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Influence of seasons and environmental variables on methane dynamics in the Muthukuda Mangrove sediments of Tamil Nadu

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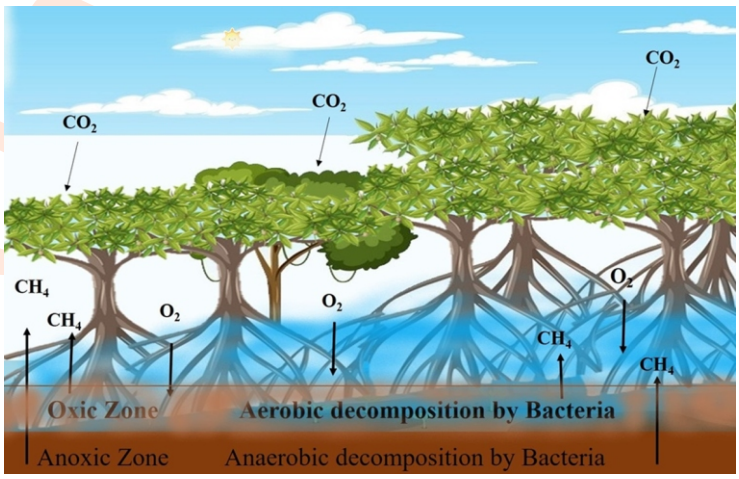
Abstract

Aim: In order to understand the methane biochemistry in the Muthukuda mangrove ecosystem, this study aimed to report on methane production, oxidation, flux, and also to investigate the effect of seasons and environmental variables on methane dynamics.

Methodology: Sediment analysis was carried out monthly for a year (2021-2022) from the Muthukuda mangrove, south-east coast of India, which is an unexplored region in terms of methane dynamics. Methane parameters methane concentration, methane oxidation, methane production and methanotrophic enumeration were analysed from the sediment samples collected by a PVC corer from the study site. The methane parameters were analysed using standard methods by gas chromatography. The physiological parameters were analysed on the site using portable probes.



Results: The physio-chemical parameters varied with the changing seasons at different depths.The concentrations of organic parameters were higher for carbohydrates, followed by lipids and proteins. LOM was higher in the surface and mid sediments in all the seasons. The concentration of methane was higher during summer in surface sediments. Generally, higher methane oxidation was observed in the mid and bottom sediments during all the seasons. Methanotrophic enumeration ranged from 0.4 ±0.7 to 4.8±2.4 x10^8 g^{-1}.

Interpretation: The results suggest that the bacterial community was involved in both methane oxidation and production, though oxidation of methane was lesser than production contributing to the atmospheric methane, methanotrophic bacteria which oxidizes methane significantly playing a major role in the regulation of methane flux.

Key words: Methane oxidation, Methanotrophs, Muthukuda mangrove

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Introduction

Mangroves are coastal ecosystems that have a distinctive blend of terrestrial and marine characteristics. They are made up of trees and other plants that can survive in brackish or salty coastal waters. The recycling of nutrients between marine and terrestrial ecosystems occurs due to high amount of organic matter. Mangrove forests are highly productive areas that sustain detritus-based food webs (Mukherjee *et al.,* 2019). Mangrove ecosystems are considered to be dynamic environments with a gradient profile of natural gasses that may be caused by tidal movements and physio-chemical factors (Qian *et al.,* 2023). Detritus and fallen leaves contribute to nutrient-rich sediment, which facilitates release of microbial nutrients and decomposition. The ecosystem's primary output is sustained by this nutrient cycle (Alongi, 2014), which is especially regulated by microorganisms producing CO_2, CH_4 and H_2S as products. Among these, methane is a potent greenhouse gas due to its radiative forcing contributing 15% to global warming and gains importance for the studies by researchers in the present scenario.

Since pre-industrial era, the concentration of methane has increased two and a half times times, as reported by the Intergovernmental Panel on Climate Change (IPCC, 2021). Methane is produced in anaerobic condition by methanogens and consumed in aerobic environments by a special group of bacteria called methanotrophs like *Methylococcus, Methylosinus and Methylocystis Methylomonas, Methylobacter* and *Methanococcus* The presence of methanotrophic bacteria relatively reduces methane emission and the net balance between the production and sink contributes to methane emission (Donato *et al.,* 2011; Das *et al.,* 2018). Methane emissions from mangroves are influenced by a number of parameters, such as nature of the sediment, salinity of the water column, tidal influx, temperature, and availability of nutrients. The environmental factors may vary with respect to seasonal changes, which could explain variance in methane emissions from mangroves. Further other than environmental conditions and seasonal changes, the ecology of mangroves along the coast is more vulnerable to anthropogenic inputs, which in turn influences methane flux (Attri and Kerkar, 2011).

The field of methane dynamics in mangroves is continuously evolving. The biochemical process in sediments involving methane is considered important and it has been documented that tropical mangrove ecosystems produce a significant amount of methane, which contributes to climate change compared to other regions (Lin *et al.,* 2020; Nazareth and Gonsalves, 2022). Hence, it is important to understand the factors that affect and influence methane, as well as the amount of methane being emitted in these environments. Also, understanding the interaction between methane emission and methanotrophs is critical for assessing potential feedback loops in response to climate change (Poffenbarger *et al.,* 2011). Muthukuda Mangrove zone near Palk Bay in India is located between Rameshwaram and Point Calimere as southern and northern borders, respectively. Despite the fact that numerous data sets are available on methane dynamics in India, there is no data on methane rates in this strategically significant location as far as our knowledge is concerned. Hence, our study specifically seeks to understand how seasonal variations and environmental factors impact the dynamics of methane in Muthukuda Mangrove sediments of Tamil Nadu.

Materials and Methods

Sampling and Study Area: Sediment samples were collected from the mangrove region located in Muthukuda, a coastal village situated in the Pudukottai district Tamil Nadu. This area is close to Mimisal town which is located in the Palk Bay area (9º 51' 37" N, 79º 8' 1.57" E) (Fig. 1). The sampling area is home to an assortment of mangroves and seagrass beds, dominated by *Avicennia marina* mangrove (Govindasamy *et al.,* 2011). A hand-held PVC corer cylinder (6.5 cm) was used to collect the samples during low tide. The cores were promptly sealed after collection, and water from the area was also collected for laboratory experiments. The temperature, pH, and redox potential (Eh) were assessed on-site with portable probes. The sediment cores were transported in ice box to the laboratory, where they were sectioned into three depths: surface (0–1 cm), mid (1-6 cm), and bottom (6–11 cm) sediments for further analysis. Samples were collected throughout the year, corresponding to the northeast monsoon in the area, encompassing four seasons: pre-monsoon (July to September), summer (April to June), post-monsoon (January to March), and monsoon (October to December). It is essential to note that the samples were handled with great care and transported under appropriate conditions to ensure the accuracy of the results.

Quantification of bacteria: Slurry was prepared using the sediment samples and sonicated to quantify the bacteria. The total and viable bacteria were measured by direct count method of acridine orange (Hobbie *et al.,* 1977).

Biochemical Parameters: Protein concentration in the sediments was determined by Lowry's Folin-Ciocalteu method using bovine serum albumin as standard (Lowry *et al*., 1951). The phenol-sulfuric acid method was used to quantify the amount of carbohydrates after an aliquot of sediment sample was extracted in 5% trichloroacetic acid (Kochert, 1978). The absorbance was read at 480 nm and expressed as glucose equivalents. Lipid extracted by, direct elution with chloroform and methanol. The acid dichromate method (Bligh and Dyer, 1959; Parsons *et al.,* 1984) was used to analyze the sediments, and the absorbance was read 440 nm.

Methane concentration in sediments: A 2.5 ml of sediment from each section was taken in a cut syringe in 30ml glass vials filled with 17.5 ml of NaOH (1M). Vials were closed with a butyl rubber stopper and sealed with aluminium crimps. The bottles were shaken to equilibrate methane into the head space (Andren *et al*., 2015). The headspace was sampled with a gas-tight syringe, and analyzed by gas chromatography using flame ionization

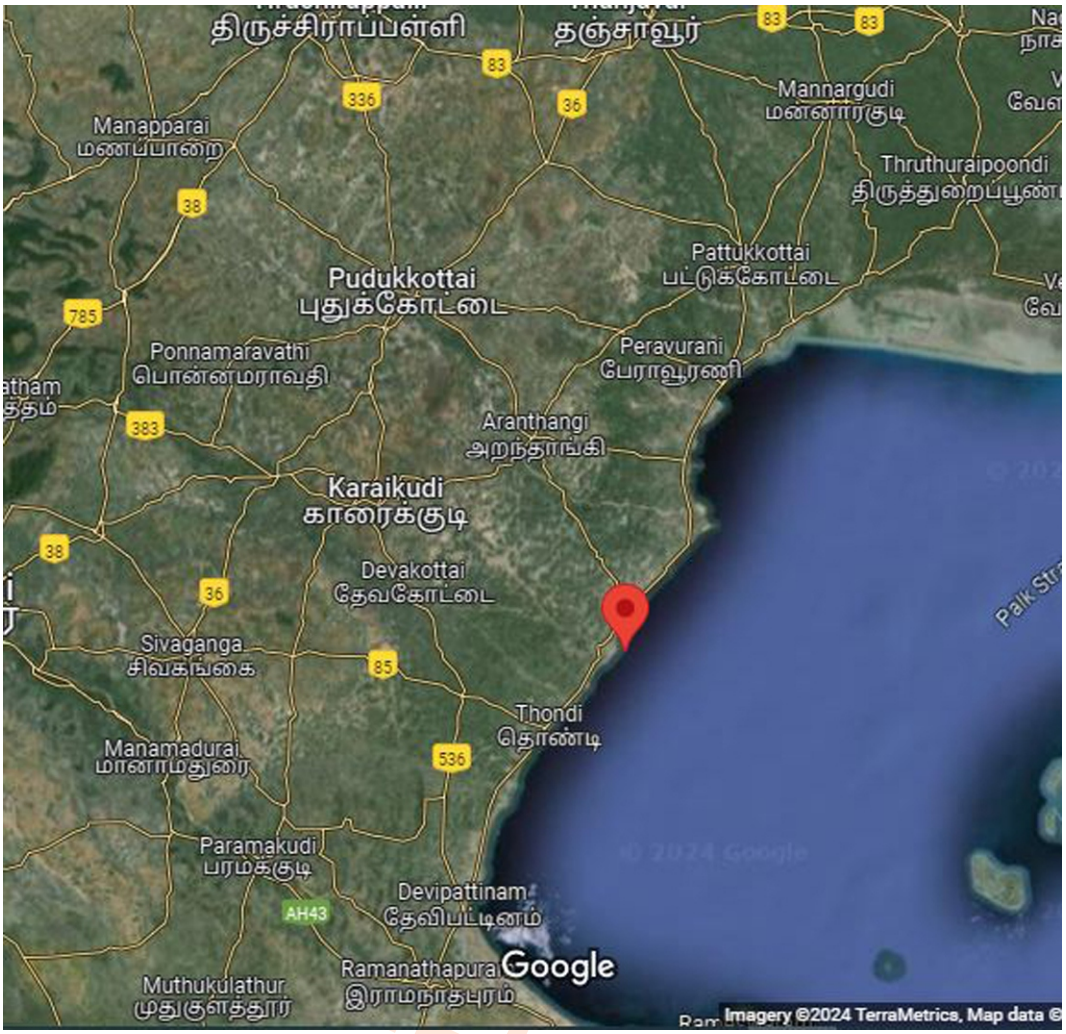

Fig. 1: Location of study area.

detector (FID) equipped with capillary column (30 m long mega bore) with a detector temperature of 150°C and injector temperature of 100°C. Nitrogen was used as a carrier gas. Methane standard with a concentration of 1-5 ppm was used for reference.

Methane production in sediments: To measure methane production in the sediment, a slurry was prepared with oxygen- and sulfate-free artificial seawater diluted (1:10 final sediment dilution) with the sediment sample. Nitrogen was flushed into the sealed vials for 10 min and an initial time point for methane production was determined. The headspace of the vacutainers was sampled by removing with a gas-tight syringe. Sediment samples were incubated in triplicates in the dark at ambient temperatures for about 62 hrs and the increase in the headspace methane was measured by gas chromatography (Sotomayor *et al.,* 1994; Schmaljohann,1996) following the conditions mentioned in previous section.

Oxidation of methane: The aerobic methane oxidation was determined by preparing slurry from the sediment subsections. Sterile glass bottle containing slurry was supplemented with methane. Methane depletion was quantified by sampling the headspace methane on the initial and the final day using gas chromatography (Schmaljohann,1996) following the specifications mentioned in previous section.

Methanotrophic counts: Slurries from the sediment sub-sample were prepared and appropriate dilutions were plated on nitrate mineral salts media of Whittenbury *et al.* (1970), solidified by adding 1.8% Bactoagar. A phosphate buffer solution was on autoclaved separately and added to the media before pouring on the plates. It was incubated at 28 ± 2°C in the dark with a 0.22 µm pore filtered gas mixture of methane and air (30:70), and observed at interval 3-7 days over a period of 3-4 weeks. Control plates incubated without methane were maintained to detect

Table 1: Physicochemical parameters of the sediments of the sampling site

Seasons	Depth	Temperature (°C)	pH	Eh (mV)
Post- Monsoon	Surface	27.20	6.96	53.00
	Mid	25.30	6.45	27.16
	Bottom	21.30	5.50	51.33
Summer	Surface	27.1	7.13	-49
	Mid	24.06	6.96	-44.83
	Bottom	21.06	5.9	-16.60
Pre-monsoon	Surface	26.5	7.1	-46
	Mid	25.8	6.8	-38.3
	Bottom	24.7	5.9	-26.5
Monsoon	Surface	27.2	6.8	-24
	Mid	24.8	6.2	-35
	Bottom	22.5	5.3	-41

colonies for non-methane oxidizing contaminants. The isolates which grew on the NMS medium were examined for further studies. The methanotrophs were enumerated and expressed as CFU g^{-1}.

Statistical Analyses: Spearman correlation analysis was carried out to explicate the influence and relationship of environmental and bacterial parameters. The correlation value which were greater than confidence level 90% were considered.

Results and Discussion

The physico-chemical parameters of the sediments at the study site are shown in Table 1. Variations in the physio-chemical parameters were reported along the depth with the seasons. A marginal decrease in temperature along the vertical profile with the seasons was observed. The average temperature during the post-monsoon was 27.2°C on the surface sediments and 21.3°C at the bottom sediments. During monsoon season the average temperature recorded was 27.2°C and 22.5°C on the surface and bottom sediments. However, during pre-monsoon and summer season the average temperature was 26.5°C and 27.1°C on the surface and 24.7°C and 22.5°C at the bottom sediments. There was a significant difference between the surface and bottom depths in all the seasons. The mean pH value during pre-monsoon and summer was 7.1 on the surface sediments, and decreased to 6.96 during post-monsoon season. In the mid-sediments, the maximum pH value of 6.96 was observed during summer, while a value of 6.8 was noted in the pre-monsoon season. The highest average redox potential during pre-monsoon was -53.6 mV on the surface sediments, while it decreased to -13 mV during post-monsoon on the surface and -9.6 mV in summer at the bottom sediments. During monsoon, it was -24 mV on the surface and -15 mV at the bottom sediments. Carbohydrates significantly correlated with methanotrophs ($r=0.66$, $p<0.05$). The concentration of organic parameters were higher for carbohydrates, followed by lipids and proteins. The maximum concentration of carbohydrates was 3.39 µg g^{-1} during monsoon on the surface, which was greater than the mid-and-bottom sediments. A minimum of 1.67 µg g^{-1} carbohydrates was observed during summer at the bottom sediments. During the post-monsoon and summer seasons, the concentrations did not fluctuate much along the depth. However, the protein concentrations were higher in the surface than mid and, bottom sediments. The maximum protein concentration was observed during summer and pre-monsoon seasons in the mid-sediments. Of all the seasons, the protein values dropped to 0.97 µg g^{-1} and 0.94 µg g^{-1} during summer and monsoon seasons in the bottom and surface sediments, respectively.

The monsoon season had the highest lipid concentration of all the seasons. A maximum of 3.9 was observed during the monsoon (~1.3 times higher), and the value dropped to 2 in the post-monsoon season. The sum of carbohydrates, proteins and lipids *i.e.,* LOM was higher in the surface and mid-sediments during all the seasons (Table 2). Temperature showed a significant positive correlation with carbohydrates ($r=0.64$, $p<0.05$). Eh was significantly correlated with protein ($r=0.59$, $p<0.05$). The methane concentration (15.2 ± 2.63 µmol) was higher during summer in the surface sediments. During monsoon, the rate of methane concentration dropped to 3.56 ± 0.9 µmol in bottom sediments. In post-monsoon season, the concentration was 13.67 ± 0.75 µmol on the surface and dropped to 8.3 ± 2.3 µmol at the bottom. In pre-monsoon season, it was 12.6 ± 1.5 µmol on the surface and 10± 2.8 µmol at the bottom sediments.

Methane concentration showed a decreasing trend throughout the year on the surface (-1.13) and in mid-layers (-0.64) whereas an increasing trend was found the bottom layer (0.089). (Fig. 2). The pH values were negatively correlated with methane concentration ($r=-0.75$, $p<0.05$). Generally, the oxidation of methane was higher in the mid and bottom sediments during all the seasons, except during pre-monsoon, which was higher on the surface (6.4 ± 2.12 µmol cm^{-2} hr^{-1}) and lower during monsoon on the surface (4.1 ± 0.6 µmol cm^{-2} hr^{-1}). During summer, the oxidation rate was (5.3 ± 1.5 µmol cm^{-2} hr^{-1}) and 6.01 ± 0.4 µmol cm^{-2} hr^{-1}) in the mid and bottom sediment, and during post monsoon season the oxidation rate was 5.34 cm^{-2} hr^{-1} and 6.2

Table 2: The mean values of sediment parameters down the core

Season	Depth	Carbohydrates (µg g^{-1})	Proteins (µg g^{-1})	Lipids (µg g^{-1})	LOM* (µg g^{-1})	Methane oxidation µmol cm^{-2} h^{-1}	Methane Concentration µmol cm^{-2} h^{-1}	Methane production µmol cm^{-2} h^{-1}	Methanotrophs x 10^4	TC x 10^8
Post- Monsoon	Surface	3.1	1.83	2.75	7.68	5.34	13.67	7.7	1.3	1.27
	Mid	3.21	1.35	2.3	6.86	6.2	13.3	7.5	3.6	1.25
	Bottom	2.3	1.4	2	5.8	6.2	8.3	13.5	4.8	1.1
Summer	Surface	1.87	1.77	2.7	6.4	5.3	15.2	9.8	0.97	1.87
	Bottom	1.67	0.97	2.13	4.77	5.7	14.3	7.85	0.4	1.37
	Mid	2.28	2.55	3.02	7.85	6.01	14.5	8.75	0.55	1.83
Pre-monsoon	Surface	2.7	1.8	2.3	6.8	6.4	12.6	12.5	2.7	1.3
	Mid	3.02	2.2	2.9	8.2	5.5	9	11.29	2.2	1.65
	Bottom	2.2	1.5	2.8	6.5	5.3	10	12.59	1.6	1.3
Monsoon	Surface	3.39	0.94	3.9	8.2	4.1	7.8	9.5	2.8	1.4
	Mid	2.38	1.6	2.8	6.8	5.5	5.5	8.7	1.8	1.2
	Bottom	2.03	1.03	2.8	5.8	5.3	3.56	8.89	1.5	1.3

*LOM - labile organic matter; n=12 (number of times the sample collected throughout the year)

cm^{-2} hr^{-1}, respectively. Methane oxidation showed a decreasing trend on the surface (-0.16), mid (-0.19) and bottom layers (-0.06). (Fig. 3). No significant difference in the methane oxidation rate, was reported, however, a marginal difference was noted during pre-monsoon season. Methane production was higher (13.5 ± 1.2 µmol cm^{-2} hr^{-1}) in the bottom sediments during post-monsoon, while during pre-monsoon season the production was 12.59 ± 1.3 µmol cm^{-2} hr^{-1}. There was no significant difference along the depth during this season. During summer, the methane production in the surface sediments was 9.8 ± 0.6 µmol cm^{-2} hr^{-1} and in monsoon it was 9.5 ± 1.9 µmol cm^{-2} hr^{-1}. Methane production was higher during pre-monsoon and monsoon seasons. Methane production showed an increasing trend on the surface (0.26) and mid layers (0.20) whereas a decreasing trend on the bottom layer (-0.25) (Fig. 4).

The total bacterial count ranged from 1.87 ± 0.15 to 1.2 ± 0.13 x 10^8 g^{-1} sediment. Methanotrophs were isolated and enumerated in all four different seasons throughout the year. The enumeration of methane-oxidizing bacteria in CFU ranged from 0.4 ± 0.7 to 4.8 ± 2.4 x 10^4 g^{-1}. The number were lower in summer (0.97 ± 0.2 on the surface and 0.4 ± 0.7 x 10^4 g^{-1} at the bottom) (Fig. 5). The highest abundance of methane oxidizing bacteria was recorded in the post-monsoon season (4.8 ± 2.4 x 10^4 g^{-1}) at the bottom and 3.6 ± 2 x 10^4 g^{-1} in mid-sediments. During pre-monsoon and monsoon seasons, the MOBs were higher in the surface sediments (Table 2). Methanotrophs showed an increasing trend on the surface (0.22) and bottom layers (0.045) with a decreasing trend in the mid layers (-0.186) (Fig. 5). The distinct colonies were selected to study the oxidation rate of each isolate, its cultural characteristics and identification by 16S rDNA sequencing (Data not shown). Carbohydrates were significantly correlated with methanotrophs ($r=0.66$, $p<0.05$). Methanotrophs showed a significant negative correlation with methane concentration ($r=-0.55$, $p<0.1$) and total count ($r=-0.53$, $p<0.1$).

Temperature is considered as one of the important physico-chemical parameter that affects and influences the growth and activity of microorganisms. Similar temperatures to those in our study were also observed by Kamaleson *et al.* (2019) in the Chorao region in Goa, and by Kathiresan *et al.* (2014) in the Pichavaram Mangrove, both in post-monsoon sediments. The marginal decrease in the pH during monsoon and post-monsoon seasons may be due to the oxidation-reduction changes due to freshwater inputs (Zingde *et al.,* 1987; Kamaleson *et al.,* 2019). The observation of lower redox potential (Eh) during wet season, was reported by Marchand *et al*. (2004) in their study on mangrove sediments, is consistent with findings from more recent research, such as that of Sugiana *et al*. (2023). This trend has significant implications for understanding the seasonal dynamics in mangrove sediments. The carbohydrate concentration was higher compared to proteins and lipids. The LOM was higher on the surface during pre-monsoon and monsoon seasons. Mangrove leaves often contain significant levels of carbohydrates, lipids, and proteins making them a vital source of sustenance for the mangrove fauna (Bhosale *et al.,* 1976). Previous reports (Renjith *et al.,* 2013; Kamaleson *et al.,* 2019) also reported similar elevated levels of carbohydrates compared to lipids and protein in Charo Mangrove and Cochin Estuarine systems. The observation made by Gremare *et al.* (1997) regarding the higher presence of proteins and carbohydrates compared to lipids in organic matter (LOM) suggests a state of freshness in the organic material as they are relatively more easily decomposed by microbial activity compared to lipids. Therefore, their higher concentration indicates recent deposition or input of fresh organic material. The influx of nutrients caused by limited rainfall during the pre-monsoon season and warmer temperature can stimulate methanotrophic activity (Dang *et al.,* 2016).

As mangrove ecosystems receive a considerable influx of organic matter from the surrounding environment, tidal inputs,

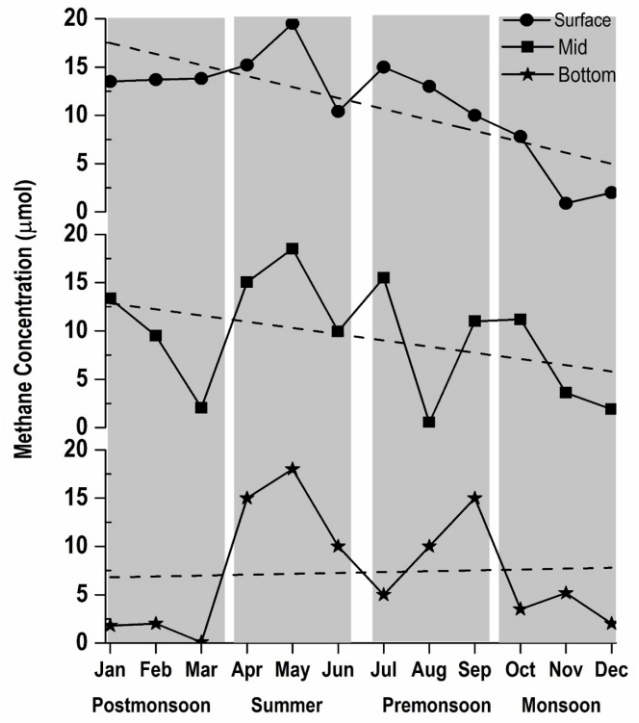

Fig. 2: Seasonal variation and vertical profile of methane concentration.

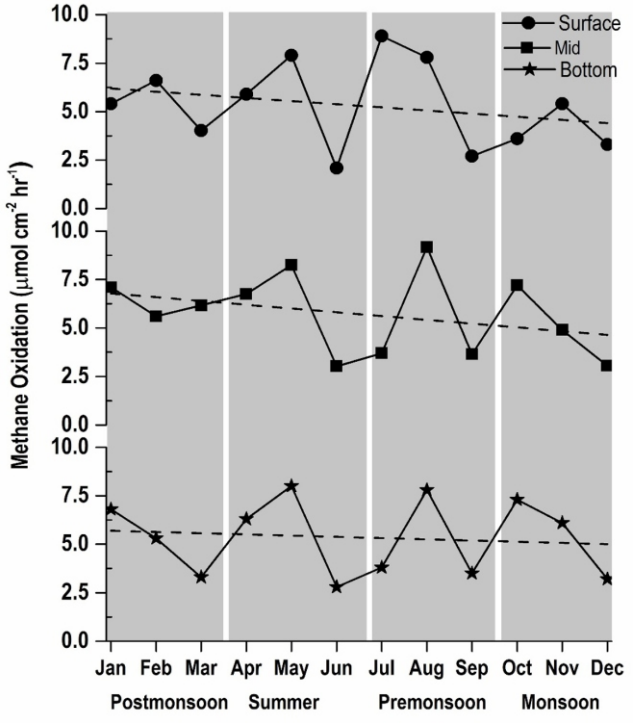

Fig. 3: Seasonal variation and vertical profile of methane oxidation.

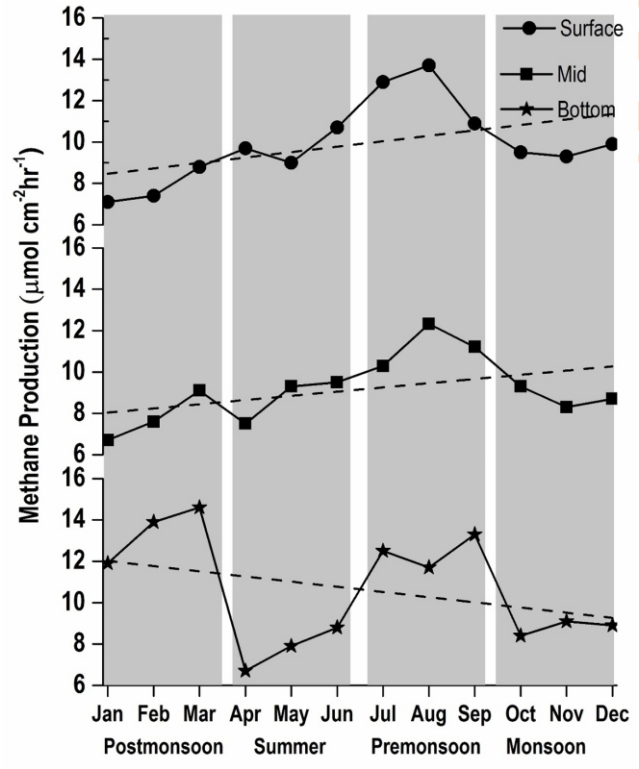

Fig. 4: Seasonal variation and vertical profile of methanotrophic bacteria.

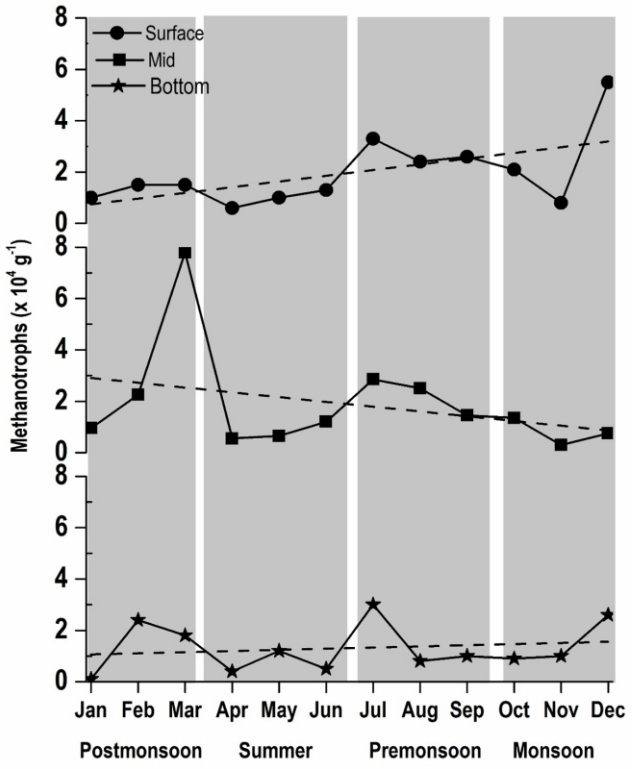

Fig. 5: Seasonal variation and vertical profile of methanotrophic bacteria.

detritus due to increased rainfall and fall of leaf litters can lead to elevated levels of organic matter in the sediments which serves as a substrate for microbial metabolism including methanogenesis (Dutta *et al.,* 2017). Nazareth *et al.* (2022) reported higher oxidation rate of methane during the pre-monsoon season in the Chorao and Betim mangrove areas in Goa. Moreover, during these seasons the waterlogged conditions can lead to oxygen limitation creating favorable condition for methanogenesis leading to elevated levels of methane (Kharitonov *et al.,* 2021).

Spearman's correlation analysis showed that the environmental factors influenced biochemical and methane parameters. Mangrove sediments harbor a variety of aerobic and anaerobic bacterial populations oxidize and reduce organic matter that regulating the nutrient cycle (Alongi, 2005; Chauhan *et al.,* 2015). Consistent with our finding, Kamaleson *et al*. (2019) also observed a higher prevalence of methanotrophic bacteria during the monsoon season. This microbial activity contributes to oxidation of methane. During these seasons, increased tidal action and anthropogenic inputs from land runoff lead to environments with elevated nutrient concentrations, creating favorable conditions for methane metabolism.

The findings of the present study highlights the important roles played by methanotrophic bacteria in regulating methane emissions, which vary with seasons and sediment depth. The link between different parameters such as methane production, oxidation, concentration and methanotrophic populations that give insight about complex relationship within mangrove ecosystems influencing methane dynamics. Sediment characteristics, water column salinity, temperature and nutrient availability were some of these factors that significantly influenced methane dynamics from mangroves. Hence this study support the view that mangrove ecosystem acts as a source of atmospheric methane.

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Authors' contribution: V.M. Sheba: Planned the study, sample collection, carried out experiments, prepared manuscript; **J. Jayaprakash:** Carried out experiments; **P.G. Nisha:** Statistical analysis of data; **B. Praath:** Sample collection idea, data curation and manuscript preparation; **S. Debora:** LOM analysis of the sample; **T.N. Begum:** Planned the study, prepared manuscript.

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Conflict of interest: The authors declare no conflict of interest.

Data availability: The data that support the findings of this study are available on request from the corresponding author.

Consent to publish: All authors agree to publish the paper in *Journal of Environmental Biology.*

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