

Influence of seasonal and environmental variables on the emission of methane from the mangrove sediments of Goa

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Abstract

Mangrove sediments are known sources for methane emission that has a very high global warming potential. The spatio-temporal emission of methane in the mangrove sediments was quantified in the present study using the static closed chamber technique. Besides, the effect of environmental parameters on methane emission were estimated at Betim (mouth), Chorao (midstream) and Volvoi (upstream) stations along the tropical Mandovi estuary. On an average, the methane emission at the upstream estuarine station at Volvoi was maximum ($1268.68 \pm 176 \text{ nM cm}^{-2} \text{ h}^{-1}$) compared to the other two stations. Annually, the methane emission was significantly influenced by physicochemical parameters like salinity at Betim and Volvoi and, the redox potential at the midstream station at Chorao. The variation of methane emission between the 3 stations ($P < 0.001$) is attributed to the variation in methanotrophy ($P < 0.05$) and methanogenesis ($P < 0.05$) influenced by differences in the concentration of nutrients ($P < 0.05$) and organic carbon ($P < 0.05$). Seasonally, the highest methane emission at Chorao was during the post-monsoon, at Betim was during the monsoon season ($1305.34 \pm 108.58 \text{ nM cm}^{-2} \text{ h}^{-1}$) and, at the upstream station at Volvoi the emission of methane was highest during the pre-monsoon season ($1514.68 \pm 130.94 \text{ nM cm}^{-2} \text{ h}^{-1}$). The influence of environmental parameters was more prominent on methane emission at the 3 stations during the monsoon season. Spearman's correlation analysis indicated that seasonal changes in methane emission is not only attributed to the influence of seasonal rainfall that leads to the fresh water input, but also to the variation in biogeochemical parameters.

Keywords: Estuary, organic matter, methane emission, nutrients, salinity

Introduction

Mangrove sediments are productive ecosystems that provide many essential services. These ecosystems are characterised by the rapid decomposition of organic matter as well as the recycling of nutrients thus creating reducing conditions (Schaeffer et al. 2002; Krupadam et al. 2007), which enhances the production of gases like methane (Krithika et al. 2008; Purvaja et al. 2004; Krupadam et al. 2007), hydrogen sulfide (Tomlinson, 1994) and nitrous oxide (Kreuzwieser et al. 2003; Allen et al. 2007). This leads to the release of 'blue carbon' hence contributing to the global climate change (Chmura et al. 2003, Rosentreter et al. 2018). Many reports confirmed that mangrove sediments are important sources of atmospheric greenhouse gases, which is further increased by the anthropogenic inputs (Purvaja and Ramesh, 2001, Muñoz et al. 2002, Kreuzwieser et al. 2003, Allen et al. 2007, Chen et al. 2011; Rosentreter et al. 2018). Methane emission levels in 2100 are projected to reach 159 Mt CH₄/year on an average (range, 78–229 Mt), less than half the emissions in 2010 (Harmsen et al. 2020). Climate change can affect wetlands by direct and indirect effects and can lead to seasonal variations that can cause altered hydrology and rising temperature which can change the biogeochemistry and function of wetlands such as mangrove ecosystems. A higher rate of decomposition than production (photosynthesis) in these ecosystems as a result of climate change might result in a shift from a sink to a source of carbon; i.e. methane emissions to the atmosphere (Laiho, 2006; Flanagan and Syed, 2011; IPCC, 2021). Also, future environmental changes like the climate warming and increased river discharge might increase CH₄ emissions and lead to a decrease in cooling effect of the mangrove ecosystem (Liu et al. 2020).

Tropical mangrove ecosystems are known to show higher contributions to the emission of methane than the other regions (Lin et al., 2020). Methane emission from the sediments of mangrove ecosystem is generally considered to be highly variable (Kristensen et al. 2008; Chen et al. 2014). Some studies report that, competitive inhibitors like high sulfate concentrations in the mangrove sediments hinder the methane emission, as the sulfur reducing bacteria outcompete methanogens for substrate. However, mangrove sediments experience tidal influence that bring in organic and nutrient inputs into the sediments and provide conducive environment for methanogenesis, which in turn impacts methane emission (Chen et al. 2010). Previous studies also report that, there was variation in methane emission due to the factors such as tidal positions, sediment temperature, pH and salinity (Wang et al. 2016). Methane emission may be 2 to 3 order lower than the CO₂ emission (Chen et al. 2010) in the mangrove ecosystem but methane has a higher radiative forcing than CO₂ which makes it an important contributor to the global climate change (Myhre et al. 2013). Under reducing

conditions and availability of substrate, the organic matter is converted into methane by the methanogens (Segers, 1998). This methane can be oxidised by aerobic methanotrophs in the aerobic zone or by the methanotrophic archaea in the anaerobic zone coupled to various electron acceptors (Schubert et al. 2010; Meulepas et al. 2010; Hinrichs et al. 2000). The interrelationship between methane production and oxidation leads to a balance in the production thus maintaining the supply of sufficient methane concentration into the ecosystem and hence, with low emission of this gas to the atmosphere (Giani et al. 1996). Sediments, rich in organic matter exhibit an increase in microbial activity and thus intensify reduction to form methane. Methane emission is proportional to the organic carbon as per Kumar et al. (2014). The proper management of nutrient, organic matter inputs and hydrological flows are promising methods for conducive mitigation of methane emissions thereby preserving the biodiversity and ecological functioning of the mangrove ecosystem (Rosentreter et al. 2021). Moreover, it is reported that altered patterns in the seasonal trend coincide with the onset of a strong linear increase in CH₄ emissions (Zhang et al. 2017). Studies show that after the 2040s, the net growth rate of CH₄ emissions from the summer to autumn seasons will be larger than that from the winter to spring seasons (Zhang et al. 2017). In the near future, seasonal variations due to the climate change, enhanced by anthropogenic activities, will be increasing heat waves, longer warm seasons and shorter cold seasons that will result in the release of significant amount of methane into the atmosphere (IPCC, 2021). In the present study on a tropical mangrove ecosystem which is located in Goa on the west coast of India, it was hypothesised that the methane emission from the sediments of the mangroves was influenced by season and biogeochemical variables.

Material and method

Study area and sampling

The study sites were located in the mangrove ecosystem along the Mandovi estuary at Betim (15°51'06" N Latitude and 73°79'19" S Longitude), Chorao (15°30'39" N Latitude and 73°51'46" E Longitude) and Volvoi (15°49'42 N Latitude and 74°01'09" E Longitude) stations (Fig. 1). The site at Betim is in close proximity (~11.99 km) to the mouth of Mandovi estuary and is highly influenced by the waters coming from the Arabian Sea. This area is characterised by anthropogenic activities such as the presence of trawlers, casinos, availability of ferry service and the terrestrial discharge. The Chorao station at the midstream is situated at the confluence of the Mandovi and Mapusa rivers. About 187 ha of mangrove forest at this site was declared as Dr. Salem Ali Bird Sanctuary that houses most of the mangroves found in Goa consisting of about 15 species (Jagtap, 1993). The

Volvo station present upstream along the river Mandovi has a lesser influence of sea water and hence has lower salinity and is relatively pristine as compared to the other sites. The emission of methane was estimated during the mid months of the three seasons at three different stations and the influence of physico-chemical and biogeochemical sediment parameters on methane emission were studied. The variation in the physico-chemical parameters was measured on the field. The sediment samples for geochemical and biological parameters were transported to the laboratory in an ice box and analysis was carried out within 24 hrs.

Physico-chemical parameters

The estimation of temperature, pH, in the surface sediments (0-5cm) was done using an Orion 4-Star Plus benchtop pH/ISE meter (Thermo Fisher Scientific Inc., MA, USA). Sediment Eh was measured using an Orion platinum redox in combination with an Ag/AgCl₂ reference electrode (Thermo Fisher Scientific Inc., MA, USA). Salinity of the pore water was measured using a handheld refractometer.

Biochemical parameters

For estimating the labile organic matter (LOM) the sediments were air dried and powdered using a mortar and pestle. Protein concentrations in sediments were estimated by Lowry's Folin Ciocalteu method (Lowry et al. 1951). Carbohydrate concentrations were estimated by phenol-sulfuric acid method (Kochert, 1978). Lipid concentrations were estimated by Bligh and Dyer method, (1959). The total concentrations of proteins, carbohydrates and lipids were summed up to give the LOM (Danovaro et al. 1993).

Total organic carbon

For the estimation of total organic carbon (TOC), sediments were oven dried (60°C) for 48 h. The sediments were then powdered using a mortar and pestle. TOC was estimated as per El Wakeel and Riley, (1957).

Nutrient analysis

For nutrient analyses, about 5-10 g of sediment was transferred to centrifuge tubes. The contents were centrifuged at 4°C for 10 min at 5000 rpm. The supernatant was withdrawn into fresh tubes and filtered through a 0.2 µm filter. Ammonium, nitrite and nitrate were measured using an autoanalyser (Skalar Analytical, The Netherlands) following standard methods (Parson et al. 1984).

Methane production and oxidation rates

To determine the methane oxidation rates, sediment slurries from the surface sediments were made in triplicate by diluting the sediment with filtered and autoclaved estuarine water in a ratio of 1:9 in gas tight vials. The vials were sealed using septa and crimped and 100 µl of 2-bromoethanesulfonate (BES) was injected into the vials as an inhibitor for methanogenesis. The vials were incubated at ambient temperatures for a week after initial readings were taken. To determine the methane production rates, sediment slurries from different sections were made in triplicate by diluting the sediment with filtered and autoclaved ambient estuarine water in a ratio of 1:9 in gas tight vials. The vials were sealed using septa and crimped and flushed with N₂ to ensure complete anaerobic conditions. The samples were injected with 1ml of acetylene (inhibitor for methanotrophy). The vials were incubated at ambient temperature for a week after initial readings were taken. Methane was estimated using a head space method on a Shimadzu 2010 Gas Chromatography (GC) and the methane concentrations were calculated according to Bastviken et al. (2002).

Methane emission

In situ methane flux measurements were made at each station covering the 3 seasons, using a static chamber technique (Sotomayor et al. 1994; Zheng et al. 2018). The static chamber was made of PVC (diameter-26 cm, length- 30 cm, volume- 20L). The open end of the chamber was inserted into the sediment at a depth of 4-5 cm to ensure minimal gas leakage. The closed end of the chamber (above the chamber) contained a controllable valve containing luer- lock, for the collection of gas. The valve was kept open for 30 min prior to the sampling which is important to inhibit the impact of root disturbance during the insertion of the chamber. Sampling was carried out after every 30 min time interval in triplicates and along 3 sites of each mangrove ecosystem. Headspace gas was collected using a 50 mL gas- tight syringe equipped with luer- lock. The gas was injected into vacutainers and further estimated in the laboratory. The methane concentration was measured using the head space method and the methane concentrations were estimated using Bunsen solubility coefficient according to Bastviken et al. (2002) and Tishchenko et al. (2005). Methane concentrations were further computed with the area of the static chamber and the time of sampling. The values were expressed as nM cm⁻² h⁻¹.

Statistical analysis

Statistical analysis was carried out using Statistica version 12. The data was log (x + 1) transformed. Spearman's correlation analysis was done to elucidate the relationship between environmental and

bacterial parameters. Analysis of variance (ANOVA) was performed to study the significant differences between the three stations.

Results and discussion

As the second most important anthropogenic greenhouse gas in the atmosphere CH₄ is strongly associated with climate feedbacks (Zhang et al., 2017) which leads to variations in season and has been reported to show to strong linear increase in methane emissions. Management of climatic change at global scale involves several quantifications of production, oxidation and emission of methane in a large variety of wetlands and studying their relationship with the environmental parameters. The present study involves one such investigation into the emission of methane from an estuarine tropical mangrove ecosystem. The average seasonal temperature at Chorao ranged from 26.5±0.19°C to 28.8±0.83°C, at Betim from 26.54±0.37°C to 29.03±1.05°C and at Volvoi the temperatures ranged from 26.70±2.60°C to 29.30±0.85°C (Table 1). Gonsalves et al. (2009) and Jagtap et al. (2006) also reported similar temperature ranges in the estuarine sediments of Mandovi. In the Pichavaram Mangrove, Tamil Nadu, Kathiresan et al. (2014) noted a temperature range from 26.51°C to 27.21°C. A significant positive relationship between the sediment temperature and methane emission was observed by Kumar et al. (2014). A rise of 3°C to 4°C in the sediment temperature led to an increase in methane emission. Duc et al. (2010) reported that an increase of 10 to 100 fold in the emission of methane in marine sediments could be due to increase in temperature. In the current study, the temperature in the upstream sediments was slightly higher as compared to the other 2 sites, which could be the reasons for a higher methane emission rate at the former site. Also seasonally, the upstream sediments at Volvoi showed maximum emission during the pre-monsoon season, which suggest the influence of sediment temperature as one of the factor on methane flux. Besides temperature other anthropogenic activities like the discharge of domestic effluents, crude oil by the cruise boats and fishing trawlers influenced the methane emission rates at Betim and Chorao sites of the Mandovi estuary. Purvaja et al. (2000) also reported that, sediment temperature controlled the methane flux except when the anthropogenic activities influence the methane emission characteristics. The average pH at Betim was near neutral (6.95±0.29) while at Chorao (6.64±0.32) and Volvoi (6.69±0.35) the pH was weakly acidic (Table 1). This variation in pH in mangrove sediments may be due to the organic compounds added by the mangrove foliage, the organisms residing within the ecosystem (Kandasamy et al. 2001) and the location of the estuary (Bhosle, 1990). The sediments were more reducing at Betim as compared to the other 2 sites which could be responsible in dictating and shaping the type of microbial communities and its activity in an

ecosystem (Rummasak et al. 2002). Also, the degradation of organic matter by aerobic bacterial groups using oxygen leads to decrease in the redox values. At the terminal step of organic matter degradation, after all the electron acceptors have been utilised, the anaerobic sediments favour the production of methane (Dutta et al. 2017). Methane production is significantly linked to Eh and this could have an impact on the methane emission. Some reports suggest that redox potential has an inverse relationship with methane emission (Yu et al. 2006; Livesley et al. 2012; Lin et al. 2020). In the present study annually the methane emission at Chorao, showed an inverse relationship with Eh ($p < 0.05$) which also contributes to a higher production of methane at the Chorao site as compared to the other 2 sites. However, the methane emission at Betim showed no significant relationship with Eh and there may have been other factors influencing it. The seasonal trend in salinity was pre-monsoon > post-monsoon > monsoon at the 3 stations (Table 1). Volvoi had minimum salinity when compared to the other 2 sites. The Mandovi estuary along the west coast of India experiences salinity gradient from 0 to 33, due to the domination of fresh waters during monsoon and saline waters during the pre-monsoon and post-monsoon due to the tidal influx (Naik et al. 2019). In the present study, the fresh water dominated site at Volvoi on an average had a maximum emission of methane suggesting the influence of salinity on methane emission rates ($p < 0.05$). This may be due to methanogenesis which is more active in freshwater sediments than in marine sediments, due to the presence of sulphate (SO_4^{2-}) in seawater (Capone and Kiene, 1988), which provides a more energetically favorable pathway for anaerobic remineralization. Besides, salinity is a proxy for sulfate availability which has an impact on methanogenesis and methanotrophy, which can in turn influence the emission of methane (Al-Haj et al. 2020). The salinity in the mangrove sediments of Sundarbans showed a negative relationship with methane concentrations indicating that salinity is one of the major governing factors for the methane concentrations in the mangrove sediments (Dutta et al. 2017). Similar reports were observed in the mangrove sediments along the Bay of Bengal (Biswas et al. 2007). Since the downstream mangrove sites are closer in proximity to the mouth of the Mandovi estuary, these sediments receive higher concentrations of sulfate due to tidal influence. Sulfate concentrations are known to decrease from the mouth towards the upper estuary as reported by Naik et al. (2019). They reported the pore water sulfate concentration at Betim ranging from 21-24 mM (salinity-33), at Amona, which is located along the middle estuary (salinity 18) from 14 to 17 mM and at the Ganjem site present along the upper estuary (salinity 1) of 0-2.4 mM. Kamaleson et al. (2019) also reported that the pore water sulfate in the Chorao sediments ranged from 9.08 ± 1.75 to 17.34 ± 4.33 mM. Also the pore water sulfate concentrations at Volvoi (salinity 14) located upstream was 11.5 mM (unpublished data). The concentrations of sulfate decreased as the salinity

decreased from the mouth to the upper part of the estuary. Thus corroborating with the effect of sulfate on methane emission at the study sites. Sulfate is known to have an inhibitory effect on methane emission (Purvaja et al. 2000) due to which methane emission rates at Betim are comparatively lesser than that at the fresh water dominated site at Volvoi.

Besides sulfate concentrations, the LOM also influences emission of methane in the mangrove sediments (Al-Haj et al. 2020). Proteins, carbohydrates and lipids make up the labile organic matter, which is easily available and rapidly utilised by the benthic fauna (Venturini et al. 2012). The mangroves accumulate LOM around their roots or rhizosphere during photosynthesis. This acts as a substrate for the production of methane (Nielsen & Boschker, 2001; Bridgham et al. 2013). On an average, labile organic matter (LOM) at Volvoi was ~2X more compared to the other 2 sites which could lead to increase methane emission as sediment rich in organic carbon exhibits an increase in the microbial activity which intensifies reduction to form methane (Jayakumar et al. 2001). Also, at Volvoi, Spearman's correlations indicated that the LOM was a non limiting factor; this could have favoured the methane emission at this site. Also reports suggest that there is a linear positive relationship between LOM and methane production (Gonsalves et al. 2011; Xiang et al. 2015). Seasonally, although the LOM was highest in Chorao ($4.34 \pm 2.81 \text{ mg g}^{-1}$) and Betim ($3.80 \pm 3.0 \text{ mg g}^{-1}$) during the pre-monsoon season, there was least methane emission was recorded (Table 2). This could probably be due to the lower methane production rates and higher oxidation potential rates which has been observed in the present study (Table 3) as, methane emission is a result of production and oxidation of methane in an ecosystem (Knittel and Boetius, 2009; Valentine, 2011). Biswas et al. (2007) reported that, the decrease in pore water methane in sediments could be attributed to the intense oxidation of methane by the methanotrophs. Also, as mentioned earlier, salinity could have been another factor that could have led to lower methane emission rates. While, at Volvoi maximum LOM ($6.74 \pm 2.80 \text{ mg g}^{-1}$) was during the post-monsoon season which favoured high emission of methane. Gonsalves et al. (2011), also reported the labile organic matter in the mangrove sediments of the Mandovi estuary to range from 3.35 mg g^{-1} to 5.43 mg g^{-1} . In the mangrove sediments the dominance of total organic carbon can be attributed to the decomposition of organic foliage and other vegetative matter which contributed to the organic carbon (Singh et al. 2005). The inherent biological productivity of the mangroves also contributes significantly to the organic content of the sediments (Prasad et al. 2008). Further, finely grained sediments favour the adsorptions of organic carbon (Ramanathan et al. 1997). Besides, the organic matter that escapes degradation in the water column gets accumulated in the sediments (Chaudhari et al. 2010). Total organic carbon (TOC) was maximum at Chorao ($6.28 \pm 1.92\%$) and minimum at Volvoi ($4.04 \pm 1.15\%$). At Chorao, maximum

TOC ($7.06 \pm 1.12\%$) was recorded during the monsoon season and at Betim ($6.45 \pm 1.58\%$) and Volvoi ($6.44 \pm 1.53\%$) the TOC was highest during the post-monsoon season (Table 1). Kamaleson et al. (2019) reported that the organic carbon in the mangrove sediments of Chorao ranged from 5.08% to 7.34%. The carbon content in the organic matter is a primary source in the formation of methane. Many reports confirm that higher the organic carbon, higher is the methane emission (Kristensen et al. 2008; Chen et al. 2010; Prayitno et al. 2016; Nóbrega et al. 2016; Lin et al. 2020). In the present study, TOC is reported to play an important role in the sediments of Betim and Chorao during the monsoon and post-monsoon seasons. As there is an increase in the emission of methane during these two seasons, which could also be attributed to the decrease in salinity, as there is influx of fresh water from the monsoon (Table 1). Besides that, at Betim salinity showed a positive relationship with methane emission ($p < 0.05$) during the monsoon season (Table 4). At Volvoi the highest organic content in the sediment was 6.44% during the post-monsoon season, which was observed to influence higher rates of methane production and favour the emission of methane. In the environment, the role of microbes (Bardgett et al. 2008) not only involve the decomposition of organic matter but also the release of nutrients (Roulet and Moore, 2006; Stets and Cotner, 2008; Corman et al. 2018). Nutrients at all the 3 stations was of an order $\text{NH}_4^+ > \text{NO}_3^- > \text{NO}_2^-$. The nitrate concentration was maximum, at Chorao during post-monsoon ($4.40 \pm 0.01 \mu\text{M}$), at Betim during the monsoon season ($5.75 \pm 3.45 \mu\text{M}$) and at Volvoi during the pre-monsoon season ($2.64 \pm 1.48 \mu\text{M}$). Nitrite concentration was maximum at Betim ($1.50 \pm 0.21 \mu\text{M}$) and Volvoi ($1.80 \pm 1.10 \mu\text{M}$) during the pre-monsoon season, while at Chorao during the monsoon season ($1.60 \pm 0.01 \mu\text{M}$) (Table 2). Divya et al. (2009) reported nitrate concentration in the Mandovi estuary during the monsoon season to range from $1.17 \mu\text{M}$ to $13.3 \mu\text{M}$ while during the non-monsoon seasons the nitrate concentrations ranged from $0.09 \mu\text{M}$ to $3.6 \mu\text{M}$. However, the nitrite concentration in the Mandovi estuary ranged from $0.72 \mu\text{M}$ to $8.9 \mu\text{M}$ during the monsoon season and $0.001 \mu\text{M}$ to $2.2 \mu\text{M}$ during the non-monsoon seasons. Ammonium concentration was highest at Chorao ($42.70 \pm 2.6 \mu\text{M}$) and Betim ($45.01 \pm 5.77 \mu\text{M}$) during the post-monsoon season and at Volvoi ($28.52 \pm 3.63 \mu\text{M}$) was during the pre-monsoon season. However, Fernandes et al. (2010) observed lower ammonium concentration in the Mandovi estuary that ranged from $15.7 \mu\text{M}$ to $31.3 \mu\text{M}$. The sustainable nutrient supply to mangrove sediments present along the banks of Mandovi estuary is due to the contributions by the rich organic matter and the mangrove litter in these estuarine systems (Wafar, 1987; Wafar et al. 1997). Also, the high nutrient supply in these estuaries is contributed from the terrestrial inputs, mangrove swamps and the iron ore extraction plant located upstream along the estuary (Qasim and Sengupta, 1981; De Souza, 1983; Sardesai and Sundar, 2007). The fertile coastal plains, along the

Mandovi supports rice cultivation and horticulture which involves the use of fertilizers that contain nitrate as a main component. Besides, mangrove ecosystems get inundated by tides and contribute to the nutrient influx into the mangrove ecosystem, which improves the microbial metabolic processes and enhances the emission of methane from the sediments to the atmosphere (Chen et al. 2010; Kreuzwieser et al. 2003). During the monsoon season, due to the land runoff, the nitrates are carried into these estuarine ecosystems. At Chorao and Betim the nitrate and ammonium concentrations were highest during the monsoon and post-monsoon seasons, which coincides with high methane emission rates during these seasons. Volvoi experienced high nutrient concentrations during the pre-monsoon season, during which the methane emission rates were observed to be maximum. An increase in the nutrient flux into the mangrove ecosystem can increase the organic load, thus reducing the oxygen concentrations in the sediments due to the aerobic decomposition (Marinho et al. 2012). This creates favourable conditions for methanogenesis and provides substrate for methanotrophs. Besides, a continuous nutrient loading in the mangrove ecosystem can lead to limited methane oxidation as a result of rapid sedimentation which can be a source for higher methane emissions in the mangrove ecosystem (Egger et al. 2016). Highest concentrations of all the 3 nutrients were recorded in the Chorao and Betim sediments, as these sites experience more anthropogenic activities in comparison to Volvoi. Purvaja et al. (2000) reported a 2 fold increase in methane emission in the anthropogenically impacted areas. Also, Purvaja and Ramesh, (2001) reported that, due to human activities, the mangrove ecosystems receive nutrients due to the discharge of sewage and agricultural activities, the availability of nutrients rises and may accelerate the emission of methane. In addition, about 24 sites of mangrove ecosystems worldwide showed that the anthropogenic activities that added on nutrients to the mangrove sediments showed 14 times increase in the methane emission as compared to the undisturbed mangrove ecosystems (Purvaja et al. 2001). Thus suggesting that mangrove ecosystem affected by the anthropogenic activities contributed largely to the climate change (Zheng et al. 2018). Climate change which in turn can lead to seasonal variations that can cause altered hydrology and rising temperature, changes in rainfall intensity and frequency, extreme climatic events such as drought, flooding and the frequency of storms which can change the biogeochemistry and function of wetlands such as mangrove ecosystems and which will impact the methane production and methane oxidation rates (Salimi et al. 2021).

Methane oxidation at the three study sites was of an order Betim > Chorao > Volvoi. At Betim the methane oxidation was higher as compared to Chorao and Volvoi by 2X and 5X respectively (Table 3). The Betim site situated at the mouth of the estuary experiences more tidal influence due to which

this site receives organic matter (Naik et al. 2019) and other electron acceptors that could have enabled higher oxidation of methane at this site. Also, human settlement areas involving industries, agricultural lands are located along the estuaries. Besides, various hotels, residents, tourist boat cruises dispose waste into the estuary which goes unchecked (Shynu et al. 2015) and which are potential contributors of CH₄ to the Betim site in the Mandovi estuary (Araujo et al. 2018). This could contribute to maximum oxidation of methane at Betim. Seasonally, at Chorao (44.07±9.8 nM g⁻¹ d⁻¹) and Betim (166.68±36.95 nM g⁻¹ d⁻¹) the highest methane oxidation activities were during the pre-monsoon, while at Volvoi highest activity was during the monsoon season (39.36±5.60 nM g⁻¹ d⁻¹). Higher CH₄ concentrations during the peak monsoon could also be largely due to runoff from the tributaries (Araujo et al. 2018). The availability of methane as substrate could have lead to higher methane oxidation rates at Volvoi during the monsoon season. Methane production at Chorao, was maximum during the monsoon season (4.51±0.26 nM g⁻¹ d⁻¹), while during the post monsoon season, the production of methane was highest at Betim (3.05±0.37 nM g⁻¹ d⁻¹) and Volvoi (2.71±1.78 nM g⁻¹ d⁻¹) respectively. In general, at Chorao the production of methane was 2.8X and 1.8X higher compared to Betim and Volvoi. The Chorao site has the maximum TOC concentration as compared to the other 2 sites. It is estimated that microbial process including decomposition of organic matter accounts for almost 70% of global methane production (Conrad, 2009). As methane is produced through anaerobic microbial decomposition of organic matter, methane production in mangrove sediment is influenced by three factors including microbial activities, environmental conditions and organic matter properties. The net flux from methane production and its oxidation will result in the amount of methane being emitted. Methane emission was of an order Volvoi > Betim > Chorao (Fig. 3) with significant spatial and temporal variations (Table 5). Methane emission, during the post-monsoon season was highest at Chorao (1145.68±201.63 nM cm⁻² h⁻¹), at Volvoi the methane emission (1514.68±130.9 nM cm⁻² h⁻¹) was maximum during pre-monsoon, while at Betim (1305.34±108.58 nM cm⁻² h⁻¹) it was maximum during the monsoon season (Fig. 2). The presence of high mangrove leaf litter-fall during the monsoon and post-monsoon seasons (Ray et al. 2011), could have led to the supply of organic matter in these sediments leading to an increase in higher CH₄ production thus influencing the emission of methane (Dutta et al. 2017). Besides, CH₄ emission during pre-monsoon season could be due to the low wind velocity, which leads to low gas transfer and low methane concentrations in the estuarine system (Abril et al. 2005). Also as described earlier, the salinity of the sediments at Chorao and Betim was maximum during this season, which is known to have an inverse relationship with methane emission. Besides that, the presence of higher concentrations of pore water sulfate in these sediments also has an impact on the methane emission

as mentioned above. The oxidation of methane at Chorao and Betim was maximum during the pre-monsoon season, hence all the above factors were collectively responsible for a decrease in methane emission during the pre-monsoon at Chorao and Betim. Mandovi being a tidal estuary, high input of suspended particulate matter in the Mandovi, enters the estuary seasonally (Rao et al. 2016). About 50% of organic matter degradation occurs in the sediments and the remaining occurs in the overlying water column (Gazeau et al. 2004). This makes processes such as ebullition (Martens and Val Klump, 1994, 1998) and tidal pumping (Bouillon et al. 2007; Kristensen et al. 2008) extremely important to manifest the release of CH₄ from the sediments to the water column and bypass the oxidation within the sediments. Therefore, the sediments are an important source of CH₄ to the estuarine waters (Araujo et al. 2018). Also, Betim and Chorao being in close proximity to the mouth of the estuary are influenced more by the tide that bring various electron acceptors enabling the oxidation of methane at the oxic and anoxic levels of the sediment. Mangrove sediments are influenced by anthropogenic activities, tidal flux and organic matter degradation which could provide various electron acceptors available for anaerobic methanotrophy (Segarra et al. 2013). This could be the probable reason for a lower emission of methane at these two sites. Also, Betim and Chorao have dense mangrove vegetation as compared to Volvoi, wherein the pneumatophores transport the gases, like CH₄ and O₂, both from the water column and the atmosphere. This promotes methane oxidation and considerably reduces the overall flux of CH₄ to the atmosphere to just over ambient levels (Kirthika et al. 2008). Thirdly, the oxidation of methane at the Volvoi site is the lowest compared to the other sites which can be mainly due to the presence of lower TOC concentrations at this site (Dutta et al. 2017).

The influence of season and environmental parameters on the emission of methane at the 3 mangrove sites was further studied by performing Spearman's correlation. The influence of environmental variables was more prominent during the monsoon season and negligible during the pre-monsoon at all the 3 stations. At Chorao, during the monsoon season, nutrients negatively influenced the emission of methane ($p < 0.05$) suggesting that the microorganisms involved in the methane cycle, utilise substrates rich in N and P and have a tendency to increase methane related activities. During the post-monsoon season, at Chorao, methane oxidation correlated positively with the emission of methane ($r = 0.769$; $p < 0.01$). Methane emission by sediments results from correlated microbial activities (Mer et al. 2001). Also, Marhino et al. (2012) observed that the large amount of organic carbon and nutrients provided by mangrove vegetation has provided plenty of material available for methanotrophy and methanogenesis. Annually, at Chorao, the methane emission was negatively influenced by the redox potential ($r = -0.409$; $p < 0.05$) and nitrite ($r = -0.400$; $p < 0.05$) (Table 4). This is

because the Eh of the sediment not only shapes the microbial community (Rummasak et al. 2002) but also enhances processes like methanogenesis (Lin et al. 2020), an important process that influences the emission of methane. This corroborates with the findings of Allen et al. (2011) who reported that the CH₄ emissions from mangroves are affected by sediment parameters like redox potential. Besides, the higher nutrient and organic loading input into the mangrove sediments also leads to decrease in oxygen concentrations which are conducive for the emission of methane (Giani et al. 1996; Purvaja and Ramesh, 2001; Kreuzwieser et al. 2003; Alongi et al. 2007). At Betim, salinity influenced methane emission positively ($p < 0.05$) and LOM ($r = -0.836$; $p < 0.01$) negatively. As salinity increases it also makes available various electron acceptors like sulfate, while availability of other electron acceptors, e.g., oxygen, nitrate, ferric oxides, and carbon dioxide, could transiently also increase (Luo et al., 2019). Annually, at Betim physicochemical parameters like temperature ($p < 0.001$), salinity ($p < 0.001$) and nutrients like nitrate ($r = 0.393$; $p < 0.05$) influenced the methane emission. Temporal variation in CH₄ emission could be explained by soil temperature, the position of the water table, and the availability of suitable substrate (Allen et al. 2007; Walter et al. 2000; Inubushi et al. 2003). Also, various studies reported that CH₄ emission varied due to factors such as tidal positions, soil temperature, salinity and pH (Chauhan et al. 2015). At Volvoi, increased methane production and minimum methane oxidation rates influenced the higher rates of methane emission ($p < 0.05$) during the monsoon and post-monsoon season respectively. The presence of sulfate and salinity of the environment is influenced by the microbial interactions for competitive substrates (Reshmi et al. 2014). Previous studies report various ecosystems in which net methane consumption is balanced by the net methane production (Yavitt et al. 1995) and both these processes influence the methane emission. So, whether a particular habitat will act as source or sink depends on the physical conditions prevailing in that ecosystem. Annually, at Volvoi methane emission was positively influenced by salinity and LOC and negatively influenced by TOC.

Conclusion

In the present study, the influence of seasonal and environmental variables on the methane emission at 3 different mangrove sediments sites was studied. The study indicates that mangrove sediments are potential sources of methane emission. The emission of methane at fresh-water dominated station at Volvoi was maximum which could be attributed to environmental factors like temperature and salinity, where higher temperatures and lower salinities increase the methane emission. Besides that, this site is at the upstream and so the tidal influence is lower as compared to the other sites as a result of which this site receives minimal amount of substrates and various electron acceptors which play

an important role in the methane cycle. Also, the slower rates of oxidation and higher rate of production of methane at this site clearly indicates that, the methane emission is being enhanced. The presence of competitive inhibitors like sulfate at Volvoi is lower in comparison to the other 2 sites which makes methanogenesis even more favourable. While at relatively downstream stations of Chorao and Betim, the methane emission increased during the monsoon and post-monsoon seasons which is attributed to the low salinity, high nutrient and TOC content in the sediments during these seasons. The influence of environmental variables like nutrients, labile organic matter, salinity and methane production during the monsoon season was more prominent as compared to the other seasons. Annually, the salinity, total organic carbon and nutrients were reported to be important parameters influencing the emission of methane. Hence we conclude that, the seasonal and biogeochemical variables have an effect on the emission of methane spatially and temporally.

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Authors' contributions –

- 1) Delcy R. Nazareth – Sampling , experimental work, data analysis and manuscript writing
- 2) Maria Judith Gonsalves – Conceptualizing the work, data validation, explication of data, finalisation of manuscript and project leader

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Legends to the figures and tables

Fig. 1 Map showing sampling sites at Betim , Chorao and Volvoi stations in a tropical estuarine system.

Fig. 2 Seasonal emission of methane at Betim, Chorao and Volvoi stations.

Fig. 3 Annual emission of methane at Betim, Chorao and Volvoi stations.

Table 1 Average values with standard deviation (Avg±SD) of physicochemical parameters of sediment samples at Betim, Chorao and Volvoi stations during the 3 seasons.

Table 2 Average values with standard deviation (Avg±SD) of biochemical, total organic carbon and nutrient parameters at Betim, Chorao and Volvoi stations during the 3 seasons.

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Table 4 Spearman's correlation analysis carried out between methane emission and the environmental variables annually (n=26) and seasonally (n=8) at Betim, Chorao and Volvoi.

Table 5 Analysis of variance of the biogeochemical variables of Betim, Chorao and Volvoi.

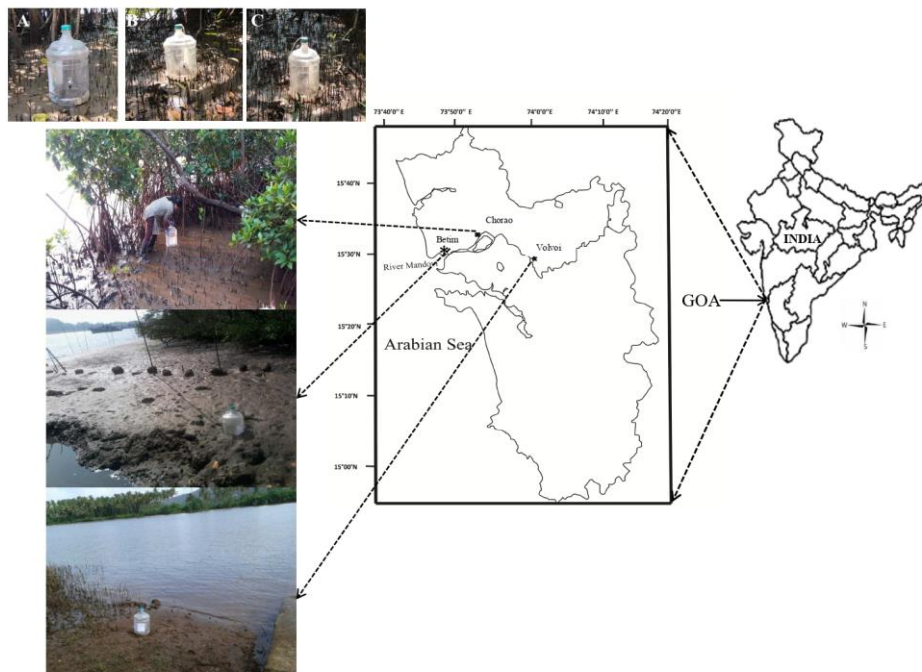


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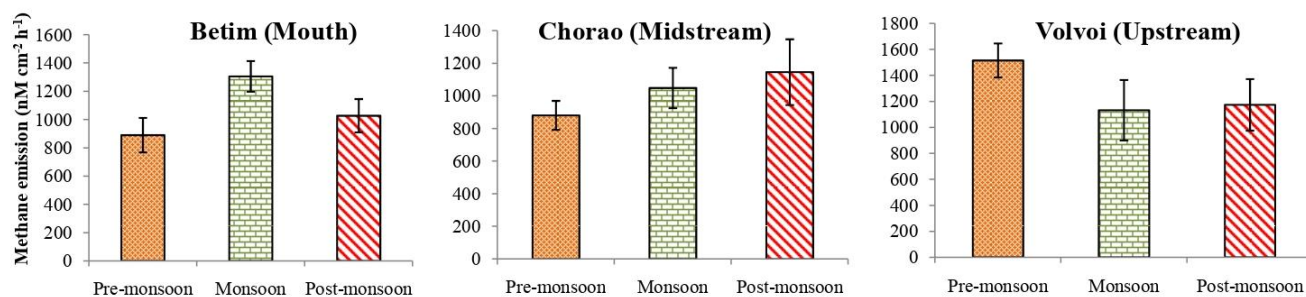


Fig. 2 Seasonal emission of methane at Betim, Chorao and Volvoi stations.

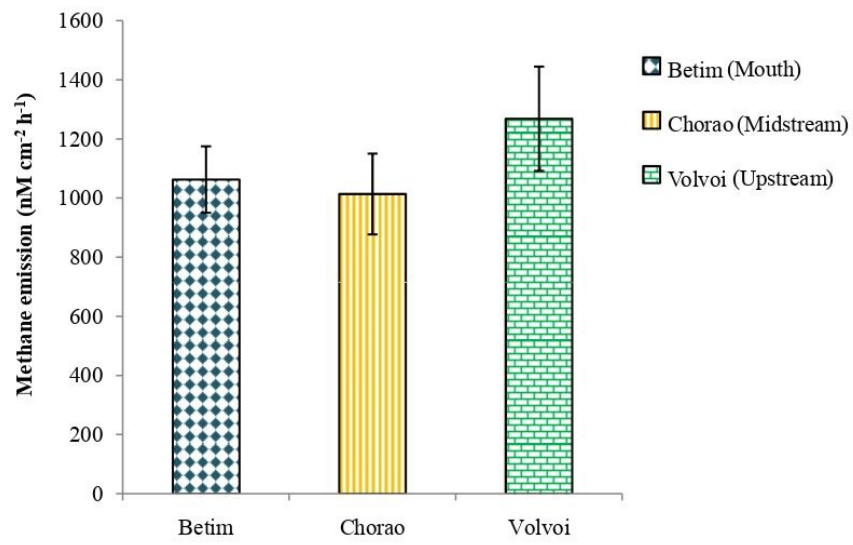


Fig. 3 Annual emission of methane at Betim, Choro and Volvoi stations.

Table 1 Average values with standard deviation (Avg±SD) of physicochemical parameters of sediment samples at Betim, Choraó and Volvoí stations during the 3 seasons.

Stations	Temp (°C)	pH	Eh (mV)	Salinity
Betim				
Pre-monsoon	29.03±1.05	7.42±0.25	-7.26±22.50	31±4.6
Monsoon	26.54±0.37	7.05±0.19	8.51±19.84	11±5.0
Post-monsoon	28.49±0.84	6.40±0.45	28.24±21.9	27±6.8
Choraó				
Pre-monsoon	28.8±0.83	6.56±0.29	31.20±15.01	35±2.0
Monsoon	26.5±0.19	6.49±0.35	32.61±14.82	10±3.0
Post-monsoon	28.2±1.83	6.89±0.33	7.54±10.75	27±4.0
Volvoí				
Pre-monsoon	29.30±0.85	6.70±0.25	13.36±17.18	14±2.0
Monsoon	27.30±1.20	6.68±0.56	5.87±15.70	2±1.0
Post-monsoon	26.70±2.60	6.71±0.26	22.67±16.18	9±6.0

Table 2 Average values with standard deviation (Avg±SD) of biochemical, total organic carbon and nutrient parameters at Betim, Chorao and Volvoi stations during the 3 seasons.

Stations	LOM (mg g⁻¹)	LOC (%)	TOC (%)	NO₃⁻ (μM)	NO₂⁻ (μM)	NH₄⁺ (μM)
Betim (mouth)						
Pre-monsoon	3.80±3.0	0.25±0.15	3.52±1.54	2.96±1.77	1.50±0.21	42.26±1.18
Monsoon	3.61±1.29	0.25±0.06	5.93±2.04	5.75±3.45	0.03±0.001	44.26±1.10
Post-monsoon	2.13±0.61	0.21±0.13	6.45±1.58	3.86±2.09	0.50±0.10	45.01±5.77
Chorao (midstream)						
Pre-monsoon	4.34±2.81	0.28±0.16	5.54±1.05	2.40±0.02	1.50±0.01	38.35±5.10
Monsoon	0.38±0.04	0.38±0.05	7.06±1.12	2.70±0.03	1.60±0.010	39.96±2.0
Post-monsoon	2.96±0.99	0.27±0.13	6.25±3.59	4.40±0.01	0.20±0.001	42.70±2.6
Volvoi (upstream)						
Pre-monsoon	4.74±2.50	0.40±0.11	1.95±0.99	2.64±1.48	1.80±1.10	28.52±3.63
Monsoon	4.19±1.12	0.29±0.12	3.75±0.95	1.40±0.37	1.80±1.03	23.60±4.52
Post-monsoon	6.74±2.80	0.21±0.10	6.44±1.53	1.87±1.03	0.30±0.01	26.40±1.86

LOM- labile organic matter; LOC- labile organic carbon; TOC- total organic carbon; NO₃⁻- nitrate; NO₂⁻- nitrite; NH₄⁺- Ammonium.

Table 3 Average values with standard deviation (Avg±SD) of methane related activities of sediment samples at Betim, Chorao and Volvoi stations during the 3 seasons.

Stations	MOA (nM g⁻¹d⁻¹)	MP (nM g⁻¹ d⁻¹, h⁻¹)	CH₄ emission (nM cm⁻² h⁻¹)
Betim (mouth)			
Pre-monsoon	166.68±36.95	0.002±0.001	889.70±122.29
Monsoon	35.41±32.13	0.64±0.53	1305.34±108.58
Post-monsoon	43.57±5.44	3.05±0.37	1027.12±117.90
Chorao (midstream)			
Pre-monsoon	44.07±9.89	3.15±0.48	880.78±88.61
Monsoon	38.09±12.62	4.51±0.26	1048.32±123.73
Post-monsoon	25.24±11.04	2.86±0.09	1145.68±201.63
Volvoi (upstream)			
Pre-monsoon	4.35±0.71	1.29±0.16	1514.68±130.94
Monsoon	39.36±5.60	1.67±0.64	1132.18±232.68
Post-monsoon	5.42±0.03	2.71±1.78	1173.80±198.43

MOA- methane oxidizing activity; MP- methane production rate

Table 4 Spearman's correlation analysis carried out between methane emission and the environmental variables annually (n=26) and seasonally (n=8) at Betim, Choraó and Volvoi.

Location	Season	Variable	r
Betim (mouth)	Pre-monsoon	ns	ns
	Monsoon	Salinity	0.717*
		LOM	-0.836**
	Post-monsoon	ns	ns
	Annual	Temp	-0.789***
		Salinity	-0.642***
		NO ₃ ⁻	0.393*
LOC		-0.741*	
Choraó (midstream)	Pre-monsoon	LOC	-0.741*
	Monsoon	NO ₂ ⁻	-0.706*
		NH ₄ ⁺	-0.640*
	Post-monsoon	MOA	0.769**
	Annual	Eh	-0.409*
		NO ₂ ⁻	-0.400*
Volvoi (upstream)	Pre-monsoon	ns	ns
	Monsoon	MP	0.675*
	Post-monsoon	MOA	0.698*
	Annual	Salinity	0.479*
		TOC	-0.428*
		LOC	0.514**

LOM-labile organic matter; MP-methane production rate; MOA-methane oxidation activity; r-correlation coefficient; ns-non-significant values; *-p<0.05; **p<0.01; ***p<0.001; Bold numbers indicates significant values.

Table 5 Analysis of variance of the biogeochemical variables of Betim, Choroao and Volvoi.

Environmental variables	Season	SS	F	P-value	F critical
Physicochemical					
Temp	Pre-monsoon	0.98	0.54	0.58	3.40
	Monsoon	3.68	3.05	0.06	3.40
	Post-monsoon	16.82	2.67	0.14	3.40
	Annual	0.88	0.15	0.85	3.11
pH	Pre-monsoon	3.77	25.12	1.3x10⁻⁶	3.40
	Monsoon	1.44	4.25	0.026	3.40
	Post-monsoon	1.09	3.92	0.033	3.40
	Annual	1.46	3.88	0.024	3.11
Eh	Pre-monsoon	6666.30	8.55	0.001	3.40
	Monsoon	3909.20	6.24	0.006	3.40
	Post-monsoon	2064.85	3.25	0.056	3.11
	Annual	2773.46	3.11	0.049	3.92
Salinity	Pre-monsoon	2323.63	105.79	1.25x10⁻¹²	3.40
	Monsoon	428.07	2.80	0.080	3.40
	Post-monsoon	1945.28	24.50	1.5x10⁻⁷	3.40
	Annual	4227.84	18.79	2.18x10⁻⁷	3.11
Biochemical & Nutrients					
LOM	Pre-monsoon	3.52	0.21	0.81	3.40
	Monsoon	75.69	34.40	8.9x10⁻⁸	3.40
	Post-monsoon	97283772	18.99	1.13x10⁻⁵	3.40
	Annual	104.60	9.45	2.1 x10⁻⁴	3.11
LOC	Pre-monsoon	0.112	2.55	0.098	3.40
	Monsoon	0.071	5.06	0.014	3.40
	Post-monsoon	0.021	0.65	0.528	3.40
	Annual	0.082	2.40	0.096	3.11
TOC	Pre-monsoon	58.24	17.96	1.7x10⁻⁵	3.40
	Monsoon	50.91	11.05	0.00039	3.40
	Post-monsoon	0.22	0.008	0.991	3.40
	Annual	59.51	6.84	0.0018	3.11
NO ₃ ⁻	Pre-monsoon	4694.09	11.67	0.00028	3.40
	Monsoon	16081.6	17.78	1.8x10⁻⁵	3.40
	Post-monsoon	6585.26	16.05	3.7x10⁻⁵	3.40
	Annual	23446.71	36.64	5.9x10⁻¹²	3.11
NO ₂ ⁻	Pre-monsoon	0.006	0.103	0.901	3.40
	Monsoon	17.51	6.25	0.0065	3.40
	Post-monsoon	0.003	0.38	0.68	3.40
	Annual	6.05	4.55	0.013	3.11
NH ₃	Pre-monsoon	90290.0	31.40	1.9x10⁷	3.40
	Monsoon	214027.5	112.66	6.3x10⁻¹³	3.40
	Post-monsoon	185140.2	246.71	9.92 x10⁻¹⁷	3.40
	Annual	472766.6	205.76	7.7 x10⁻³²	3.11
Methane activity					
MOA	Pre-monsoon	125875.6	2.98	0.069	3.40
	Monsoon	73.09	0.02	0.97	3.40

	Post-monsoon	6552.88	1.99	0.158	3.40
	Annual	61134.08	3.41	0.037	3.11
MP	Pre-monsoon	45.13	3.89	0.034	3.40
	Monsoon	72.36	5.05	0.014	3.40
	Post-monsoon	0.50	0.03	0.96	3.40
	Annual	74.67	5.28	0.007	3.11
CH ₄ emission	Pre-monsoon	1.09	90.11	6.9x10⁻¹²	3.40
	Monsoon	0.14	5.70	0.0093	3.40
	Post-monsoon	0.05	1.74	0.19	3.40
	Annual	0.43	9.99	0.00013	3.11

LOM-labile organic matter; LOC-labile organic carbon; TOC-total organic carbon; MOA-methane oxidation activity; MP-methane production rate; ss-sum of squares; F- variation in samples; P-level of significance (P<0.05); Numbers in bold indicate significant values; Df-degree of freedom=2.