

Effect of sediment composition on methane concentration and production in the transition zone of a mangrove (Sepetiba Bay, Rio de Janeiro, Brazil)

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Abstract

The aim of this research was to evaluate the effect of sediment composition on methane (CH₄) dynamics in sediments of different areas in the transition zone between a mangrove and the sea. This research was conducted in a mangrove at Coroa Grande, on the southern coast of Rio de Janeiro. Samples were collected at three stations: (1) region colonised by *Rhizophora mangle* L. on the edge of the mangrove, (2) region colonised by seagrasses and (3) infra-littoral region without vegetation. Samples were collected from the surface layer of the sediment to determine the concentrations of nutrients (C, N and P) and CH₄ concentration and production. We observed that concentrations of CH₄ and carbon (C) were significantly higher ($p < 0.05$) in station 1 than station 3. The molar ratios (C:N, C:P and N:P) suggest that the origin of the substrate is mainly autochthonous. Methanogenesis was initially low, possibly due to competition between methanogens and sulfate reducers, and increased significantly ($p < 0.05$) on the twenty-sixth day in the sediment of station 1, probably due to higher organic matter (OM) availability in this region. Results indicate that methanogenic activity observed herein is not regulated by the amount or quality of OM, but by other factors. The concentration of CH₄ in the sea-land ecotone at Mangrove Coroa Grande is a function of available OM suggesting a possible inhibition of methanotrophy by intense oxygen consumption in the soil surface covered by detritus of *Rhizophora mangle* vegetation.

Keywords: ecotone, methanogens, OM, macrophytes, Rhizophora.

Efeito da composição do sedimento sobre a concentração e a produção do metano na zona de transição de um mangue tropical, na Baía de Sepetiba-RJ, Brasil

Resumo

O objetivo desta pesquisa foi avaliar a dinâmica do metano (CH₄) no sedimento em diferentes áreas na região de transição entre a borda do manguezal e o mar, em função da composição do sedimento. A pesquisa foi realizada no Manguezal de Coroa Grande, no litoral sul do Estado do Rio de Janeiro. Foram coletadas amostras em três estações: (1) região colonizada por *Rhizophora mangle* L. na borda do mangue, (2) região colonizada por macrófitas, e (3) região sem vegetação, permanentemente inundada pelo mar. Foram coletadas amostras da fração superficial do sedimento para determinação da concentração de nutrientes (C, N e P), e da concentração e da produção de CH₄. Foi observado que as concentrações de CH₄ e carbono (C) foram significativamente maiores ($p < 0,05$) na estação 1, em relação à estação 3. Com relação às razões molares (C:N, C:P e N:P), estas indicam que a origem da composição do substrato é principalmente autóctone. Quanto à metanogênese, foram observados, inicialmente, baixos valores de CH₄ produzido, indicando provável competição entre metanogênicos e sulfatorredutores. Seguiu-se, então, um significativo aumento ($p < 0,05$) da metanogênese no 26º dia, no sedimento da estação 1, provavelmente em função da maior disponibilidade de matéria orgânica (MO) nessa região. Os resultados mostram que a atividade metanogênica observada pela presente pesquisa não é regulada pela quantidade e a qualidade da MO e sim por outros fatores. A concentração de CH₄ no ecótono mar-terra no manguezal de Coroa Grande, ocorre em função da MO disponível, sugerindo possível inibição da metanotrofia, em razão de intenso consumo de oxigênio na superfície do solo coberto pelo detrito da vegetação de *Rhizophora mangle*.

Palavras-chave: ecótono, metanogênicos, MO, macrófitas, Rhizophoras.

1. Introduction

Methane is considered one of the most important greenhouse effect gases due to its high global warming potential compared to CO₂ (IPCC, 2001). Methane presents capacity of infrared radiation absorption 21 times higher than CO₂ per mass unit (El-Fadel and Massoud, 2001). According to the IPCC (2007), the concentration of atmospheric methane increased from 715 ppb in the pre-industrial period to 1732 ppb in the early 1990, and 1774 ppb in 2005.

Methanogenesis is a microbiological degradation of organic compounds into methane, controlled by the absence of oxygen and substrate availability (Segers, 1998). This nutrient cycling is performed by strict anaerobe organisms that belong to the *Archea* domain, known as methanogens (Mohanraju et al., 1997), which metabolise simple organic substrates. Most of them use only one or two types of substrates (Ferry, 1993), with two main substrates: (1) carbon dioxide with molecular hydrogen, (2) formate and (3) acetate (Mohanraju et al., 1997).

Several factors may influence methanogenesis, such as salinity, temperature, pH, substrate availability and microbial interaction with other anaerobic metabolic groups, such as iron (Fe³⁺) reducing bacteria, acetogenic and sulfate reducing bacteria. Methanogens may, for example, compete for substrate with sulfate-reducers in the presence of sulfate (Ferry, 1993), that in coastal environments tend to have higher concentrations due to the proximity to the sea. In these ecosystems, sulfate-reduction may be favoured in detriment of methanogenesis, because it is more energetically advantageous in relation to substrates common to both types of microorganisms (Lyimo et al., 2002a). However, these processes may coexist in presence or absence of non-competitive substrates, which are organic osmoregulators that tend to occur as an adaptative response to environments with high salinity (Ferry, 1993). Substrate availability may also be high enough to maintain both processes (Purvaja et al., 2004). This is common in environments altered by anthropogenic activities, which have increased capacity of methane production by having higher amounts of organic matter (OM), increasing bacterial metabolism in the sediment (Strangmann et al., 2008). According to Schubert et al. (2010) methane produced in anaerobic sediments of the ecosystem can be oxidised in the aerobic zone by methanotrophic bacteria, or even in anaerobiosis by *Archea* methanogens, often related to sulfate reduction (Meulepas et al., 2010; Hinrichs et al., 2000). Thus, the relation between production and consumption of methane allows a system to have an intensive production without great concentration, and hence, with low emission of this gas to the atmosphere (Giani et al., 1996).

Mangroves are coastal ecosystems which occur in the areas of transition between marine and terrestrial environments subjected to tidal variation (Schaeffer-Novelli, 1995). They are characterised by low flora diversity, with species that are adapted to the salinity fluctuation common to mangrove forests (Tomlinson, 1994), and by high

production and accumulation of OM. This makes mangroves highly important in the maintenance of food chains that support intense microbial activity in sediment, formed by autochthonous or allochthonous fine-grained material, water and salts (Schaeffer-Novelli, 1995). Due to this composition, the sediment is generally organic, not very consistent and with little or no oxygen (Schaeffer-Novelli et al., 2002), making fermentation the main process of OM decomposition (Krupadam et al., 2007), which provides production of hydrogen sulfide (Tomlinson, 1994), nitrous oxide and methane (Kreuzwieser et al., 2003; Allen et al., 2007; Krithika et al., 2008; Purvaja et al., 2004; Krupadam et al., 2007). Although the aspect of the mangrove forest soil seems quite homogeneous, areas with different vegetation covers have different compositions (Lacerda et al., 1995). According to Nguyen et al. (2010), sediments rich in labile organic matter tend to have higher methane production than refractory substrates formed by OM. According to the same author, sediments that have C:N ratio <10 have a higher potential for methane formation when compared to those with C:N ratio >10.

In the present research, conducted in a mangrove forest located in southeast Brazil, we hypothesised that the methanogenic activity in sediment of the mangrove transition zone with the sea is controlled by the amount of organic matter in sediment, depending on the concentration of nutrients and its quality, through the molar ratios between nutrients. To test this hypothesis, we: (1) determined C, N and P and their the molar ratios C:N, C:P and N:P in sediments colonised by different types of vegetation and in sediments without vegetation in the mangrove-sea transition area, (2) evaluated the dynamics of methane in the sediment in these same regions, through the determination of its concentration and production.

2. Methodology

2.1. Study area

This study was conducted in Coroa Grande mangrove, Itaguaí district, located along the northern coast of Sepetiba Bay, about 100 km from the city of Rio de Janeiro, southeastern Brazil. Sepetiba Bay has an area of 447 km² with an average depth of about 6.0 m. The tides vary between 0.6 m and 2.6 m.

The border area of the mangrove forest is mainly colonised by *Rhizophora mangle* L., a few individuals of *Laguncularia racemosa* Gaertn. and *Avicennia schaueriana* Saptf and Leech. The inner part of the mangrove is colonised by *Laguncularia racemosa* and *Avicennia schaueriana* only (Coimbra, 2003), and the area above the edge of the mangrove is colonised by seagrasses. The sediment is characterised by fractions of sand in the region without vegetation (an area of transition from sea to mangrove) and silty-clay in the area of mangrove forest, with the highest percentage of organic matter in the latter (Coimbra, 2003).

The number of inhabitants of Sepetiba Bay has increased greatly in recent years due to industrial growth in the

region over the last decades, which has been reported as the main cause of deterioration of the bay water quality (Molisani et al., 2004). This large demographic expansion raises environmental pressures such as high inputs of domestic sewage and of agriculture effluents, sand extraction and others, affecting the bay's ecosystem, including mangroves (Coimbra, 2003).

2.2. Sampling

Sampling was carried out in May 2008, during low tide through a 50 m transect perpendicular to the sea. Along the transect, three sampling stations were established: (1) edge of the area colonised by *Rhizophora mangle* mangrove, (2) area colonised by seagrasses, and (3) infra-littoral sediment (see Figure 1). Samples were collected from the surface layer of sediment (3 cm) to determine the concentration of nutrients, i.e. carbon (C), nitrogen (N) and phosphorus (P) and CH₄, and determination of methanogenic activity.

2.3. Concentration of methane in the sediment

In the field, 5 mL of sediment were placed in 25 mL glass vials (n = 3), containing 5 mL of concentrated sodium chloride solution to expel methane contained in the sediment. The vials were sealed and transported to the laboratory, where 1 mL of the headspace of the bottle was removed with a syringe to determine methane concentration by gas chromatography. Methane was quantified on a Varian Star 3400 (Varian Co., USA) gas chromatograph equipped with a FID detector (200 °C and injector at 120 °C) and a Poropak-Q 1 m (60/100 mesh) column (65 °C) with N₂ gas as carrier gas.

2.4. Methanogenesis

In the laboratory, 5 g of sediment were incubated in 25 mL glass vials (n = 3) with 5 mL of water of the mangrove, previously filtered through 0.2 µm membrane filters. The vials were closed and sealed, and N₂ was flowed for 2 minutes to establish an anoxic environment and expel the methane present in the vial. The concentrations of CH₄ in the vial headspace were monitored regularly

according to the methodology described above, until CH₄ concentrations stabilised.

2.5. Concentration of nutrients in the sediment

For determination of C, N and P in sediment samples were dried at 50 °C until constant weight. After maceration, the total nitrogen content was determined according to Allen et al. (1974) and total phosphorus according to Fassbender (1973). For total carbon determination, we used the solid unit of a 5000 TOC Analyzer (Shimadzu Co., Japan).

2.6. Statistical analysis

The results of methane concentration in the sediment and nutrients, as well as methanogenesis, were analysed using the nonparametric Kruskal-Wallis and Dunn's post-test. We used a significance level of 95%. Tests were performed using GraphPad InStat 3.0 (GraphPad Software Co.).

3. Results and Discussion

The highest concentration of methane in the sediment was found at station 1 and it was significantly higher ($p < 0.05$) than at station 3 (see Figure 2a). Results obtained in other degraded mangroves have shown high concentrations of methane compared to non-degraded mangroves (see Table 1). However, although the Coroa Grande mangrove ecosystem is impacted by the uncontrolled urban expansion that Sepetiba Bay has suffered, the values of methane concentration found were low compared to other environments altered by human activities. The difference between the concentration of methane found in this study and those of other studies in mangroves can be attributed to the sampling site, which was restricted to the transition area from the sea to the mangrove forest, contrary to most studies that consider the interior mangrove, where there is less leaching of litter and soil oxygenation by tides and higher values of carbon, nitrogen and phosphorus (Sherman et al., 1998). The substrate of the inner area of the mangrove forest is usually composed of large amounts

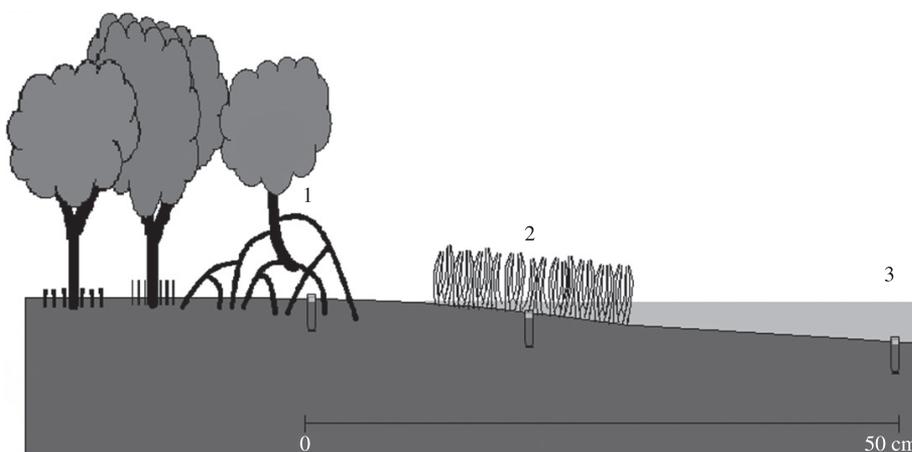


Figure 1. Scheme of sampling area along the transect in Coroa Grande mangrove. Sampling stations: Within mangrove vegetation (1), area colonised by macrophytes (2) and bare soil (3).

Table 1. Concentration and production of methane in sediment of mangroves differentes.

Site	Comment	Fraction (cm)	Concentration (μM)	Production		Reference	
				$\text{nmol.g}^{-1}\text{FW.day}^{-1}$	$\text{nmol.g}^{-1}\text{DW.day}^{-1}$		
Mangrove Coroa Grande, Brazil	Sewage effluent	0-3	0.049-6.11	1.8-2.1	5.4-10.4	2.7-3.12	Present study
Mangrove Mandovi-Zuari system, India		0-4		± 100			Gonsalves et al. (2011)
Mangrove La Paz, Baja California Sur, México	Preserved	0-7	2.83			$1.1 \times 10^3 - 1.6 \times 10^3$	Strangmann et al. (2008)
Mangrove La Paz, Baja California Sur, México	Aquaculture waste	0-5	28.88			$48 \times 10^3 - 378 \times 10^3$	Strangmann et al. (2008)
Mangrove La Paz, Baja California Sur, México	Sewage effluent	0-3	22.27			$13 \times 10^3 - 127 \times 10^3$	Strangmann et al. (2008)
Lothian Island, Sundarban mangrove, India		0-30	0.16-5.77				Biswas et al. (2007)
Mtoni Manguove, Tanzania	Deforested	0-2		12			Lyimo et al. (2002b)
Island of Hainan, China		20-30			0.63		Lu et al. (1999)
Mangrove Ensenada La Paz, Baja California Sur, Mexico	Sewage effluent	0-10	0.48				Giani et al. (1996)
Mangrove Balandra, Baja California Sur, Mexico	Deforested	0-10	33			98×10^3	Giani et al. (1996)
Mangrove Balandra, Baja California Sur, Mexico	Preserved	0-10	<0.2-2.09			1.83×10^3	Giani et al. (1996)
Mangrove La Parguera, Porto Rico	Sewage effluent	0-20	$\pm 8.3 \text{ a} \pm 75$				Sotomayor et al. (1994)

of silt and clay that act as filters retaining OM (>5% of particulate organic carbon), while the border area has little OM (<3% particulate organic carbon) and higher sand content (Kristensen, 2007). Bernini et al. (2006) analysed the texture of mangrove sediments at the Rio São Mateus estuary, Espírito Santo, Brazil, finding the highest OM% in sediments with smallest particle size, while the more sandy sediment had lower levels of nutrients. Sotomayor et al. (1994) studied the mangrove forest of La Parguera, Puerto Rico, and found low methane concentrations in its border area. Lu et al. (1999) also found lower values of methane production at the sediment surface area at the edge of a mangrove forest in Hainan Island, China. This result was attributed to the more recent origin of the superficial part of sediment from the border area, which suffers more tidal influence than the transition area and the inside of the mangrove. Another relevant question regards the sampled sediment portion: while our study used the 0-3 cm layer of the sediment, many of the studies presented in Table 1 were performed in the 0-30 cm layer of sediment. As the concentration of methane in sediment is directly related to the competition between methanogens and sulfate reducers and denitrifiers, methane concentrations tend to increase with sediment depth.

The origin of C in mangrove ecosystems varies according to tidal cycles, at high tide there is a greater contribution of marine material, while at low tide the autochthonous C prevails, thus influencing the quality of the MO present in the sediment surface (Lacerda et al., 1988). According to Lacerda et al. (1988), from the $\delta^{13}\text{C}$ analysis of the material in suspension and different components of the Coroa Grande mangrove, it was observed that mangrove leaves are the main source of sediment C and suspended matter of mangrove. In this study, the C concentration showed the same pattern obtained for the methane concentration with significant difference ($p < 0.05$) between stations 1 and 3 (see Figure 2b). The values of N (see Figure 2c) and P (see Figure 2d) concentrations were not significantly different between seasons ($p > 0.05$). Regarding the molar ratios higher values of C:N, C:P and N:P were observed in a season. Confirming the results found by Lacerda et al. (1988), the contents of C, N and P indicate a greater predominance of autochthonous material in the composition of Coroa Grande mangrove substrate, characteristic of the period of low tide. Most mangrove sediments show C:N ratio >10, indicating that this sediment is composed mostly of autochthonous material (Kristensen et al., 2008), however, when by human interference these ecosystems receive additional nutrients such as sewage and chemical fertilizers used

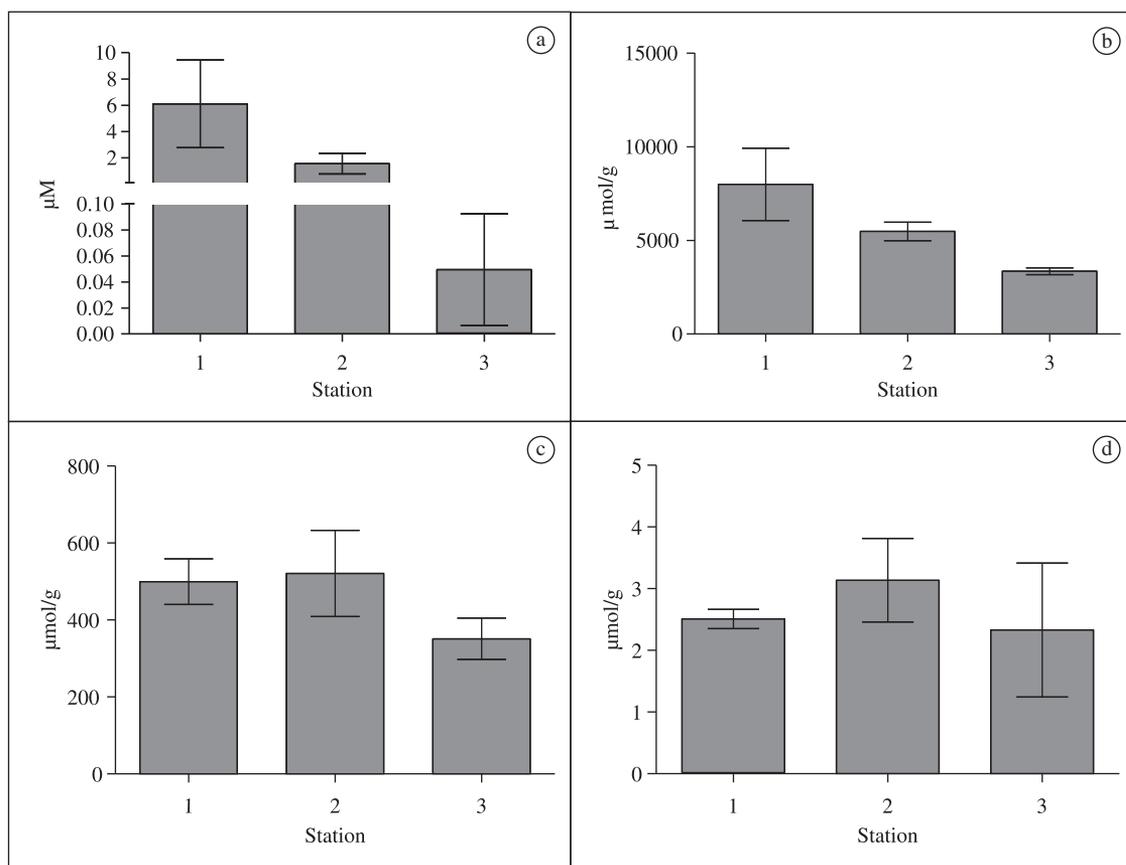


Figure 2. Concentration of Methane (a), Carbon (b), Nitrogen (c) and Phosphorus (d) in the sediment at three sampling stations in Coroa Grande mangrove.

in agriculture, the availability of nutrients rises and may exacerbate the emission of methane (Purvaja and Ramesh, 2001). Although according to the literature, substrates rich in N and P have a tendency to have higher methanogenic activity, in the present study we observed that the highest methane concentrations occurred at stations that had the greatest C:N and C:P (see Table 2), suggesting that the large amount of organic carbon provided by mangrove vegetation has provided plenty of material available for methanogenesis, with OM quantity becoming more important than its quality to maintain the process. This higher C concentration in the sediment of station 1 (see Figure 2b) reflects the contribution of biomass that tends to be relatively higher near the ground and lower toward the sea (Komiya et al., 2008), making the substrate a limiting factor for methanogenesis due to the low OM availability (Sotomayor et al., 1994).

The key factors for methanogenesis are the absence of oxygen and OM availability, so variations in these factors depending on the different sites may have contributed to differences in results between the stations. As a consequence of the large supply of OM in a station, depending on the values of C, this area may have a reduced oxygen concentration in the subsurface due to intense aerobic decomposition of OM, creating conditions for methanogenesis, and inhibiting the oxidation of methane by methanotrophic bacteria. But the availability of OM in station 1 was not significantly different ($p > 0.05$) between sampling stations, as can be seen in the early days in the curve of methane production (see Figure 3), likely due to competition between methanogens and sulfate reducers. The probable

cause for the different values of methane concentration between stations would be a greater distance from station 1 over the sea, which provides less frequent floods, and through evaporation, increases the concentration of salt in the substrate (Bouillon et al., 2007). This leads to a lower solubility of gases in the pore water (Minello, 2004), such as oxygen, and therefore decreases methanotrophy. Moreover, this distance decreases the uptake of sulfate reducing anaerobic oxidation of methane through the consortium with sulfate reducing organisms, contributing to the highest concentration of methane found at this station.

Sulfate and salinity have negative effect on methanogenesis (Lu et al., 1999), so environments that have a strong marine influence, such as mangroves, are not considered major sources of methane because of the high concentration of salt and sulfate from sea water (Purvaja and Ramesh, 2001). Despite being higher than the values found for stations 2 and 3, methane concentration at station 1 is not considered high. Low concentrations of methane were also observed in a study conducted in an anthropogenically disturbed mangrove at La Paz, Mexico, indicating that methanogenesis is balanced by the processes of oxidation of methane in the sediment (Giani et al., 1996). In a pristine mangrove at Bengal Bay, India, the maximum methane concentration of the pore water ($5.769 \mu\text{M}$) was low, suggesting intense competition between oxidation and methanogenic and sulfate-reducing bacteria and nitrate (Biswas et al., 2007).

Though the initial values of methanogenesis were not significantly different ($p > 0.05$), there was a greater production of methane in sediments from stations 1 and 2 (see Figure 3). The salinity in this case does not appear to interfere with the results, since the incubation of all the sediments was done with water collected from the same point, equaling salinities. Considering the presence of sulfate ions, the values of methane produced during the incubation period of sediment at station 1 indicates a possible competition for substrate in the early days between methanogenic and sulfate reducing organisms. In the early days of incubation the interaction between organisms seems to favour sulfate reduction. As sulfate-reducing bacteria are more competitive than the methanogens for substrates that are common to both organisms, they maintain low concentrations of these substrates for methanogens (Lyimo et al., 2002a). However, after the second week, there was a probable decline in the amount of sulfate available that provided a significant increase at methanogenesis ($p < 0.05$) observed in the twenty-sixth day of the experiment (see Figure 3), especially in relation to station 1, probably due to increased availability of OM. Experiments developed by Lyimo et al. (2002a) show that although acetate and

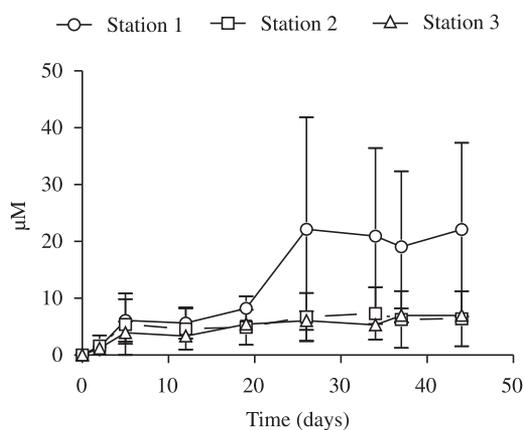


Figure 3. Variation of concentrations of methane in the headspace of vials during the incubation period to determine potential methane production in sediment Coroa Grande mangrove.

Table 2. Molar ratios C:N, C:P and N:P in the sediment at sampling stations of Mangrove Coroa Grande.

Station	Feature	C:N	C:P	N:P
1	Presence of mangrove vegetation	16	3185	199
2	Colonised by aquatic macrophytes	10.5	1747	166
3	Bare soil and and permanently flooded	9.6	1439	151

formate have stimulated methanogenesis and sulfate reduction, only some of these substrates were converted into methane. However, inhibition of bacterial sulfate reduction resulted in complete conversion of substrates to methane. Mohanraju et al. (1997) reported that methane produced in the mangrove sediments is probably derived from methylated amines, compounds that are not used by sulfate reducing bacteria.

4. Conclusion

This study provided results regarding the production and concentration of methane in a coastal ecotone in southeastern Brazil. The research hypothesis was rejected, because the quantity and quality of OM were not the main factors in increasing the activity of methanogens in the sediment sampling stations. The results indicate a gradient of methane dynamics in the sampling stations, where other factors may regulate methanogenesis. This study concludes that the concentration of methane in the transition area from sea to land in the Coroa Grande mangrove is a function of the amount of C available in the various sampling sites, determined mainly by the presence of vegetation, indicating a possible negative effect on methanotrophy. Facing the progressive unregulated population growth in the region of the Sepetiba Bay that contributes to a continuous increase of OM in this ecosystem by the disposal of raw sewage into water bodies bordering mangroves, the methanogenesis regulating factors tend to be less expressive.

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