

Abundance and activity of methane-oxidizing bacteria in commercial shrimp aquaculture

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Introduction

- Aquaculture is a rapidly expanding food industry and contributing significantly to the protein-rich food supply to the world.
- Expansion has resulted in the conversion of coastal regions into aquaculture farms with serious alteration of the ecosystem, habitat destruction and marine pollution.
- Intensive aquaculture operations can also lead to eutrophication that receives higher amounts of nitrogen (NH_4^+ , NO_2^- and NO_3^-) and phosphorus (PO_4^{3-}) as nutrients. The flushing of eutrophied water into nearby coastal or river waters can cause harmful algal blooms leading to oxygen depletion and toxicity to organisms.
- One major challenge is to overcome environmental concerns causing CH_4 emissions, a potent greenhouse gas (GHG) 34 times more potent than CO_2 .
- Aquaculture development threatens the GHG sinks & reservoirs.
- The objective of the current study was to get insights on the role of methanotrophs and the relative methane activities in tropical shrimp pond sediments growing *Litopenaeus vannamei*.

Methodology

- The study was conducted in a tropical bio-secured zero-water exchange commercial shrimp farm (Karnataka; 14.42° N and 74.40° E).
- Water & sediment samples were collected at time intervals for one crop cycle (96 days).
- Consistent management practices for pH, dissolved oxygen, feed and probiotic applications for shrimp health.
- Physicochemical & biological parameters include pH, temperature, salinity, total suspended solids (TSS), dissolved oxygen (DO), biochemical oxygen demand (BOD), chlorophyll *a* (Chl *a*), phaeophytin (Phaeo), dissolved nutrients (APHA 2005).
- Sediment samples were analyzed for carbohydrates (Kochert 1978), protein (Lowry et al., 1951), lipids (Bligh and Dyer, 1959), total organic carbon (TOC) (El Wakeel and Riley, 1957), total nitrogen (TN) (Bremner 1965).
- Culturable bacterial parameters: total heterotrophic bacteria (THB) (Rodina 1972), methane-oxidizing bacteria (MOB) (Whittenbury et al., 1970), total culturable anaerobic bacteria (TAB) (Himedia) in water and sediment.
- During the estimation of CH_4 production for the pond sediments, acetylene was used as an inhibitor for CH_4 oxidation.
- For the analysis of potential CH_4 oxidation, Sodium 2-bromoethanesulphonate was used as the inhibitor to arrest the CH_4 production in the sediments.
- Potential CH_4 oxidation & production was detected using gas chromatograph with Flame Ionization Detector (FID).
- Spearman's rank correlation test done for statistical analysis.

Conclusion

The CH_4 oxidation & production rates are influenced by the *in situ* environmental variables. This oxidation and production rates are in turn influencing the emission of CH_4 into the atmosphere.

Acknowledgment

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Figure 1

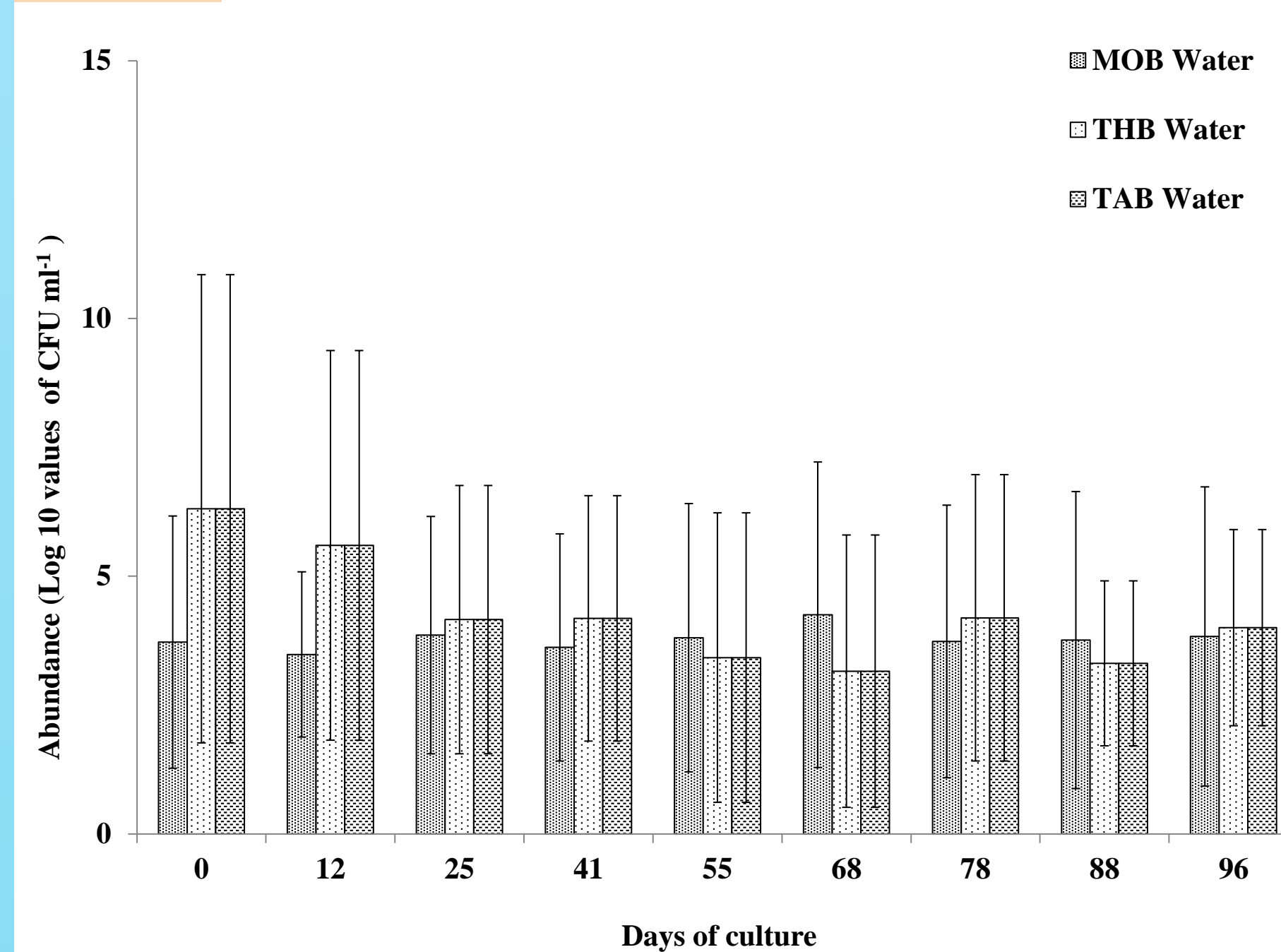


Figure 2

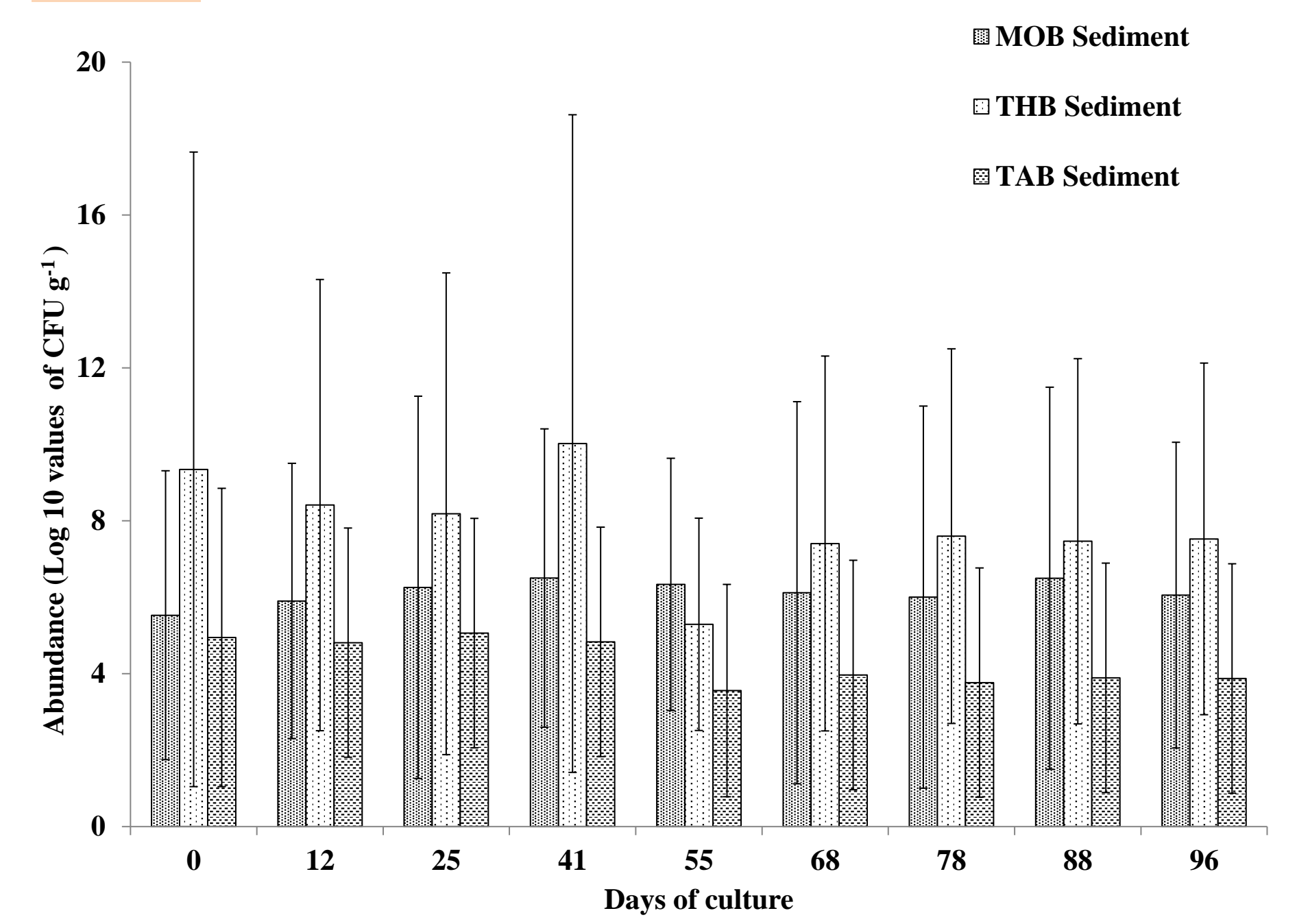


Figure 3

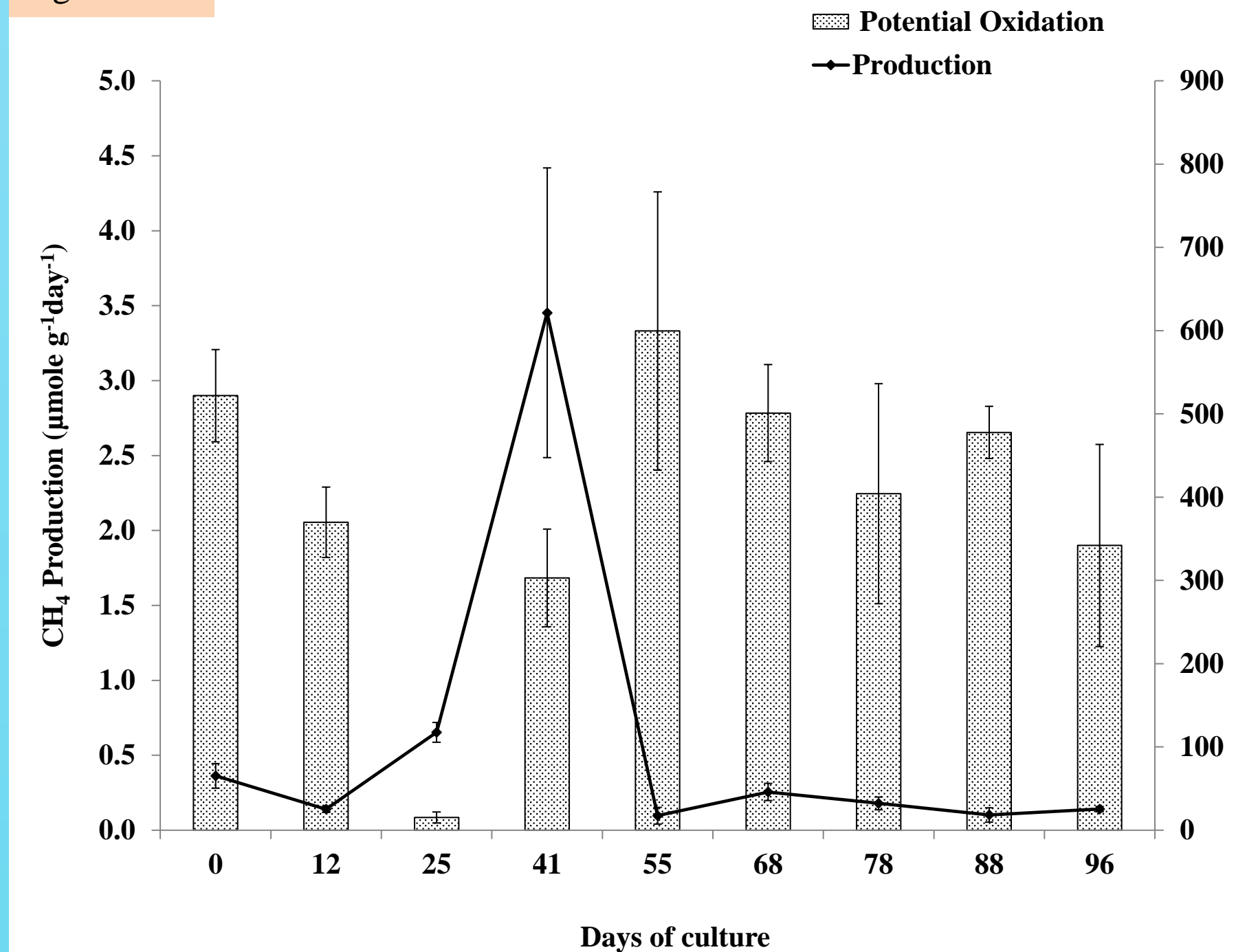
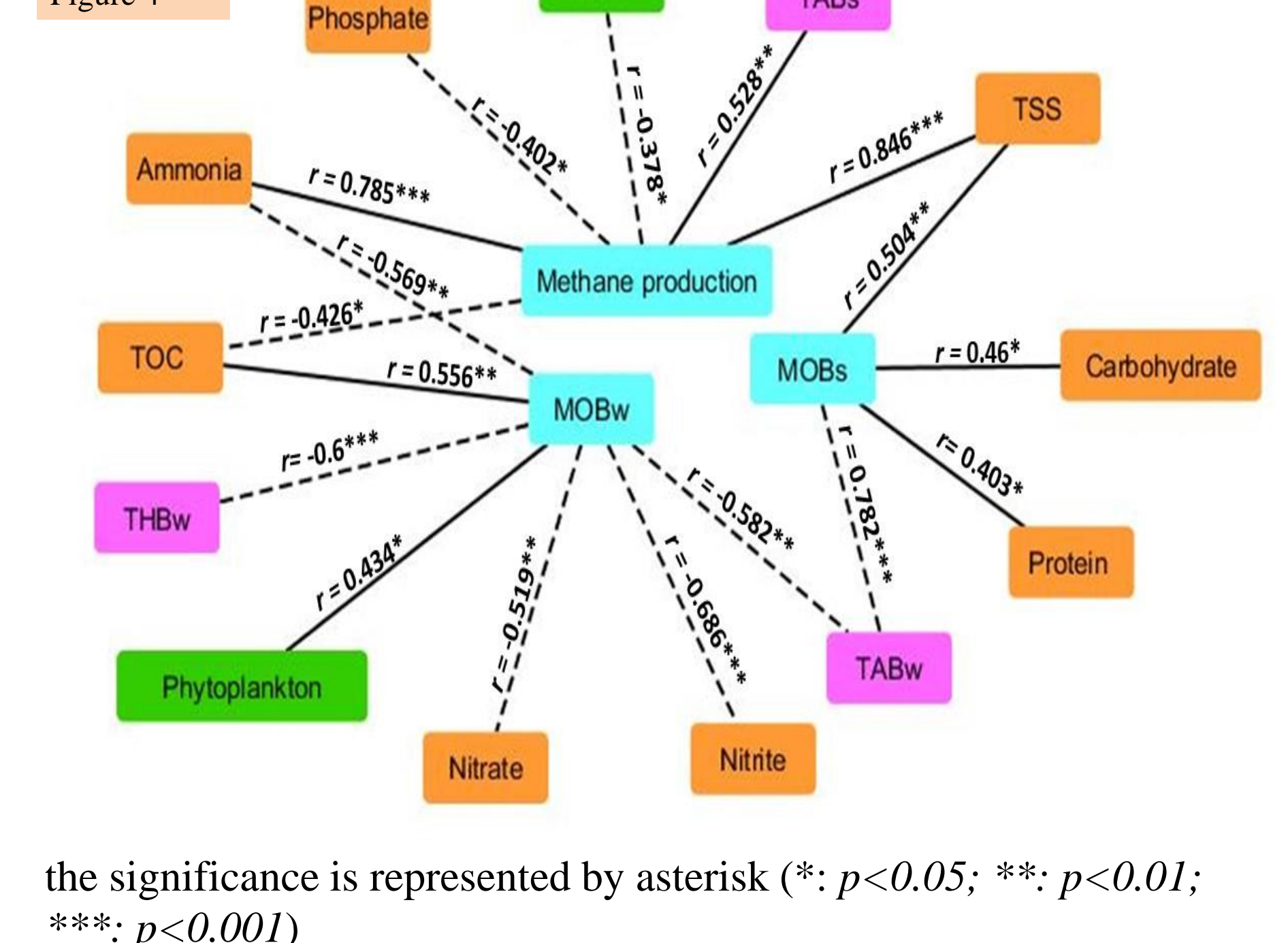


Figure 4



the significance is represented by asterisk (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$)

Results and Discussion

- Figure 1 & 2 represents the abundance of bacterial groups (THB, TAB & MOB) in water and sediment for one production cycle respectively.
- MOB ranged between 4.16×10^3 CFU ml^{-1} to 6.8×10^3 CFU ml^{-1} in water and 3.36×10^5 CFU g^{-1} to 3.17×10^6 CFU g^{-1} sediment.
- The average CH_4 production of 0.60 ± 0.30 $\mu\text{mole/g/day}$ and average potential CH_4 oxidation of 392.71 ± 53.06 $\mu\text{mole/g/day}$ for one shrimp production cycle (Figure 3).
- Figure 4 depicts the significant interrelationships obtained from the Spearman's rank correlation for the MOB in water and sediment along with the CH_4 production during the shrimp production cycle.
- There was a positive correlation of MOB with the phytoplankton abundance ($p < 0.05$) and negative correlation of CH_4 production with Chl *a*. Dimethylsulfoniopropionate (DMSP)—a constituent of phytoplankton leachates and debris, acting as a substrate for production of CH_4 (methanogenesis) and its subsequent oxidation by MOB.
- The negative relationship of MOB with NH_4^+ and NO_2^- may explain their competitive inhibitory effect on methane monooxygenase (MMO)—the major enzyme involved in CH_4 oxidation.
- The accumulation of organic matter enhances the microbial activity. Suspended particles serve as microniches for anaerobes thus supporting the CH_4 production.
- Gonsalves et al. (2011) reported CH_4 production rates of 3.1 $\mu\text{g/g/day}$ in the shallow Arabian Sea and 216 ng/g/day in the mangrove sediments. Kamalason et al. (2019) reported a CH_4 oxidation activity of 110.94 mM/day for the tropical mangrove sediments.
- The average CH_4 production for the one shrimp production cycle was 0.037 ± 0.018 ng/g/day with the highest value of 0.215 ng/g/day on 41st day. This is still lower than the reported values for marine sediments.
- CH_4 oxidation rates in the pond sediments were comparatively lower than the wetland regions.
- CH_4 is effectively oxidized within the system and this is supported by the efficient bio-secured commercial pond management practices including frequent/continuous aeration supporting the effective pond water mixing and constant addition of various probiotics.
- Thus making this system to act as CH_4 sink than an emission source compared to other wetland regions.
- Within the system, there exists complex interactions between the environmental variables of water and sediment.
- Due to the significance of CH_4 in global warming, it is utmost important to monitor and estimate the contributions of fast growing aquaculture industry situated along the coastal regions.
- Further studies for the long term monitoring of greenhouse gases for different stocking densities, effluent discharge points, specific aquaculture pond management practices are required.