <https://doi.org/10.1038/srep25701>
- Marinho, C. C., Campos, E. A., Guimarães, J. R. D., & Esteves, F. A. (2012). Effect of sediment composition on methane concentration and production in the transition zone of a mangrove (Sepetiba Bay, Rio de Janeiro, Brazil). *Brazilian Journal of Biology*, 72(3), 429–436. <https://doi.org/10.1590/S1519-69842012000300003>
- Martinez-Cruz, K., Gonzalez-Valencia, R., Sepulveda-Jauregui, A., Plascencia-Hernandez, F., Belmonte-Izquierdo, Y., & Thalasso, F. (2017). Methane emission from aquatic ecosystems of Mexico City. *Aquatic Sciences*, 79(1), 159–169. <https://doi.org/10.1007/s00027-0160487-y>
- McGraw, W., Teichert-Coddington, D. R., Rouse, D. B., & Boyd, C. E. (2001). Higher minimum dissolved oxygen concentrations increase penaeid shrimp yields in earthen ponds. *Aquaculture*, 199(3-4), 311-321. [https://doi.org/10.1016/S0044-8486\(01\)00530-0](https://doi.org/10.1016/S0044-8486(01)00530-0)
- Moriarty, D. J. (1997). The role of microorganisms in aquaculture ponds. *Aquaculture*, 151(1-4), 333-349. [https://doi.org/10.1016/S0044-8486\(96\)01487-1](https://doi.org/10.1016/S0044-8486(96)01487-1)
- Muralidhar, M., Vasanth, M., Chitra, V., Thulasi, D., Kathyayani, S., & Jannathulla, R. (2017). Greenhouse Gases Emission and Mitigation from Brackish Water Aquaculture Systems. *Agriculture under Climate Change: Threats, Strategies and Policies*, 1, 268.
- Nakayama, T., Lu, H., & Nomura, N. (2009). Inhibitory effects of Bacillus probionts on growth and toxin production of *Vibrio harveyi* pathogens of shrimp. *Letters in Applied Microbiology*, 49(6), 679–684. <https://doi.org/10.1111/j.1472-765X.2009.02725.x>
- Neue, H. U., Gaunt, J. L., Wang, Z. P., Becker-Heidmann, P., & Quijano, C. (1997). Carbon in tropical wetlands. *Geoderma*, 79(1–4), 163–185. [https://doi.org/10.1016/S0016-7061\(97\)00041-4](https://doi.org/10.1016/S0016-7061(97)00041-4)
- Nyerges, G., & Stein, L. Y. (2009). Ammonia cometabolism and product inhibition vary considerably among species of methanotrophic bacteria. *FEMS Microbiology Letters*, 297(1), 131-136. <https://doi.org/10.1111/j.1574-6968.2009.01674.x>
- Nyerges, G., Han, S. K., & Stein, L. Y. (2010). Effects of ammonium and nitrite on growth and competitive fitness of cultivated methanotrophic bacteria. *Applied and Environmental Microbiology*, 76(16), 5648-5651. <https://doi.org/10.1128/AEM.00747-10>
- Pack, M. A., Heintz, M. B., Reeburgh, W. S., Trumbore, S. E., Valentine, D. L., Xu, X., & Druffel, E. R. (2011). A method for measuring methane oxidation rates using low levels of ¹⁴C-labeled methane and accelerator mass spectrometry. *Limnology and Oceanography: Methods*, 9(6), 245-260 <https://doi.org/10.4319/lom.2011.9.245>
- Parsons, T.R., Maita, Y. and Lalli, C.M. (1984). *A Manual of Chemical and Biological Methods for Seawater Analysis*. Pergamon, New York, pp. 52-71.

- Ponce-Palafox, J. T., Pavia, Á. A., López, D. G. M., Arredondo-Figueroa, J. L., Lango-Reynoso, F., del Refugio Castañeda-Chávez, M., & Peraza-Gómez, V. (2019). Response surface analysis of temperature-salinity interaction effects on water quality, growth and survival of shrimp *Penaeus vannamei* postlarvae raised in biofloc intensive nursery production. *Aquaculture*, 503, 312–321. <https://doi.org/10.1016/j.aquaculture.2019.01.020>
- Purvaja, R., & Ramesh, R. (2001). Natural and anthropogenic methane emission from coastal wetlands of South India. *Environmental Management*, 27(4), 547-557. <https://doi.org/10.1007/s002670010169>
- Queiroz, H. M., Artur, A. G., Taniguchi, C. A. K., da Silveira, M. R. S., do Nascimento, J. C., Nóbrega, G. N., & Ferreira, T. O. (2019). Hidden contribution of shrimp farming effluents to greenhouse gas emissions from mangrove soils. *Estuarine, Coastal and Shelf Science*, 221, 8–14. <https://doi.org/10.1016/j.ecss.2019.03.011>
- Raul, C., Pattanaik, S. S., & Prakash, S. (2020). Greenhouse Gas Emissions from Aquaculture Systems. *WORLD AQUACULTURE*, 57.
- Ray, A. J., Dillon, K. S., & Lotz, J. M. (2011). Water quality dynamics and shrimp (*Litopenaeus vannamei*) production in intensive, mesohaline culture systems with two levels of biofloc management. *Aquacultural Engineering*, 45(3), 127-136. <https://doi.org/10.1016/j.aquaeng.2011.09.001>
- Ray, A. J., Seaborn, G., Leffler, J. W., Wilde, S. B., Lawson, A., & Browdy, C. L. (2010). Characterization of microbial communities in minimal-exchange, intensive aquaculture systems and the effects of suspended solids management. *Aquaculture*, 310(1–2), 130–138. <https://doi.org/10.1016/j.aquaculture.2010.10.019>
- Rodina, A. G. (1972). *Methods in aquatic microbiology*. (ed. y RR. Colwell MS. Zambruski), University Park Press, Maryland. <https://doi.org/10.1002/jobm.19730130834>.
- Sabu, E. A., Gonsalves, M. J., Sreepada, R. A., Shivaramu, M. S., & Ramaiah, N. (2021). Evaluation of the Physiological Bacterial Groups in a Tropical Biosecured, Zero-Exchange System Growing Whiteleg Shrimp, *Litopenaeus vannamei*. *Microbial Ecology*, 1–12. <https://doi.org/10.1111/are.12152>
- Sahu, M. K., Swarnakumar, N. S., Sivakumar, K., Thangaradjou, T., & Kannan, L. (2008). Probiotics in aquaculture: importance and future perspectives. *Indian Journal of Microbiology*, 48(3), 299-308. <https://doi.org/10.1007/s12088-008-0024-3>
- Seo, J., & Boyd, C. E. (2001). Effects of bottom soil management practices on water quality improvement in channel catfish *Ictalurus punctatus* ponds. *Aquacultural Engineering*, 25(2), 83-97. [https://doi.org/10.1016/S0144-8609\(01\)00077-2](https://doi.org/10.1016/S0144-8609(01)00077-2)
- Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B & Ideke T (2003). Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. *Genome Research*, 3:2498–2504. <https://doi.org/10.1101/gr.1239303>

- Shariff, M., Yusoff, F. M., Devaraja, T. N., & Rao, P. S. (2001). The effectiveness of a commercial microbial product in poorly prepared tiger shrimp, *Penaeus monodon* (Fabricius), ponds. *Aquaculture Research*, 32(3), 181–187. <https://doi.org/10.1046/j.1365-2109.2001.00543.x>
- Sosa, O. A., Casey, J. R., & Karl, D. M. (2019). Methylphosphonate oxidation in Prochlorococcus strain MIT9301 supports phosphate acquisition, formate excretion, and carbon assimilation into purines. *Applied and Environmental Microbiology*, 85(13), e00289-19. <https://doi.org/10.1038/ngeo234>
- Stein, L. Y., & Klotz, M. G. (2011). Nitrifying and denitrifying pathways of methanotrophic bacteria. *Biochemical Society Transactions*, 39(6), 1826–1831. <https://doi.org/10.1042/BST20110712>
- Tacon, A. G., Jory, D., & Nunes, A. (2013). Shrimp feed management: issues and perspectives. *On-farm feeding and feed management in aquaculture*, 583, 481-488.
- Tendencia, E. A., Bosma, R. H., Verdegem, M. C., & Verreth, J. A. (2015). The potential effect of greenwater technology on water quality in the pond culture of *Penaeus monodon* Fabricius. *Aquaculture Research*, 46(1), 1-13. <https://doi.org/10.1111/are.12152>
- Tishchenko, P., Hensen, C., Wallmann, K., & Wong, C. S. (2005). Calculation of the stability and solubility of methane hydrate in seawater. *Chemical Geology*, 219(1-4), 37-52. <https://doi.org/10.1016/j.chemgeo.2005.02.008>
- Tranvik, L. J., Downing, J. A., Cotner, J. B., Loiselle, S. A., Striegl, R. G., Ballatore, T. J., & Kortelainen, P. L. (2009). Lakes and reservoirs as regulators of carbon cycling and climate. *Limnology and Oceanography*, 54(6part2), 2298-2314. https://doi.org/10.4319/lo.2009.54.6_part_2.2298
- Trotsenko, Y. A., & Murrell, J. C. (2008). Metabolic aspects of aerobic obligate methanotrophy. *Advances in Applied Microbiology*, 63, 183–229. [https://doi.org/10.1016/S0065-2164\(07\)00005-6](https://doi.org/10.1016/S0065-2164(07)00005-6)
- Wang, F., Cao, M., Wang, B., Fu, J., Luo, W., & Ma, J. (2015). Seasonal variation of CO₂ diffusion flux from a large subtropical reservoir in East China. *Atmospheric Environment*, 103, 129–137. <https://doi.org/10.1016/j.atmosenv.2014.12.042>
- Whittenbury R, Phillips KC, Wilkinson JF (1970). Enrichment, isolation and some properties of methane utilizing bacteria. *Microbiology* 61:205–218. <https://doi.org/10.1099/00221287-61-2-205>
- Xiong, Y., Wang, F., Guo, X., Liu, F., & Dong, S. (2017). Carbon dioxide and methane fluxes across the sediment-water interface in different grass carp *Ctenopharyngodon idella* polyculture models. *Aquaculture Environment Interactions*, 9, 45–56. <https://doi.org/10.3354/aei00214>
- Yang, P., Bastviken, D., Lai, D. Y. F., Jin, B. S., Mou, X. J., Tong, C., & Yao, Y. C. (2017a). Effects of coastal marsh conversion to shrimp aquaculture ponds on CH₄ and N₂O emissions. *Estuarine, Coastal and Shelf Science*, 199, 125–131. <https://doi.org/10.1016/j.ecss.2017.09.023>

- Yang, P., Lai, D. Y., Huang, J. F., & Tong, C. (2018). Effect of drainage on CO₂, CH₄, and N₂O fluxes from aquaculture ponds during winter in a subtropical estuary of China. *Journal of Environmental Sciences*, 65, 72–82. <https://doi.org/10.1016/j.jes.2017.03.024>
- Yang, P., Lai, D. Y., Jin, B., Bastviken, D., Tan, L., & Tong, C. (2017b). Dynamics of dissolved nutrients in the aquaculture shrimp ponds of the Min River estuary, China: Concentrations, fluxes and environmental loads. *Science of the Total Environment*, 603, 256–267. <https://doi.org/10.1016/j.scitotenv.2017.06.074>
- Yang, P., Lai, D. Y., Yang, H., Tong, C., Lebel, L., Huang, J., & Xu, J. (2019c). Methane dynamics of aquaculture shrimp ponds in two subtropical estuaries, southeast China: dissolved concentration, net sediment release, and water oxidation. *Journal of Geophysical Research: Biogeosciences*, 124(6), 1430–1445. <https://doi.org/10.1029/2018JG004794>
- Yang, P., Zhang, Y., Bastviken, D., Lai, D. Y., Yang, H., Zhang, Y. F., ... & Tong, C. (2020). Large increase in diffusive greenhouse gas fluxes from subtropical shallow aquaculture ponds during the passage of typhoons. *Journal of Hydrology*, 583, 124643. <https://doi.org/10.1016/j.jhydrol.2020.124643>
- Yang, P., Zhang, Y., Yang, H., Guo, Q., Lai, D. Y., Zhao, G., & Tong, C. (2020). Ebullition was a major pathway of methane emissions from the aquaculture ponds in southeast China. *Water Research*, 116176. <https://doi.org/10.1016/j.watres.2020.116176>
- Yang, P., Zhang, Y., Yang, H., Zhang, Y., Xu, J., Tan, L., Tong, C., & Lai, D. Y. (2019b). Large fine-scale spatiotemporal variations of CH₄ diffusive fluxes from shrimp aquaculture ponds affected by organic matter supply and aeration in Southeast China. *Journal of Geophysical Research: Biogeosciences*, 124(5), 1290–1307. <https://doi.org/10.1029/2019JG005025>
- Yang, Z., Tang, C., Li, X., Zhang, H., & Cai, Y. (2019a). Dynamics of dissolved greenhouse gas response to seasonal water mixing in subtropical reservoirs. *Environmental Monitoring and Assessment*, 191(10), 639. <https://doi.org/10.1007/s10661-019-7772-x>
- Yuan, J., Liu, D., Xiang, J., He, T., Kang, H., & Ding, W. (2021). Methane and nitrous oxide have separated production zones and distinct emission pathways in freshwater aquaculture ponds. *Water Research*, 190, 116739. <https://doi.org/10.1016/j.watres.2020.116739>
- Yuan, J., Xiang, J., Liu, D., Kang, H., He, T., Kim, S., & Ding, W. (2019). Rapid growth in greenhouse gas emissions from the adoption of industrial-scale aquaculture. *Nature Climate Change*, 9(4), 318–322. <https://doi.org/10.1038/s41558-019-0425-9>
- Zar, J.H., 1996. Biostatistical Analysis. Prentice Hall, Upper Saddle River, New Jersey,

Figures and Tables

Fig. 1. Temporal variation in the abundance (CFU mL⁻¹) of physiological bacterial groups: total heterotrophic bacteria (THBw), total anaerobic bacteria (TABw) and methane-oxidizing bacteria (MOBw) in the pond water during the cultivation of *Litopenaeus vannamei* in zero-exchange shrimp system. Data presented is Mean \pm SD (n = 3).

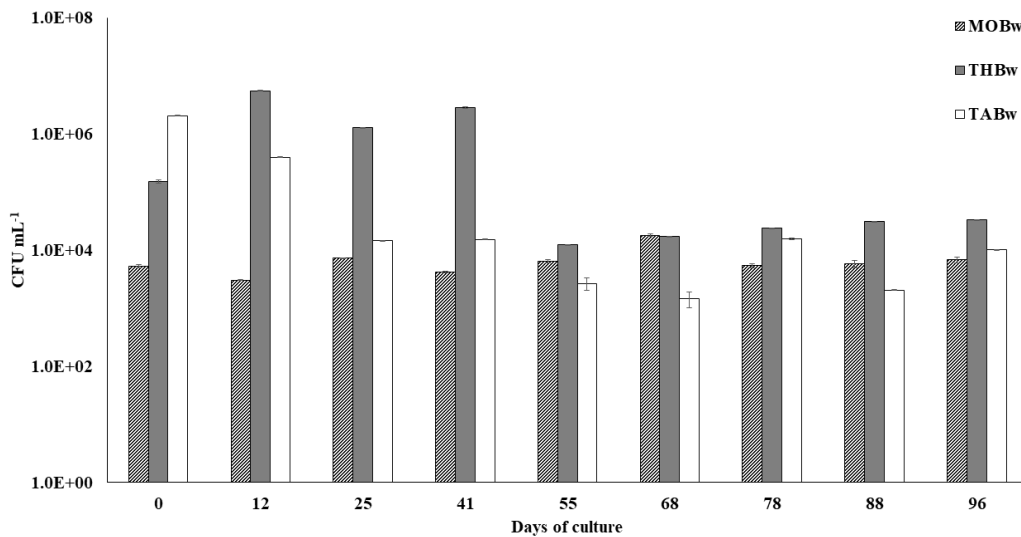


Fig. 2. Temporal variation in the abundance (CFU g⁻¹) of physiological bacterial groups: total heterotrophic bacteria (THBs), total anaerobic bacteria (TABs) and methane-oxidizing bacteria (MOBs) in the pond sediment during the cultivation of *Litopenaeus vannamei* in zero-exchange shrimp system. Data presented is Mean \pm SD (n = 3).

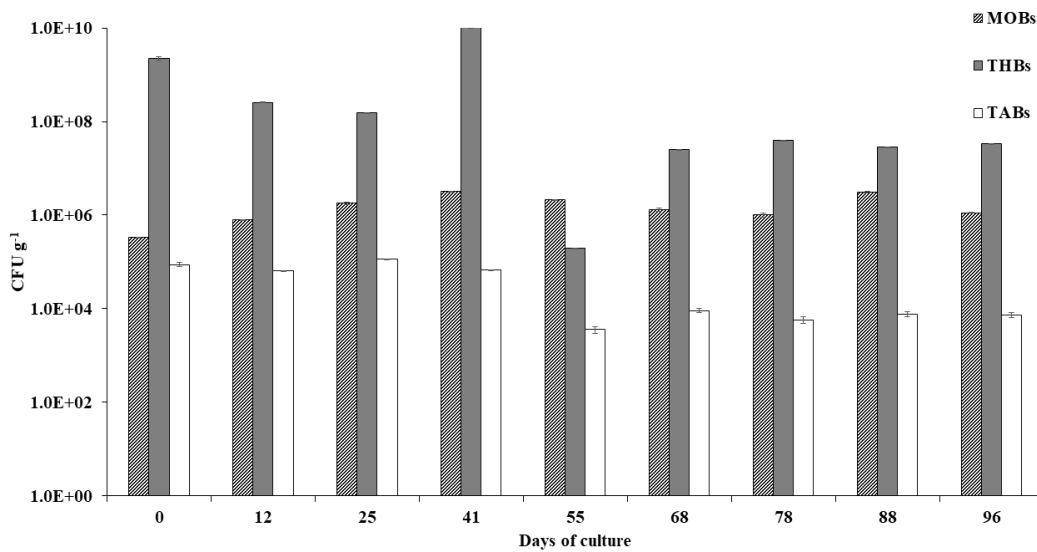


Fig. 3. Methane production (MPA) and potential methane oxidation rates (MOA) in ($\mu\text{mole g}^{-1} \text{d}^{-1}$) in pond sediments during the cultivation of *Litopenaeus vannamei* in a zero-exchange shrimp culture system.

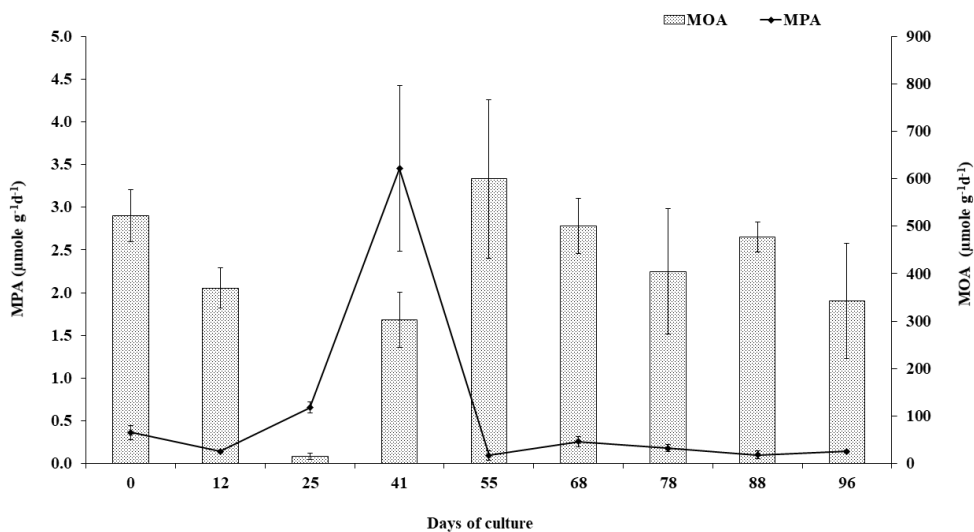
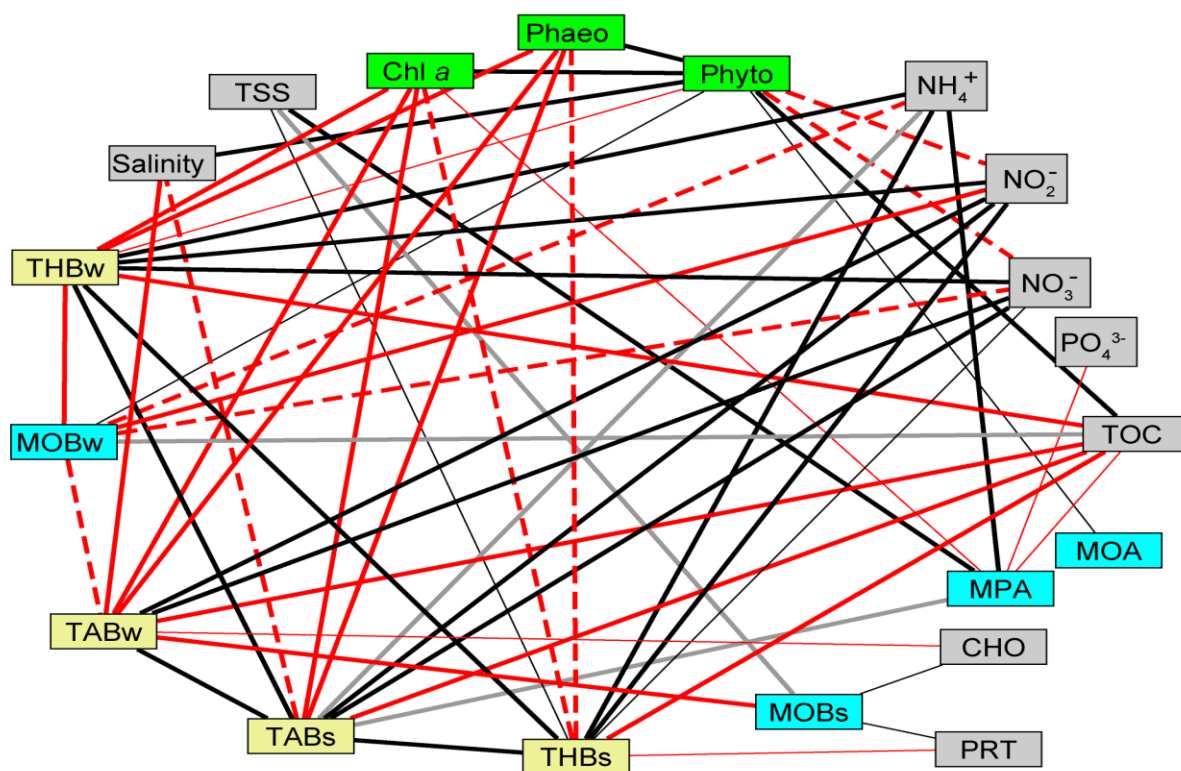
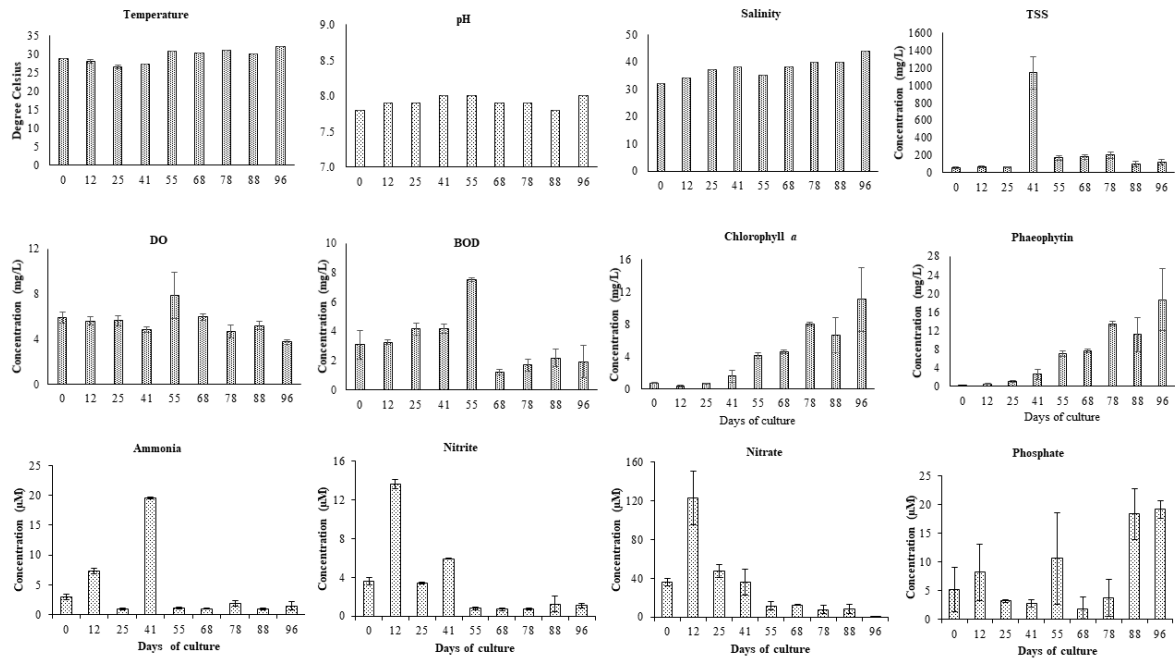


Fig. 4. Network of interactions between the methane related variables with various environmental parameters in the shrimp pond. The relationships with only significant correlation coefficient (r) were plotted ($p \leq 0.05$).



Note: In the above diagram, the positive correlations are denoted by bold black line ($p < 0.001$), bold grey line ($p < 0.01$) and thin black line ($p < 0.05$). The negative correlations are denoted by bold red line ($p < 0.001$), bold dash red line ($p < 0.01$) and thin red line ($p < 0.05$). THB - total heterotrophic bacteria, TAB - total anaerobic bacteria, Phyto - phytoplankton, Chl *a* - chlorophyll *a*, Phaeo - phaeophytin, TSS - total suspended solids, TOC - total organic carbon, CHO - carbohydrates, PRT - proteins, Temp - temperature, MOB - methane-oxidizing bacteria, MOA - methane oxidation activity, MPA - methane production activity. The subscript 'w' and 's' denote water and sediment as the sampling sources for the bacterial groups.

Supplementary Fig. S1. Temporal variation in the environmental parameters in the pond during the cultivation of *Litopenaeus vannamei* in zero-exchange shrimp culture system. Data shown is Mean \pm SD (n = 3).



Note: TSS - total suspended solids, DO - dissolved oxygen, BOD - biochemical oxygen demand.

PC	Eigen values	Variation (%)	Cumulative Variation (%)		
1	11.2	40	40		
2	5.18	18.5	58.5		
3	3.42	12.35	70.85		
4	2.98	10.55	81.40		
5	1.65	5.9	87.30		
Eigenvectors					
Variables	PC1	PC2	PC3	PC4	PC5
pH	-0.05	-0.29	0.18	-0.04	-0.34
Salinity	-0.23	-0.19	-0.17	-0.13	0.09
Temperature	-0.26	0.09	-0.04	-0.05	-0.11
TSS	0.042	-0.398	0.024	-0.049	-0.019
NH ₄ ⁺	0.189	-0.268	-0.041	-0.199	-0.01
NO ₂ ⁻	0.261	-0.007	-0.007	-0.216	0.089
NO ₃ ⁻	0.272	0.043	0.082	-0.07	0.174
PO ₄ ³⁻	-0.139	0.151	0.014	-0.44	-0.196
DO	0.062	0.161	0.423	0.196	-0.076
BOD	0.114	-0.029	0.404	-0.043	-0.365
Chl <i>a</i>	-0.286	-0.04	-0.078	-0.105	0.016
Phaeo	-0.283	-0.083	-0.02	-0.119	0.082
TN	0.174	-0.03	0.182	-0.016	0.331
TP	0.069	-0.095	0.294	-0.382	0.084
TOC	-0.27	-0.066	0.081	0.056	0.199
CHO	-0.082	-0.372	-0.005	0.037	-0.026
LIP	-0.105	0.024	-0.32	-0.32	0.185
PRT	-0.042	0.115	0.302	-0.143	0.498
MPA	0.166	-0.333	0.00	0.008	-0.132
MOA	0.004	0.335	0.046	-0.251	-0.013
THB _w	0.259	-0.115	-0.039	-0.136	0.078
TAB _w	0.22	0.163	-0.209	-0.051	-0.195
MOB _w	-0.162	0.014	-0.019	0.387	0.1
THB _s	0.203	-0.137	-0.344	-0.047	0.063
TAB _s	0.269	-0.024	-0.156	0.039	-0.023
MOB _s	-0.087	-0.261	0.258	-0.144	0.178
Phyto	-0.194	-0.244	0.025	0.114	0.073

Table 1 Summary of principal component analysis (PCA) of environmental variables in shrimp aquaculture system. Loadings $\geq \pm 0.2$ are represented in bold.

Note: TSS - total suspended solids, DO - dissolved oxygen, BOD - biochemical oxygen demand, Chl *a* - chlorophyll *a*, Phaeo - phaeophytin, TN - total nitrogen, TP - total phosphorus, TOC - total organic carbon, CHO - carbohydrates, LIP - lipids, PRT - proteins, MPA - methane production activity, MOA - methane oxidation activity, THB - total heterotrophic bacteria, TAB - total anaerobic bacteria, MOB - methane-oxidizing bacteria, Phyto - phytoplankton abundance. The subscript 'w' and 's' denote water and sediment as the sampling sources for the bacterial groups.