

## **Impact of COVID-19 lockdown on methane related activities in a tropical estuarine mangrove ecosystem.**

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### **Abstract**

The COVID-19 pandemic led to an immediate lockdown declared by the government of India (March to April 2020) which saw a decrease in public activities. In view of this, a comparative study was carried out on the influence of environmental parameters on methane activities between the pre-lockdown and lockdown periods along a tropical estuarine mangrove ecosystem. Significant changes in the studied parameters specially in the organic matter and methane production rates ( $P < 0.05$ ) were observed. During the lockdown as compared to the pre-lockdown period, the downstream site was impacted more than those at the upstream site with an average methane production decrease by 60X and 1.5X respectively. Both, oxidation and production of methane decreased during the lockdown and was related to organic matter availability ( $P < 0.05$ ). Thus, the findings elucidate the positive impact of lockdown on the model ecosystem and the reduction in greenhouse methane activities.

**Keywords** lockdown, mangrove ecosystem, sediment, methane, organic matter, tropical

## 1. Introduction

Coastal ecosystems are threatened by extensive human activities that lead to conditions like sedimentation, eutrophication, water pollution, overexploitation of fish resources and transportation by waterways (Halpem et al., 2008; Jones et al., 2018; Masood et al., 2016; Wu et al., 2017). One of such coastal ecosystem that is highly impacted by anthropogenic inputs is the mangrove ecosystem. Besides, these ecosystems receive organic matter and other substrates through tidal influence, anthropogenic activities and agricultural runoff. The high degradation rates by the microbial activities create a reduced environment in these ecosystems (Fernandes et al., 2016). Mangrove ecosystems are responsible for the ecological sustainability, economic prosperity, environmental security and cultural values (Nanjo et al., 2014; Sandilyan et al., 2012). This ecosystem supports unique microbiota by providing habitats, nurseries, shelter and feeding areas for various animals such as reptiles, birds, mammals, shrimps and fish (Nagelkerken et al., 2008). Various studies report that, about 50% of the fish harvest is associated with the mangrove ecosystem (Nanjo et al., 2014; Sandilyan et al., 2012). However, mangrove ecosystems are known to be one of the important sources of methane production. Since methane is a potent greenhouse gas with a global warming potential of 34 times more than CO<sub>2</sub>, it is important to control the concentration of methane before its emission into the atmosphere (Yang et al., 2019). About 90% of methane gets oxidised and plays an important role in the global climate change (Cui et al., 2020; Krause et al., 2017). Hence, any activity towards mediating the reduction of greenhouse gas could contribute to the sustainability of the tropical estuarine mangrove ecosystems (Adame et al., 2021).

The outbreak of SARS-CoV-2 as a pandemic on the 11<sup>th</sup> March 2020 (<https://www.who.int/news-room/detail/27-04-2020-who-timeline—covid-19>) led the government of India to impose a lockdown from 24<sup>th</sup> March 2020 for 21 days and again thereafter in phases till 8<sup>th</sup> June 2020 ([https://www.business-standard.com/article/current-affairs/here-s-a-timeline-of-events-since-lockdown-was-imposed-in-india-120070201413\\_1.html](https://www.business-standard.com/article/current-affairs/here-s-a-timeline-of-events-since-lockdown-was-imposed-in-india-120070201413_1.html)). The lockdown brought about a restriction in the movement of people, industrial activities, hotels/restaurants, transportation, schools/ colleges and various other offices. Most importantly there was improvement in the air quality (Bauwens et al., 2020; Dantas et al., 2020; Lokhandwala and Gautam, 2020; Mahato et al., 2020; Muhammad et al., 2020; Shi and Brasseur, 2020; Siciliano et al., 2020; Zangari et al., 2020) and water quality (Agarwal et al., 2020; Link, 2020; Selvam et al., 2020; Yunus et al., 2020; Zambrano-Monserrate et al., 2020). Due to the halt in the level of anthropogenic material input into the coastal waters there has been a significant impact on the coastal water quality of several regions of the world ocean such as the Gulf of Mannar (India), Tangier (Morocco) and Jakarta (Indonesia) (Adwibowo, 2020; Cherif

et al., 2020; Edward et al., 2021; Yunus et al., 2020). Studies conducted by Garg et al. (2020) concluded that the suspended particulate matter and turbidity of the Ganga and Yamuna rivers was considerably lower during the lockdown. Also, Edward et al. (2021) reported that, the turbidity, nutrient concentration and microbial levels had decreased during the lockdown, while the dissolved oxygen, growth of phytoplankton and fish densities increased. Also reduction in Chl a concentration during the pre-monsoon in the Indian waters was attributed to the decline in the anthropogenic nutrient input during the lockdown in April 2020 (Mishra et al., 2020). In addition, remote sensing observations showed that decrease in the quantity of anthropogenic influx into the coastal waters led to an improvement in water quality, during lockdown phases of COVID-19 (Lotliker et al., 2021). Due to the lockdown, the concentration of microplastics in Tuticorin coastal waters was also reported (Edward et al., 2021) to be reduced. It was observed that during the lockdown human stressors on all the beaches had reduced, dune vegetation and the numbers of crab burrows increased. Besides, the beach litter, noise and foul odours reduced due to the lockdown (Soto et al., 2021; Zambrano-Monserrate et al., 2020). At the Goan beaches, there was a significant reduction in beach litter during the lockdown period (Nigam et al., 2022). Karnan et al. (2021) reported that the atmospheric and water quality parameters at the Zuari estuary and Indian coastal region showed significant positive impacts of lockdown. Enforcement of lockdown led to positive effects on water quality of River Damodar, India by showing a decrease in heavy metal index (Chakraborty et al., 2021). Comparison between the pre-lockdown and lockdown periods at Ghogla beach and Somnath beach, India showed a drastic decrease in pathogenic bacterial counts of *Vibrio sp.* from 7733 CFU/mL to 70 CFU/mL (Sahoo et al., 2022). Various studies have reported that the implementation of lockdown had a positive impact on the various ecosystems and associated biodiversity around the globe (Henriques, 2020; Mani, 2020; Stone, 2020). This led to healthier environment in the aquatic ecosystems (Mandal, 2020; Mitra, 2019). Dias et al. (2021) also reported that the COVID-19 imposed lockdown drastically controlled the anthropogenic activities that led to the build-up of coloured dissolved organic matter in the estuarine ecosystem of Goa and had a positive effect on the quality of air over India (Arora et al., 2020). In the coastal state of Goa which is a tourism hub, the lockdown led to a standstill in activities such as the operation of casinos, recreational activities, hospitality industry, industrial and other social activities. The present study highlights the impact that the lockdown brought, in the reduction of anthropogenic inputs which lead to lowering the levels of methane production and oxidation. Here, a tropical estuarine mangrove ecosystem is used as a model site for the study of the impact of lockdown on methane related activities at the upstream and downstream sites of a tropical estuary, in Goa on the west coast of India.

## **2. Material and methods**

### **2.1. Sampling site and sampling**

The sediment sampling was carried out at the downstream (15°30'39" N and 73°51'46" E) and upstream (15°49'42" N and 74°01'09" E) sites located along the tropical estuarine mangrove ecosystem in the Mandovi estuary in Goa, India (Graphical abstract). Mandovi estuary is known to be the lifeline of the state of Goa, as it provides waterways for transportation for the mining industry, tourism, fishing and passengers ferrying across the river. Improper management of the above activities has led to the deterioration of the water quality of the Mandovi estuary (Araujo et al., 2018; Maya et al., 2011). The downstream study site is situated in close proximity (~5.82 Km) to the mouth of the Mandovi estuary and consists of an undisturbed and conserved mangrove forest (2.5 km<sup>2</sup>), the Dr. Salim Ali Bird Sanctuary (Department of Forests, Government of Goa; <http://www.forest.goa.gov.in>). This site has more tidal and anthropogenic influence (Fernandes et al., 2022; Nazareth and Gonsalves, 2022). The second site is located upstream ~28.6 Km from the mouth of the Mandovi estuary. This site is freshwater dominated, influenced by sand mining activities (Sm, 2018) and has a comparatively less mangrove cover as that of the downstream site. The mining industry and ferry services are also in close proximity to this site.

Sediment cores were collected using an acrylic core liner (inner diameter 7.5 cm, 15 cm length) with pre marked depth intervals during the pre-lockdown and lockdown periods. The outer side of the core had holes coinciding with the depths, which were sealed prior to sampling using a water proof tape. On retrieving the core containing sediments, the liners were capped at both ends and the tape was removed to measure the physicochemical parameters like temperature, pH and Eh at site using a multiprobe (Knab et al., 2009; Lopes et al., 2011). After the measurements, the cores were re-sealed, placed in an ice box and transported to the laboratory. Six core liners were collected from each site; three cores were used to measure the physico-chemical and biochemical parameters and 3 cores were used to study the methane activities. All the samples were processed within 24 hrs of sampling.

### **2.2. Physico-chemical and biochemical parameters**

The sediment temperature, pH and Eh was measured using an IQ Scientific Waterproof ISFET pH/ion kit (Cole-Parmer-99361-12). Total sediment proteins were estimated using Lowry's Folin Ciocalteu method (Lowry et al., 1951). Sediment carbohydrates were measured using the phenol sulfuric acid method (Kochert, 1978). Sediment lipids were measured by Bligh and Dyer method (1959). The sum of concentrations of proteins, carbohydrates and lipids was calculated to derive the labile organic matter concentrations (LOM). To calculate the labile organic carbon, LOM was

converted to carbon equivalents using the individual conversion factors and expressed in mg C (Fabiano and Danovaro, 1994; Fichez, 1991). Total organic carbon (TOC) in the sediment was estimated using the wet oxidation method (El-Wakeel and Riley, 1957). Recalcitrant carbon was calculated by subtracting the TOC from the LOC (Sarkar et al., 2021).

### **2.3. Methane activities**

The 3 sediment cores were processed aseptically in the microaerophilic workstation (model: C400M, Ruskinn). The sealed sediment core was subsampled by inserting a 5 mL cut-off syringe into holes coinciding with the depth (Knab et al., 2009; Lopes et al., 2011). For methane production activity, 1 cm<sup>3</sup> of sediment was dispensed into 20 mL gas tight vials containing 9 mL filter sterilized sea water. The vials were immediately sealed using septa and crimped and vortexed. The vials were flushed with nitrogen for 2 min and 1 mL acetylene was used as an inhibitor for methanotrophy. The samples were kept in dark for a period of 4 days, after taking the 0 day readings (Kamaleson et al., 2019; Krause et al., 2015). Methane was quantified using Shimadzu 2010 gas chromatography (GC) (Gonsalves et al., 2011; Kamaleson et al., 2019; Sabu et al., 2022). The procedure for sampling of sediment and dispersing into vials for the methane oxidation activity was same as above. The vials containing the samples were injected with 2-bromoethanesulfonate (BES) which is a methanogenic inhibitor. Vials were incubated in the dark for 4 days after taking the 0 day readings and the methane concentration was measured as above. Heat killed controls were maintained in case of both the activities.

### **2.4. Enumeration of total prokaryotic cells**

The cores were sectioned aseptically as described above. Sub-samples of ~1 g wet weight sediment were diluted with 9 mL of sterile estuarine water. The sediments were vortexed thoroughly and allowed to settle. One mL of sample was transferred to sterile eppendorf tubes and fixed with buffered formalin at a final concentration of 2%. The samples were stored in 4 °C until further processing. During the enumeration of total prokaryotic cells, the stored samples were sonicated at 15 hertz for 15 s. The samples were stained with 50 µL of 0.01% acridine orange for 10 min in the dark and filtered on 0.22 µm black polycarbonate filter paper (Millipore, USA), using a Millipore filtration unit. The cells were enumerated using the epifluorescence microscope (Nikon Eclipse E50i microscope) by the acridine orange direct count (AODC) method as described by Hobbie et al. (1977). Cells were counted from 10 random microscopic fields. The total prokaryotic cells were expressed as cells g<sup>-1</sup> of dry weight of the sediment.

### **2.5. Statistical analysis**

Regression analysis was done to elucidate the relationship between the environmental parameters and methane activities. The t-test was performed using statistical package for social sciences (SPSS) to determine the deviation of values between pre- lockdown and lockdown periods.

### **3. Results & discussion**

This study compares the upstream and the downstream sites of a tropical mangrove ecosystem. It highlights the positive impact of lockdown in the mangrove ecosystem due to reduction in methane related activities (Graphical abstract). The variations in environmental parameters before and after the lockdown are given in table 1. Among the physicochemical parameters, temperature showed significant difference during the pre-lockdown and lockdown ( $P < 0.05$ ) (Table 4). The pH decreased down the core and showed a minor increase during the lockdown period. The sediments during the lockdown were comparatively 2X oxic showing an increase in the redox potential as compared to that in the pre-lockdown (Table 1). During the lockdown, there was a reduction in various anthropogenic activities such as release of industrial effluent into the water bodies and discharge of oil from trawlers during fishing and movement of ferries to and fro. This could have contributed to increase levels of oxygen and reduced the BOD levels in the sediments (Edward et al., 2021; Mitra, 2019). Also, the Chl a values reported during the lockdown were comparatively lower than that reported during the pre-lockdown period (Karnan et al., 2021). This can also impact the turnover of organic matter which will have an effect on the green house gas methane production rates (Bhattacharyya et al., 2023).

In the sediments, at both the sites, during the lockdown, depth wise decrease in the redox values by 13X and 11X respectively could be due to higher bacterial activity that led to reduction in the Eh of the sediments in the deeper depths (Rummasak et al., 2002). While, during the pre-lockdown, the Eh fluctuated along the sediment depths (Table 1). This could be due to the disturbances caused by the influx of anthropogenic activities such as discharge of urban, agricultural, livestock and industrial waste water into the wetlands (Lai, 2004; Zinn et al., 2005). Besides, although tides bring in freshwater into the estuary, a major part of organic matter received into the estuary leads to increased oxygen demand. Hence, organic matter are the key determinants in the oxidation-reduction potential thereby establishing changes in the redox potential in the mangrove ecosystem (Mitsch et al., 2009).

Mangrove sediments are characterised by high concentrations of organic matter ranging from 0.93% to 8.91% (Fernandes et al., 2022; Kamaleson et al., 2019) which is deposited through different sources (Valiela and Cole, 2002). Prior to the lockdown, the tropical mangrove ecosystem was prone to various anthropogenic activities such as fishing, water ways transport via ferry boats, hotel outlets

into the waters and the presence of casinos, shipbuilding/repair yards, sand mining, small/ large scale industries, mine waste rejects and transportation of mineral ores by barges (Araujo et al., 2018; Dias et al., 2021; Shynu et al., 2015). All the above activities contributed to the influx and deposition of organic matter with a concentration of TOC ranging from 1.95 to 6.44 % into the mangrove sediments during the pre-lockdown at the present study locations (Nazareth and Gonsalves, 2022). In the present study, the downstream site which is in close proximity to the mouth of the Mandovi estuary showed significant variation in the labile organic matter ( $P < 0.05$ ) during the lockdown and pre-lockdown with the former having lower values of LOM (Table 4). This may be due to the standstill in the anthropogenic activities (Cotano and Villate, 2006; Liu et al., 2020). Previous studies report that LOM at the same study sites ranged from 0.38 to 6.74 mg g<sup>-1</sup> during the pre-lockdown period (Nazareth and Gonsalves, 2022). Also, in the upstream site, there was no significant changes in the LOM concentrations, however, minimum changes were observed specially in the protein concentrations (Table 2). This may be because of increased light intensity due to clearer waters during the lockdown thereby enhancing the growth of phytoplankton (Edward et al., 2021; Lotlikar et al., 2021). This is corroborated by the higher turbidity values at the downstream site as compared to the upstream site as reported by Fernandes et al. (2018). Recently, in a study along the coastal environment of the anthropogenically impacted Tuticorin, a positive effect of lockdown was observed (Edward et al., 2021). It was reported that a comparison between pre-lockdown and post-lockdown data showed that the water parameters such as turbidity, nutrient concentrations and microbial levels decreased from pre-lockdown to post-lockdown period and parameters such as dissolved oxygen levels, phytoplankton and fish densities had improved. He also reported that fish densities in the reef areas increased from 406 no. 250 m<sup>-2</sup> to 510 no. 250 m<sup>-2</sup>.

The TOC at the downstream site decreased by 1.9X during the lockdown period as compared to the pre-lockdown (Table 2) with an increase in the bacterial abundance from 10<sup>8</sup> g<sup>-1</sup> to 10<sup>9</sup> g<sup>-1</sup> respectively. This can be attributed to the decrease in the supply of anthropogenic inputs (Singaraja et al., 2014). The low organic carbon during the lockdown could be due to sudden standstill of floating casinos, water transport systems, shipbuilding yard and commercial and domestic activities following the government-imposed lockdown (Dias et al., 2021). Other reason could be due to the low productivity at this site and hence low input from autochthonous production (Dias et al., 2021). In the present study, the downstream site had more mangrove cover and is in close proximity to the mouth and has 3.8X higher organic carbon as compared to the upstream site. The downstream site had 2.4X higher TOC during the lockdown as compared to the pre-lockdown, which could be due to significant variations ( $P < 0.001$ ) in the recalcitrant organic matter in the pre-lockdown (Table 4).

Depth wise the maximum TOC was recorded in the bottom sediments (Table 2) during the lockdown. This could be due to the preserved organic carbon which is yet to get decomposed probably due to the minimum exposure to oxic conditions (Chapman et al., 2019; Spivak et al., 2019; Trevathan-Tackett et al., 2017). Organic carbon decomposition is also dependent on microbial enzyme kinetics (Davidson and Janssens, 2006). The maximum total prokaryotic counts in these sediments was recorded during pre-lockdown. At the downstream site, during pre-lockdown the total prokaryotic counts ranged from  $7.5 \times 10^8 \text{ g}^{-1}$  to  $1.63 \times 10^9 \text{ g}^{-1}$  and at the upstream site it ranged from  $1.38 \times 10^9 \text{ g}^{-1}$  to  $1.78 \times 10^9 \text{ g}^{-1}$ . While, at the downstream site during lockdown the total prokaryotic counts ranged from  $1.35 \times 10^8 \text{ g}^{-1}$  to  $1.78 \times 10^8 \text{ g}^{-1}$  and at the upstream site the counts ranged from  $1.23 \times 10^9 \text{ g}^{-1}$  to  $1.48 \times 10^9 \text{ g}^{-1}$ . Microbial groups in the mangrove ecosystem play an important role in decomposition of organic matter (Keuskamp et al., 2015). Moreover, mangroves sediments play an important role in the carbon sequestration, one of the important sources being from the autochthonous organic matter, which represents one-third of the mangrove vegetation net production. Mangrove sediments are known to be potential sources of methane. The sediments in these ecosystems being reducing and the presence of high organic matter favour the production of methane (Prayitno, 2016). Various studies revealed the importance of macromolecules such proteins, carbohydrates and lipids in the production of methane (Gonsalves et al., 2011; Prayitno, 2016). In the present study, methane production at both the sites reduced drastically during the lockdown ( $P < 0.05$ ) (Table 3 & 4). During the lockdown, some environmental variables like the redox potential ( $r = -0.956$ ;  $p < 0.01$ ), LOM ( $r = 0.722$ ;  $p < 0.01$ ), TOC ( $r = 0.674$ ;  $p < 0.05$ ) and methane oxidation activity ( $r = -0.668$ ;  $p < 0.05$ ) had an influence on the production of methane (Table 5). Methane production initiates at the terminal step of organic matter mineralisation where all the terminal electron acceptors have been utilised. Also, as the consumption of oxygen increases due to the rapid organic matter degradation, the redox potential also decreases (Kumar and Ramanathan, 2014). Methanogens are strictly anaerobes and are sensitive to the presence of oxygen (Jing et al., 2016) and hence methane production occurs at an Eh of  $< 150 \text{ mV}$  (Dutta et al., 2017). In the present study, the Eh ranged from 1.84 to 100.44 thus making methane production favourable (Table 1). Three important factors that control the production of methane include the presence of microbes, environmental conditions and the presence of organic matter. The different forms of organic matter that influence the methane formation are total organic carbon, carbon/ nitrogen ratio and macromolecules (Prayitno, 2016). However, during the lockdown there was a decrease in total organic carbon which could have attributed to lower methane concentrations during the period (Kumar and Ramanathan, 2014). Also, studies report that since the organic matter plays a significant role in the production of



methane, the manipulation of the former can be used to reduce the methane production thus controlling the emission into the atmosphere. In the present study during the lockdown period there has been a decline in such anthropogenic activities thus controlling the production of methane. Another important parameter that controls the escape of methane into the atmosphere is the presence of methanotrophs that act as biofilters/sink for methane. In the present study, methane oxidation was negatively correlated to methane production thus indicating the consumption of methane by the methanotrophs thus aiding in mediating the emission of methane into the atmosphere (Jiang et al., 2010; Strong et al., 2017). Methane oxidation can serve as an important sink for methane (Dutta et al., 2017). Studies in temperate regions reported an average methane turnover time of 65 days in the coastal sediments (Osudar et al., 2015), while, in tropical Sundarbans report an average turnover time of 3.77 days. The turnover of methane is also dependent on environmental variables like salinity (negative effect), temperature (positive effect), suspended particulate matter, oxygen and nutrients like nitrite and nitrate which enhanced the oxidation of methane (Osudar et al., 2015). In the present study, the downstream site had comparatively higher salinity, lower temperature and higher organic matter than those at the upstream site. However, the turnover rates of methane could be faster at the downstream site than the upstream site due to higher methane oxidation rates, thus serving as a better sink for methane than the upstream site. Also, during the pre-lockdown period, the production was strongly influenced by parameters (Table 5) like temperature ( $r=0.847$ ;  $p<0.05$ ) and lipids ( $r=-0.847$ ;  $p<0.05$ ). In the present study, the temperatures were within the optimum range as was also reported by Torres-Alvarado et al. (2013) in a tropical mangrove ecosystem where the methanogenic process is optimum at temperatures ranging from 26 to 30°C. Also various studies observed that there was an increase in the rate of methane production and methanogens with an increase in seasonal sediment temperatures in the mangrove ecosystem (Lyimo et al., 2009; Zeikus and Winfrey, 1976). In addition, organic matter inputs in the form of terrestrial influx, waste disposal from the fishing and standing casinos, domestic waste and other sources could have been deposited in these mangrove ecosystem during the pre-lockdown periods, leading to reducing conditions and availability of substrates thus providing conducive environment for the production of methane (Araujo et al., 2018; Dias et al., 2021).

Methane that is produced in the deeper sediments is aerobically or anaerobically oxidised in sediments by methanotrophs before it escapes into the atmosphere (Abril et al., 2007; Hinrichs et al., 2000; Meulepas et al., 2010). About 90% of methane is known to be oxidised by the methanotrophs (Dutta et al., 2017; Kankaala et al., 2006). In the present study, during the lockdown, oxidation of methane was negatively influenced by the production of methane ( $r=-0.668$ ;  $p<0.05$ ) indicating the

utilisation of methane by methanotrophs (Table 5). Besides methane, lipid concentration is another factor that influenced methane oxidation ( $p < 0.05$ ) (Table 5) during the pre-lockdown. The methane oxidation rates were 16X higher (Table 3) during the pre-lockdown as compared to the lockdown period suggesting the presence of higher substrate (methane) and labile organic matter during the former period. It has also been reported (Kravchenko et al., 2005) that, the supply of methane to the methanotrophs is regulated by the organic matter that restricts the diffusion of methane. Besides, methanogenesis utilises organic matter for the production of methane which is made available to the methanotrophs, thereby influencing the methanotrophic activity (Steinkamp et al., 2001b). This study provides insights on management policies for lowering of the anthropogenic inputs that can lead to a more sustainable ecosystem.

#### **4. Conclusion**

The tropical mangrove ecosystem of Goa along the west coast of India, has been a lifeline to various occupations for many decades. It accommodates various activities such as fishing, transportation using the water ways (public transport and mining activities), floating casinos, presence of hotels on river banks and shipyards. All the above activities have been a source of organic matter and other anthropogenic inputs to the mangrove sediments which had enhanced the production of methane during the pre-lockdown period. Methane is a greenhouse gas and as a matter of concern, it is important to control the emission of methane into the atmosphere. The present study highlights lower methane oxidation rates and lower methane production rates during the lockdown as compared to that in the pre-lockdown period which was more evident in the downstream site which has more anthropogenic influence. This will also have an influence on the methane turnover time. The lockdown period has provided the environment to self heal which is visibly observed in the present study where there has been significant decrease in the organic matter concentrations and methane production rates. This positive scenario has considerably enhanced the recovery of the mangrove sediments which will in turn improve the ecosystem. This study provides insights that the controlled anthropogenic inputs into an ecosystem can lead to lower levels of greenhouse gas methane and better sustainability of the mangrove ecosystem.

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Table 1 Down-core variation in the physicochemical parameters at downstream and upstream sites in a tropical estuary during the pre-lockdown and lockdown period.

Pre-lockdown				Lockdown		
Downstream						
Depth (cm)	Temp (°C)	pH	Eh (mV)	Temp (°C)	pH	Eh (mV)
0-1	27.60±0.00	6.92±0.02	5.40±0.75	29.0±0.00	7.67±0.60	100.44±76.42
3-4	27.90±0.01	7.12±0.02	12.20±1.01	29.0±0.00	7.09±0.41	58.22±8.79
6-8	27.90±0.02	6.68±0.01	20.50±1.35	28.0±0.00	6.81±0.39	70.11±2.19
10-12	28.10±0.01	6.47±0.01	35.90±2.97	28.0±0.00	6.76±0.62	27.00±0.33
14-15	28.30±0.01	6.41±0.02	37.90±6.06	27.0±0.00	6.76±0.10	7.66±0.37
Upstream						
0-1	30.20±0.00	6.97±0.02	8.00±4.00	30.0±0.00	7.26±0.18	21.00±11.06
3-4	30.90±0.00	6.92±0.13	2.43±1.75	30.0±0.00	7.05±0.13	16.20±6.94
6-8	29.90±0.00	6.89±0.02	36.00±1.10	28.0±0.00	6.78±0.16	9.15±3.81
10-12	29.80±0.01	6.51±0.02	0.50±2.00	28.0±0.00	6.78±0.04	6.32±2.00
14-15	28.40±0.00	6.47±0.02	- 85.00±1.93	28.0±0.00	6.75±0.06	1.84±0.58

Table 2 Down-core variation in the biochemical parameters at downstream and upstream sites in a tropical estuary during the pre-lockdown and lockdown period.

Depth (cm)	Pre-lockdown							Lockdown						
	PRT (mg g <sup>-1</sup> )	LIP (mg g <sup>-1</sup> )	CHO (mg g <sup>-1</sup> )	LOM (mg g <sup>-1</sup> )	LOC (%)	TOC (%)	Recalcitrant carbon (%)	PRT (mg g <sup>-1</sup> )	LIP (mg g <sup>-1</sup> )	CHO (mg g <sup>-1</sup> )	LOM (mg g <sup>-1</sup> )	LOC (%)	TOC (%)	Recalcitrant carbon (%)
	Downstream													
0-1	1.52± 0.01	0.03± 0.01	1.29± 0.01	1.57± 1.09	0.18±0 .01	5.08± 0.20	4.9± 0.10	0.58± 0.036	0.06± 0.007	0.25± 0.43	0.910± 0.040	0.43± 0.017	2.61± 0.33	2.18± 0.17
3-4	1.57± 0.01	0.02± 0.00	1.36± 0.01	1.64± 1.13	0.12±0 .01	4.20± 0.72	4.08± 0.36	0.62± 0.020	0.07± 0.006	0.27± 0.35	0.979± 0.029	0.47± 0.011	2.23± 0.14	1.76± 0.07
6-8	1.31± 0.01	0.04± 0.00	1.16± 0.01	1.39± 0.97	0.26±0 .02	4.15± 0.07	3.89± 0.04	0.49± 0.076	0.07± 0.003	0.17± 0.900	0.740± 0.160	0.36± 0.071	1.76± 0.27	1.40± 0.14
10-12	1.68± 0.01	0.04± 0.01	1.33± 0.01	1.69± 1.17	0.22±0 .01	3.46± 0.06	3.24± 0.03	0.56± 0.069	0.07± 0.006	0.20± 0.009	0.845± 0.072	0.41± 0.035	2.37± 0.14	1.96± 0.08
14-15	1.98± 0.01	0.03± 0.01	1.30± 0.02	1.84± 1.27	0.28±0 .01	4.41± 0.30	4.13± 0.15	0.42± 0.044	0.07± 0.003	0.11± 0.180	0.614± 0.057	0.30± 0.027	2.24± 0.22	1.94± 0.12
	Upstream													
0-1	0.27± 0.00	0.03± 0.00	1.20± 0.00	1.15± 1.13	0.39±0 .12	1.94± 0.19	1.55± 0.15	1.03± 0.06	0.06± 0.009	0.33± 0.020	1.43± 0.087	0.64± 0.04	2.18± 0.10	1.54± 0.07
3-4	0.42± 0.01	0.04± 0.00	1.92± 0.00	1.35± 0.97	0.33±0 .10	1.21± 0.06	0.88± 0.08	0.76± 0.16	0.07± 0.005	0.26± 0.042	1.10± 0.120	0.50± 0.050	2.69± 0.46	2.19± 0.25
6-8	1.30± 0.01	0.05± 0.00	1.81± 0.00	1.50± 0.78	0.22±0 .10	0.93± 0.17	0.71± 0.13	0.72± 0.07	0.06± 0.001	0.48± 0.058	1.28± 0.081	0.56± 0.030	2.67± 0.45	2.11± 0.24
10-12	1.36± 0.00	0.04± 0.00	0.54± 0.01	1.18± 0.92	0.48±0 .08	1.21± 0.04	0.73± 0.06	1.08± 0.10	0.05± 0.006	0.52± 0.103	1.66± 0.156	0.73± 0.064	3.03± 0.62	2.30± 0.34
14-15	1.31± 0.01	0.05± 0.00	1.66± 0.01	1.43± 0.73	0.13±0 .17	0.23±0. 05	0.10±0.0 1	1.24± 0.14	0.07± 0.002	0.55± 0.129	1.86± 0.254	0.83± 0.100	2.58± 0.42	1.75± 0.26

\*PRT-proteins; CHO-carbohydrates; LIP-lipids; LOM-labile organic matter; LOC-labile organic carbon; TOC-total organic carbon

Table 3 Down-core variation in methane activities at downstream and upstream sites in a tropical estuary during the pre-lockdown and lockdown period.

Pre-lockdown			Post-lockdown	
Downstream				
Depth (cm)	MOA (nM g <sup>-1</sup> d <sup>-1</sup> )	MP (nM g <sup>-1</sup> d <sup>-1</sup> )	MOA (nM g <sup>-1</sup> d <sup>-1</sup> )	MP (nM g <sup>-1</sup> d <sup>-1</sup> )
0-1	95.38±5.24	3.88±1.96	18.47±1.19	0.09±0.01
3-4	84.04±8.29	5.90±0.09	16.99±3.58	0.575±0.23
6-8	1.59±0.024	8.83±0.38	9.96±2.96	0.223±0.12
10-12	14.09±0.14	10.20±0.33	17.67±2.33	0.003±0.001
14-15	77.01±17.95	25.20±2.34	10.70±2.77	0.020±0.001
Upstream				
0-1	2.83±0.56	0.21±0.001	0.45±0.14	0.08±0.001
3-4	9.28±1.70	0.10±0.05	0.96±0.023	0.01±0.002
6-8	18.49±0.83	7.1±1.49	0.0056±0.004	1.83±0.250
10-12	0.18±0.06	1.37±0.63	0.49±0.13	1.80±0.890
14-15	1.726±0.03	0.81±0.73	0.31±0.19	2.48±0.170

\*MOA-methane oxidation activity; MP-methane production

Table 4 t-Test for deviation in the environmental variables and methane activities during the pre-lockdown and lockdown period at downstream and upstream sites in a tropical estuary.

Environmental variables	t Stat	P(T<=t) one-tail	P(T<=t) two-tail
<b>Physicochemical</b>			
Temperature	<b>-2.91</b>	<b>4.00x10<sup>-3</sup></b>	<b>9.20x10<sup>-3</sup></b>
pH	-0.75	0.23	0.46
Eh	-1.43	0.08	0.16
<b>Biochemical</b>			
PRT	<b>2.74</b>	<b>6.00x10<sup>-3</sup></b>	<b>0.01</b>
CHO	<b>7.88</b>	<b>1.52x10<sup>-7</sup></b>	<b>3.04x10<sup>-7</sup></b>
LIP	<b>-7.47</b>	<b>3.17x10<sup>-7</sup></b>	<b>6.34x10<sup>-7</sup></b>
LOM	<b>2.26</b>	<b>1.79x10<sup>-2</sup></b>	<b>0.03</b>
LOC	<b>-4.08</b>	<b>3.46x10<sup>-4</sup></b>	<b>6.92x10<sup>-4</sup></b>
TOC	0.43	0.33	0.66
Recalcitrant organic matter	<b>5.79</b>	<b>5.7x10<sup>-4</sup></b>	<b>1.1x10<sup>-3</sup></b>
<b>Methane activities</b>			
MOA	1.83	0.04	0.08
MP	<b>2.34</b>	<b>0.01</b>	<b>0.03</b>

\*PRT-proteins; CHO-carbohydrates; LIP-lipids; LOM-labile organic matter; LOC-labile organic carbon; TOC-total organic carbon; MOA-methane oxidation activity; MP-methane production; P-level of significance; Numbers in bold indicate significant values; Df- degree of freedom=18.

Table 5 Regression analysis showing the influence of the environmental variables on methane activities during the pre-lockdown and lockdown at downstream and upstream sites in a tropical estuary.

Environmental variables	SS	F	Significance F	P	R <sup>2</sup>
Pre-lockdown					
MOA x Temperature	0.859	3.69	0.090	8.85x10 <sup>-15</sup>	0.315
MOA x PRT	0.726	3.17	0.112	7.05x10 <sup>-4</sup>	0.284
MOA x LIP	0.0003	7.77	0.023	5.46x10 <sup>-7</sup>	0.492
MOA x LOM	0.204	7.15	0.028	5.00x10 <sup>-8</sup>	0.472
MOA x LOC	0.025	2.27	0.170	1.20x10 <sup>-4</sup>	0.221
MOA x TOC	15.05	9.47	0.015	1.20x10 <sup>-2</sup>	0.542
MPx Temperature	1.780	15.15	0.004	7.46x10 <sup>-16</sup>	0.654
MP x pH	184.68	4.46	0.067	0.05	0.358
MP x Eh	166.31	3.81	0.086	0.01	0.322
MP x PRT	246.42	7.32	0.026	0.02	0.478
MP x LOM	315.65	12.64	0.007	0.01	0.612
MP x TOC	156.68	3.49	0.098	0.02	0.303
Lockdown					
MOA x PRT	300.97	8.64	0.018	3.32x10 <sup>-3</sup>	0.519
MOA x CHO	259.26	6.47	0.034	4.56x10 <sup>-3</sup>	0.447
MOA x LOM	305.26	8.90	0.017	3.23x10 <sup>-3</sup>	0.526
MOA x LOC	278.46	7.40	0.026	5.68x10 <sup>-3</sup>	0.480
MOA x MP	182.74	3.68	0.091	4.75x10 <sup>-3</sup>	0.315
MP xEh	1.79	2.27	0.169	2.12x10 <sup>-2</sup>	0.221
MP x CHO	6.81	43.23	0.0001	6.80x10 <sup>-3</sup>	0.843
MP x LOM	5.14	14.00	0.005	4.64x10 <sup>-2</sup>	0.636
MP x LOC	5.05	13.34	0.006	4.10x10 <sup>-2</sup>	0.625
MP x TOC	1.51	3.43	0.10	0.01	0.329
MP x MOA	2.20	3.00	0.121	2.11x10 <sup>-2</sup>	0.272

\*PRT-proteins; CHO-carbohydrates; LIP-lipids; LOM-labile organic matter; LOC-labile organic carbon; TOC-total organic carbon; MOA-methane oxidation activity; MP-methane production; SS-sum of squares; F-statistical significance; P-level of significance; R<sup>2</sup>-coefficient of determination; Df-degree of freedom=1.

\* Statistically significant values are shown in the table.