#### Greenhouse gas fluxes in mangrove forest soil in an Amazon estuary 1

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- 11 Abstract: Tropical mangrove forests are important carbon sinks, the soil being the main
- 12 carbon reservoir. Understanding the variability and the key factors that control fluxes is
- 13 critical to accounting for greenhouse gas (GHG) emissions, particularly in the current
- 14 scenario of global climate change. This study is the first to quantify methane (CH<sub>4</sub>) and
- 15 carbon dioxide (CO<sub>2</sub>) emissions using a dynamic chamber in a natural mangrove soil of
- 16 the Amazon. The plots for the trace gases study were allocated at contrasting
- 17 topographic heights. The results showed that the mangrove soil of the Amazon estuary
- is a source of  $CO_2$  (6.66 g  $CO_2$  m<sup>-2</sup> d<sup>-1</sup>) and  $CH_4$  (0.13 g  $CH_4$  m<sup>-2</sup> d<sup>-1</sup>) to the atmosphere. 18
- The CO<sub>2</sub> flux was higher in the high topography (7.858 g CO<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup>) than in the low 19
- topography (4.734 g CO<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup>) in the rainy season, and CH<sub>4</sub> was higher in the low 20
- topography  $(0.128 \text{ g CH}_4 \text{ m}^{-2} \text{ d}^{-1})$  than in the high topography  $(0.014 \text{ g CH}_4 \text{ m}^{-2} \text{ d}^{-1})$  in 21
- 22 the dry season. However, in the dry period, the low topography soil produced more
- 23 CH<sub>4</sub>. Soil organic matter, carbon and nitrogen ratio (C/N), and redox potential
- 24 influenced the annual and seasonal variation of CO<sub>2</sub> emissions; however, they did not
- affect CH<sub>4</sub> flux. The mangrove soil of the Amazon estuary produced 35.4 Mg CO<sub>2-eq</sub> ha 25
- <sup>1</sup> y<sup>-1</sup>. A total of 2.16 kg CO<sub>2</sub> m<sup>-2</sup> y<sup>-1</sup> needs to be sequestered by the mangrove ecosystem 26
- to counterbalance CH<sub>4</sub> emissions. 27

#### Introduction

- 29 The mangrove areas are estimated to be the main contributors to greenhouse gas
- 30 emissions in marine ecosystems (Allen et al., 2011; Chen et al., 2012). However,
- 31 mangrove forests are highly productive due to a high nutrient turnover rate (Robertson
- 32 et al., 1992) and have mechanisms that maximize carbon gain and minimize water loss
- 33 through plant transpiration (Alongi and Mukhopadhyay, 2015). A study conducted in 25
- 34 mangrove forests (between 30° latitude and 73° longitude) revealed that these forests

36 which 49 to 98% is present in the soil (Donato et al., 2011). The estimated soil CO<sub>2</sub> outgassing, in tropical estuarine areas is 16.2 Tg C y<sup>-1</sup> (Alongi, 37 38 2009). However, soil efflux measurements from tropical mangroves revealed emissions ranging from 2.9 to 11.0 g CO<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup> (Castillo et al., 2017; Chen et al., 2014; Shiau 39 40 and Chiu, 2020). In situ CO<sub>2</sub> production is related to the water input of terrestrial, riparian, and groundwater brought by rainfall (Rosentreter et al., 2018b). Due to the 41 42 periodic tidal movement, the mangrove ecosystem is daily flooded, leaving the soil 43 anoxic and consequently reduced, favoring methanogenesis (Dutta et al., 2013). Thus, 44 estuaries are considered hotspots for CH<sub>4</sub> production and emission (Bastviken et al., 45 2011; Borges et al., 2015). The organic material decomposition by methanogenic 46 bacteria in anoxic environments, such as sediments, inner suspended particles, 47 zooplankton gut (Reeburgh, 2007; Valentine, 2011), and the impact of freshwater 48 should change the electron flow from sulfate-reducing bacteria to methanogenesis 49 (Purvaja et al., 2004), which also results in CH<sub>4</sub> formation. On the other hand, an 50 ecosystem with salinity levels above 18 ppt may show an absence of CH<sub>4</sub> issions (Poffenbarger et al., 2011), since me ne dissolved in pores is typically oxidized 51 52 anaerobically by sulfate (Chuang et al., 2016). Currently the uncertainty in emitted CH<sub>4</sub> 53 values in vegetated coastal wetlands is approximately 30% (EPA, 2017). Soil flux 54 measurements from tropical mangroves revealed emissions ranging from 0.3 to 4.4 mg CH<sub>4</sub> m<sup>-2</sup> d<sup>-1</sup> (Castillo et al., 2017; Chen et al., 2014; Kreuzwieser et al., 2003). 55 56 The production of greenhouse gases from soils is mainly driven by biogeochemical 57 processes. Microbial activities and gas production are related to soil properties, 58 including total carbon and nitrogen concentrations, moisture, porosity, salinity, and 59 redox potential (Bouillon et al., 2008; Chen et al., 2012). Due to the dynamics of tidal 60 movements, mangrove soils may become saturated and present a reduced oxygen 61 availability, or suffer total aeration caused by the ebb tide. Studies attribute soil carbon 62 flux responses to moisture perturbations because of seasonality and flooding events 63 (Banerjee et al., 2016), with fluxes being dependent on tidal extremes (high tide and low 64 tide), and flood duration (Chowdhury et al., 2018). In addition, phenolic compounds inhibit microbial activity and help keep organic carbon intact, thus leading to the 65 66 accumulation of organic matter in mangrove forest soils (Friesen et al., 2018).

are the richest in carbon storage in the tropics, containing on average 1,023 Mg C ha<sup>-1</sup> of

The Amazonian coastal areas in the State of Pará (Brazil) cover 2,176.8 km<sup>2</sup> where mangroves develop under the macro-tide regime (Souza Filho, 2005), representing approximately 85% of the entire area of Brazilian mangroves (Herz, 1991). The objective of this study is to investigate the monthly flux of CO<sub>2</sub> and CH<sub>4</sub> from the soil, at two topographic heights, in a pristine mangrove area in the Mojuim River Estuary, belonging to the Amazon biome. The gas fluxes were studied together with the analysis of the vegetation structure and soil physical-chemical parameters.

#### 2 Material and Methods

## **75 2.1 Study site**

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76 This study was conducted in the Amazonian coastal zone, Macaca Island (-0.746491 77 latitude and -47.997219 longitude), located in the Mojuim River estuary, at the 78 Mocapajuba Marine Extractive Reserve, municipality of São Caetano de Odivelas 79 (Figure 1), state of Pará (Brazil). The Macaca island has an area of 1,322 ha of pristine mangroves, which belongs to a mangrove area of 2,177 km<sup>2</sup> in the state of Pará (Souza 80 81 Filho, 2005). The climate is type Am (tropical monsoon) according to the Köppen 82 classification (Peel et al., 2007). The climatological data were obtained from the 83 Meteorological Database for Teaching and Research of the National Institute of 84 Meteorology (INMET). The area has a rainy season from January to June (2,296 mm of 85 precipitation) and a dry season from July to December (687 mm). March and April were 86 the rainiest months with 505 and 453 mm of precipitation, while October and November 87 were the driest (53 and 61 mm, respectively). The minimum temperatures occur in the 88 rainy period (26 °C) and the maximum in the dry period (29 °C). The Mojuim estuary 89 has a macrotidal regime, with an average amplitude of 4.9 m during spring tide and 3.2 90 m during low tide (Rollnic et al., 2018). During the wet season the Mojuim River has a flow velocity of 1.8 m s<sup>-1</sup> at the ebb tide and 1.3 m s<sup>-1</sup> at the flood tide. During the dry 91 season, the maximum currents reach 1.9 m s<sup>-1</sup> at the flood and 1.67 m s<sup>-1</sup> at the ebb tide 92 93 (Rocha, 2015). The annual mean salinity is 26.95 PSU (Valentim et al., 2018).

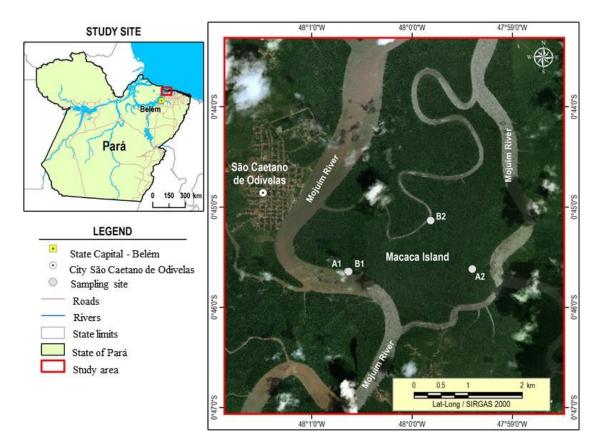


Figure 1. The Macaca Island located in the mangrove coast of Northern Brazil, Municipality of São Caetano de Odivelas (state of Pará), with sampling points at low (plot B1 and plot B2) and high topographies (plot A1 and plot A2). Image Source: © Google Earth

The Mojuim River region is geomorphologically formed by partially submerged river basins consequent of the increase in the relative sea level during the Holocene (Prost et al., 2001) associated with the formation of mangroves, dunes, and beaches (El-Robrini et al., 2006). This river forms the entire watershed of the municipality of São Caetano de Odivelas and borders the municipality of São João da Ponta (Figure 1). Before reaching the estuary, the Mojuim River crosses an area of a dryland forest highly fragmented by family farming, forming remnants of secondary forest (< 5.0 ha) of various ages (Fernandes and Pimentel, 2019). The population economically exploited the estuary, primarily by artisanal fishing, crab (*Ucides cordatus* L.) extraction, and oyster farms.

The flora of the mangrove area of Macaca Island is little anthropized and comprises the plant genera *Rhizophora*, *Avicenia*, *Laguncularia*, and *Acrostichum* (Ferreira, 2017; França et al., 2016). The estuarine plains are influenced by macrotide dynamics and can

be physiographically divided into four sectors (França et al., 2016). The Macaca Island is ranked as being from the fourth sector, which implies having woods of adult trees of the genus *Ryzophora* with an average height of 10 to 25 m, being located at an elevation of 0 to 5 m, and having silt-clay soil (França et al., 2016).

Four sampling plots were selected in the Macaca Island (Figure 1) on 19/05/2017, when the moon was in the waning quarter phase: two plots where flooding occurs every day (plots B1 and B2; Figure 1), called low topography (Top\_Low), and two plots where flooding occurs only at high tides during the solstice and on the high tides of the rainy season of the new and full moons (plots A1 and A2; Figure 1), called high topography (Top\_High).

#### 2.2 Greenhouse gas flux measurements

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In each plot, eight Polyvinyl Chloride rings with 0.20 m diameter and 0.12 m height were randomly installed within a circumference with a diameter of 20 m. The rings had an area of 0.028 m<sup>-2</sup> (volume of 3.47 L), were fixed 0.05 m into the ground, and remained in place until the study was completed. Once a month, the Greenhouse gas flux was measured during periods of waning or crescent moon, as these are the times when the soil in the low topography is more exposed. To avoid the influence of mangrove roots on the gas fluxes, the rings were placed in locations without any seedlings or aboveground mangrove roots. CO<sub>2</sub> and CH<sub>4</sub> concentrations (ppm) were measured using the dynamic chamber methodology (Norman et al., 1997; Verchot et al., 2000), sequentially connected to a Los Gatos Research portable gas analyzer (Mahesh et al., 2015). The device was calibrated monthly with a high quality standard gas (500 ppm CO<sub>2</sub>; 5 ppm CH<sub>4</sub>). The rings were sequentially closed for three minutes with a PVC cap, being connected to the analyzer through two 12.0 m polyethylene hoses. The gas concentration was measured every two seconds and automatically stored by the analyzer. CO<sub>2</sub> and CH<sub>4</sub> fluxes were calculated from the linear regression of increasing/decreasing CO<sub>2</sub> and CH<sub>4</sub> concentrations within the chamber, usually between one and three minutes after the ring cover was placed (Frankignoulle, 1988; McEwing et al., 2015). The flux is considered zero when the linear regression reaches an R<sup>2</sup> < 0.30 (Sundqvist et al., 2014). However, in our analyses, most regressions reached  $R^2$  > 0.70, and the regressions were weak and considered zero in only 6% of the samples. At the end of each flux measurement, the height of the ring above ground was measured at

- 144 four equidistant points with a ruler. The seasonal data were analyzed by comparing the
- average monthly fluxes in the wet season and dry season separately.

# 146 **2.3 Vegetation structure and biomass**

- The floristic survey was conducted in October 2017 using circular 1,256.6 m<sup>2</sup> plots
- 148 (Kauffman et al., 2013) divided into four 314.15 m<sup>2</sup> subplots, which is the equivalent to
- 0.38 ha (Figure 1), at the same topographies as the gas flux analysis. We recorded the
- diameter above the aerial roots, the diameter of the stem, and total height of all trees
- with DBH (diameter at breast height; m) greater than 0.05m. The allometric equations
- 152 (Howard et al., 2014) to calculate tree biomass (aboveground biomass; AGB) were:
- AGB =  $0.1282 * DBH^{2.6}$  (R<sup>2</sup> = 0.92) for *R. mangle*; AGB =  $0.140 * DBH^{2.4}$  (R<sup>2</sup> = 0.97)
- for A. germinans; and Total AGB =  $0.168 * \rho * DBH^{2.47}$  (R<sup>2</sup> = 0.99), where  $\rho_{R. mangle}$  =
- 155 0.87;  $\rho_{A. \text{ germinans}} = 0.72$  ( $\rho = \text{wood density}$ ).

# 156 **2.4** Soil sampling and environmental characterization

- Four soil samples were collected with an auger at a depth of 0.10 m in all the studied
- plots for gas flux measurements in July 2017 (beginning of the dry season) and January
- 159 2018 (beginning of the rainy season; Figure 1). Before the soil samples were removed,
- pH and redox potential (Eh; mV) were measured with a Metrohm 744 equipment by
- inserting the platinum probe directly into the intact soil at a depth of 0.10 m (Bauza et
- al., 2002). The soil samples collected in the field were transported to the laboratory
- 163 (Chemical Analysis Laboratory of the Museu Paraense Emílio Goeldi) in thermal boxes
- 164 containing ice. The soil samples were analyzed on the day after collection at the
- laboratory, and the samples were kept in a freezer. Salinity (Sal; ppt) was measured with
- PCE-0100, and soil moisture (Sm; %) by the residual gravimetric method (EMBRAPA,
- 167 1997).
- Organic Matter (OM; g kg<sup>-1</sup>), Total Carbon (T<sub>C</sub>; g kg<sup>-1</sup>) and Total Nitrogen (T<sub>N</sub>; g kg<sup>-1</sup>)
- were calculated by volumetry (oxidoreduction) using the Walkley-Black method
- 170 (Kalembasa and Jenkinson, 1973). Microbial carbon (C<sub>mic</sub>; mg kg<sup>-1</sup>) and microbial
- nitrogen (N<sub>mic</sub>; mg kg<sup>-1</sup>) were determined through the 2.0 min of Irradiation-extraction
- method of soil by microwave technique (Islam and Weil, 1998). Microwave heated soil
- extraction proved to be a simple, fast, accurate, reliable, and safe method to measure
- soil microbial biomass (Araujo, 2010; Ferreira et al., 1999; Monz et al., 1991). The C<sub>mic</sub>
- was determined by dichromate oxidation (Kalembasa and Jenkinson, 1973; Vance et al.,

- 176 1987). The N<sub>mic</sub> was analyzed following the method described by Brookes et al. (1985),
- changing fumigation to irradiation, which uses the difference between the amount of T<sub>N</sub>
- in irradiated and non-irradiated soil. We used the flux conversion factor of 0.33
- 179 (Sparling and West, 1988) and 0.54 (Almeida et al., 2019; Brookes et al., 1985), for
- carbon and nitrogen, respectively. Particle size analysis was performed separately on
- four soil samples collected at each flux plot, in the two seasons (October 2017 and
- March 2018), according to EMBRAPA (1997).
- At each gas flux measurement, environmental variables such as air temperature (T<sub>air</sub>,
- 184 °C), relative humidity (RH, %), and wind speed (W<sub>s</sub>, m s<sup>-1</sup>) were quantified with a
- portable thermo-hygrometer (model AK821) at the height of 2.0 m above the soil
- surface. Soil temperature (T<sub>s</sub>, °C) was measured with a portable digital thermometer
- 187 (model TP101) after each gas flux measurement. Daily precipitation was obtained from
- an automatic precipitation station installed at a pier on the banks of the Mojuim River in
- 189 São Caetano das Odivelas (coordinates: -0.738333 latitude; -48.013056 longitude).

#### 2.5 Statistical analyses

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- On the Macaca Island, two treatments were allocated (low and high topography), with
- two plots in either. In each plot, eight chambers were randomly distributed, which were
- 193 considered sample repetitions. The normality of the data of FCH<sub>4</sub>, FCO<sub>2</sub>, and soil
- 194 physicochemical parameters was evaluated using the Shapiro-Wilks method. The soil
- 105 CO<sub>2</sub> and CH<sub>4</sub> flux showed a non-normal distribution. Therefore, we used the non-
- parametric ANOVA (Kruskal-Wallis, p < 0.05) to test the differences between the two
- 197 treatments among months and seasons. The physicochemical parameters were normally
- distributed. Therefore, a parametric ANOVA was used to test the statistical differences
- (p < 0.05) between the two treatments among months and seasons. Pearson correlation
- 200 coefficients were calculated to determine the relationships between soil properties and
- 201 gas fluxes in the months (dry and wet season) when the chemical properties of the soil
- were analyzed at the same time as gas fluxes were measured. Statistical analyses were
- 203 performed with the free statistical software Infostat 2015®.

#### 3 Results

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#### 3.1 Carbon dioxide and methane fluxes

- $CO_2$  fluxes differed significantly between topographies only in January (H = 3.915; p =
- 207 0.048), July (H = 9.091; p = 0.003), and November (H = 11.294; p < 0.000) (Figure 2;

Supplementary Information, SI 1), with generally higher fluxes at the high topography than at the low topography. At the high topography,  $CO_2$  fluxes were significantly higher (H = 24.510; p = 0.011) in July compared to August and December, March, October, and May, not differing from the other months of the year. Similarly, at the low topography,  $CO_2$  fluxes were statistically higher (H = 19.912; p = 0.046) in September and February than in January and November, not differing from the other months. We found a mean monthly flux of  $327.9 \pm 78.0$  mg  $CO_2$  m<sup>-2</sup> h<sup>-1</sup> (mean  $\pm$  standard error) and  $217.2 \pm 51.0$  mg  $CO_2$  m<sup>-2</sup> h<sup>-1</sup> at the high and low topographies, respectively.

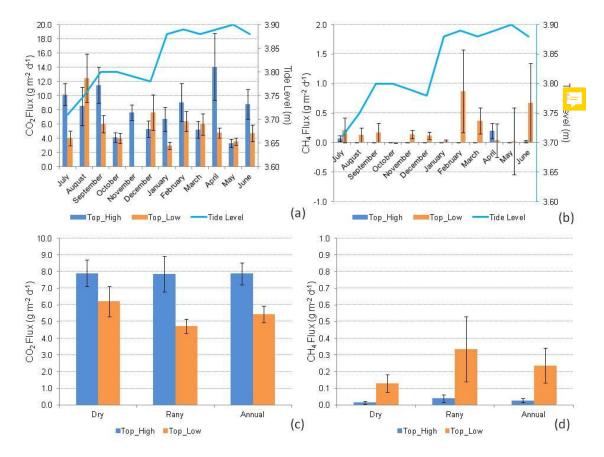


Figure 2. CO<sub>2</sub> (a) and CH<sub>4</sub> (b) fluxes (g CO<sub>2</sub> or CH<sub>4</sub> m<sup>-2</sup> d<sup>-1</sup>) monthly (July 2018 to June 2019) (n = 16). Seasonal (Dry and Rainy) and annual fluxes of CO<sub>2</sub> (c) and CH<sub>4</sub> (d), at high (Top\_High) and low (Top\_Low) topographies (n = 96), in a mangrove forest soil compared to tide level (Tide Level). The bars represent the standard error of the mean.

The CH<sub>4</sub> fluxes were statistically different between topographies only in November (H = 9.276; p = 0.002) and December (H = 4.945; p = 0.005), with higher fluxes at the low topography (Figure 2; SI 1). At the high topography, CH<sub>4</sub> fluxes were significantly (H = 40.073; p < 0.001) higher in April and July compared to the other months studied, and in November CH<sub>4</sub> was consumed from the atmosphere (Figure 2; SI 1). Similarly, CH<sub>4</sub>

- fluxes at the low topography did not vary significantly among months (H = 10.114; p =
- 227 0.407).

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- 228 Greenhouse gas fluxes (Figure 2) were only significantly different between
- 229 topographies in the dry season (Figure 3), period when CO<sub>2</sub> fluxes were higher (H =
- 7.378; p = 0.006) at the high topography and CH<sub>4</sub> fluxes at the low topography (H =
- 8.229; p < 0.001). In the Macaca Island, the mean annual fluxes of  $CO_2$  and  $CH_4$  were
- 232  $6.659 \pm 0.419 \text{ g CO}_2 \text{ m}^{-2} \text{ d}^{-1} \text{ and } 0.132 \pm 0.053 \text{ g CH}_4 \text{ m}^{-2} \text{ d}^{-1}, \text{ respectively.}$

#### 3.2 Weather data

- There was a marked seasonality during the study period (Figure 2), with 2,155.0 mm of
- precipitation during the rainy period and 1,016.5 mm during the dry period. The highest
- 236 tides occurred in the period of greater precipitation (Figure 3) due to the rains. However,
- 237 the rainfall distribution was different from the climatological normal (Figure 3). The
- precipitation in the rainy season was 553.2 mm below and in the dry season was 589.1
- 239 mm above the climatological normal. Thus, in the period studied, the dry season was
- 240) rainier and the rainy season drier than the climatological normal, which may be a
- consequence of global climate change.

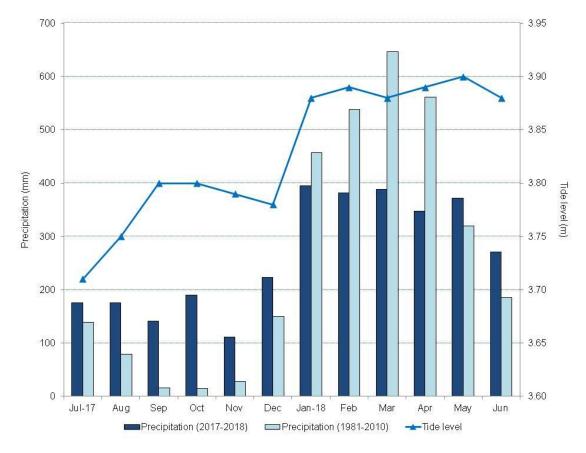


Figure 3. Monthly climatological normal in the municipality of Soure (1981-2010, mm), monthly precipitation (mm), and maximum tide height (m) from 2017 to 2018, in the municipality of São Caetano de Odivelas (PA).

 $T_{air}$  was significantly higher (LSD = 0.72, p = 0.01) at the high (31.24  $\pm$  0.26 °C) than at the low topography (30.30  $\pm$  0.25 °C) only in the rainy season (Figure 4a). No significant variation in  $T_s$  was found between topographies in either season (Figure 4b). RH was significantly higher (LSD = 2.55, p = 0.01) at the high topography (70.54  $\pm$  0.97%) than at the low topography (66.85  $\pm$  0.87%) only in the rainy season (Figure 4c).  $W_s$  (Figure 4d) was significantly higher (LSD = 0.15, p < 0.00) at the low (0.54  $\pm$  0.06 m s<sup>-1</sup>) than at the high topography (0.24  $\pm$  0.04 m s<sup>-1</sup>) also in the rainy season.

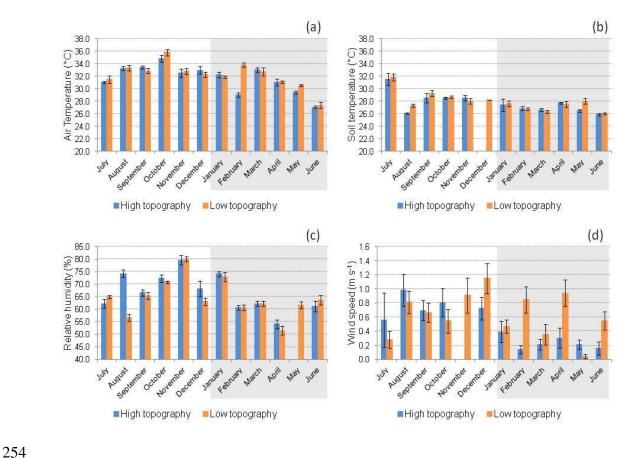


Figure 4. a) Air temperature (°C), b) soil temperature (°C), c) relative humidity (%), and d) wind speed (m s<sup>-1</sup>) at high and low topographies, from July 2017 to June 2018 in a mangrove area in the Mojuim River estuary. Bars highlighted in grey correspond to the rainy season (n = 16). The bars represent the standard error.

#### 3.3 Soil characteristics

Silt concentration was higher at the low topography (LSD: 14.763; p=0.007) and clay concentration was higher at the high topography plots (LSD: 12.463; p=0.005), in both seasons studied (Table 1). Soil particle size analysis did not differ statistically (p>0.05) between the two seasons (Table 1). Soil moisture did not vary significantly (p>0.05) between topographies at each season, or between seasonal periods at the same topography (Table 1). The pH varied statistically (LSD: 5.950; p=0.006) only at the low topography when the two seasons were compared, being more acidic in the dry period (Table 1). The pH values were significantly (LSD: 0.559; p=0.008) higher in the dry season (Table 1). No variation in Eh was identified between topographies and seasons (Table 1), although it was higher in the dry season than in the rainy season. However, Sal values were higher (LSD: 3.444; p=0.010) at the high topography than at

- the low topography in the dry season (Table 1). In addition, Sal was significantly higher
- in the dry season than in the rainy season, in both high (LSD: 2.916; p < 0.001) and low
- 273 (LSD: 3.003; p < 0.001) topographies (Table 1). (Table 1).

Table 1. Analysis of Sand (%), Silt (%), Clay (%), Moisture (%), pH, Redox Potential (Eh, mV) and salinity (Sal; ppt) in the mangrove soil of high and low topographies, and in the rainy and dry seasons (Macaca island, São Caetano das Odivelas). Numbers represent the mean  $\pm$  standard error of the mean. Lower case letters compare topographies in each seasonal period and upper-case letters compare the same topography between seasonal periods. Different letters indicate statistical difference (LSD, p < 0.05).

	7T 1	Sand	Silt	Clay	Moisture	TT	Eh	Sal
Season	Topography	(%)	(%)	(%)	(%)	pН	(mV)	(ppt)
	High	12.1±1.4 <sup>aA</sup>	41.8±3.3 <sup>bA</sup>	46.1±2.6 <sup>aA</sup>	73.1±6.6 <sup>aA</sup>	5.5±0.2 <sup>aA</sup>	190.25±45.53 <sup>aA</sup>	35.25±1.11 <sup>aA</sup>
Dry	Low	$9.7{\pm}2.5^{\mathrm{aA}}$	63.6±6.1 <sup>aA</sup>	$26.6 \pm 5.2^{bA}$	86.9±3.4 <sup>aA</sup>	5.3±0.3 <sup>aA</sup> 106.38±53.76 <sup>aA</sup>		$30.13\pm1.16^{bA}$
	Mean	10.9±1.4 <sup>A</sup>	52.7±4.4 <sup>A</sup>	36.4±3.8 <sup>A</sup>	80.0±4.0 <sup>A</sup>	5.4±0.2 <sup>A</sup>	148.31±35.71 <sup>A</sup>	32.69±1.02 <sup>A</sup>
Rainy	High	12.1±1.4 <sup>aA</sup>	41.8±3.3 <sup>bA</sup>	46.1±2.6 <sup>aA</sup>	88.9±3.5 <sup>aA</sup>	$4.9{\pm}0.4^{aA}$	92.50±56.20 <sup>aA</sup>	7.50±0.78 <sup>aB</sup>
	Low	$9.7{\pm}2.5^{\mathrm{aA}}$	63.6±6.1 <sup>aA</sup>	$26.6\pm5.2^{bA}$	88.6±3.7 <sup>aA</sup>	$4.4\pm0.1^{aB}$	36.25±49.97 <sup>aA</sup>	$8.13\pm0.79^{aB}$
	Mean	10.9±1.4 <sup>A</sup>	52.7±4.4 <sup>A</sup>	36.4±3.8 <sup>A</sup>	88.7±2.5 <sup>A</sup>	$4.6\pm0.2^{B}$	64.38±37.04 <sup>A</sup>	7.81±0.54 <sup>B</sup>

279 The C<sub>mic</sub> did not differ between topographies in the two seasons (Table 2). However, T<sub>C</sub> 280 was significantly higher in the low topography in the dry season (LSD: 5.589; p < 281 0.000) and in the rainy season (LSD: 5.777; p = 0.024). In addition,  $C_{mic}$  was higher in 282 the dry season in both the high (LSD: 11.325; p < 0.010) and low (LSD: 9.345; p < 283 0.000) topographies (Table 2). N<sub>mic</sub> did not vary between topographies seasonally. 284 However,  $N_{mic}$  in the high (LSD: 9.059; p = 0.013) and low topographies (LSD: 4.447; 285 p = 0.001) was higher during the dry season (Table 2). The C/N ratio (Table 2) was 286 higher in the low than in the high topography in both the dry (LSD: 3.142; p < 0.000) 287 and rainy seasons (LSD: 3.675; p = 0.033). However, only in the low topography was 288 the C/N ratio higher (LSD: 1.863; p < 0.000) in the dry season than in the rainy season 289 (Table 2). Soil OM was higher at the low topography in the rainy (LSD: 9.950; p = 290 0.024) and in the dry seasons (LSD: 9.630; p < 0.000). However, only in the lowland 291 topography was the OM concentration higher in the dry season than in the rainy season 292 (Table 2).

Table 2. Seasonal and topographic variation in microbial Carbon ( $C_{mic}$ ; mg kg<sup>-1</sup>), microbial Nitrogen ( $N_{mic}$ , mg kg<sup>-1</sup>), Total Carbon ( $T_C$ ; g kg<sup>-1</sup>), Total Nitrogen ( $N_T$ ; g kg<sup>-1</sup>), Carbon/Nitrogen ratio (C/N) and Soil Organic Matter (OM; g kg<sup>-1</sup>). Numbers represent the mean ( $\pm$ standard error). Lower case letters compare topographies at each season, and upper-case letters compare the topography between seasons.

Season	Topography	$C_{mic}$	$N_{\mathrm{mic}}$	$T_{\rm C}$	$T_{N}$	C/N	OM	
		mg kg <sup>-1</sup>	mg kg <sup>-1</sup>	g kg <sup>-1</sup>	g kg <sup>-1</sup>	C/N	g kg <sup>-1</sup>	
	High	22.12±5.22 <sup>aA</sup>	$12.76\pm4.20^{aA}$	14.12±2.23 <sup>bA</sup>	$1.43\pm0.06^{aA}$	$9.60\pm1.20^{bA}$	24.35±3.84 <sup>bA</sup>	
Dry	Low	26.34±4.23 <sup>aA</sup>	±4.23 <sup>aA</sup> 10.34±2.05 <sup>aA</sup> 2		$26.44{\pm}1.35^{aA} \qquad 1.56{\pm}0.04^{aA}$		45.59±2.32 <sup>aA</sup>	
	Mean	24.23±3.29 A	11.55±2.28 <sup>A</sup>	20.28 ±2.03 <sup>A</sup>	1.49±0.04 <sup>A</sup>	13.29±1.19 <sup>A</sup>	34.97±3.50 A	
	High	$7.40\pm0.79^{aB}$	0.75±0.41 <sup>aB</sup>	11.46±2.48 <sup>bA</sup>	1.32±0.04 <sup>aA</sup>	8.42±1.70 <sup>bA</sup>	19.75±4.27 <sup>bA</sup>	
Rainy	Low	$5.95\pm1.06^{aB}$	$1.23\pm0.28^{aB}$	$18.27 \pm 1.06^{aB}$	$1.46\pm0.06^{aA}$	$12.47\pm0.22^{aB}$	$31.51\pm1.83^{aB}$	
	Mean	6.68±0.67 <sup>B</sup>	0.99±0.25 <sup>B</sup>	14.86 ±1.57 <sup>B</sup>	1.39±0.04 A	10.44±0.98 <sup>A</sup>	25.63±2.71 <sup>B</sup>	

# 3.4 Vegetation structure and biomass

Only the species R. mangle and A. germinans were found in the floristic survey carried out. The DBH did not vary significantly between the topographies for either species (Table 3). However, R. mangle had a higher DBH than A. germinaris at both high (LSD: 139.304; p = 0.037) and low topographies (LSD: 131.307; p = 0.001). The basal area (BA) and AGB did not show significant variation (Table 3). A total aboveground biomass of  $322.1 \pm 49.6$  Mg ha<sup>-1</sup> was estimated.

G :	T 1	N ha <sup>-1</sup>	DBH	BA	AGB	
Specie	Topography		(cm)	$(m^2 ha^{-1})$	(Mg ha <sup>-1</sup> )	
Rhizophora	High	302.4±20.5	238.8±24.9 <sup>aA</sup>	$17.3\pm2.0^{aA}$	219.3±25.7 <sup>aA</sup>	
mangle	Low	310.4±37.6	283.5±45.0 <sup>aA</sup>	24.2±4.3 <sup>aA</sup>	338.7±62.9 <sup>aA</sup>	
Avicennia	High	47.7±20.5	86.8±51.2 <sup>aB</sup>	13.8±9.2 <sup>aA</sup>	135.3±94.7 <sup>aA</sup>	
germinans	Low	15.9±9.2	$46.1\pm29.3^{aB}$	11.8±8.8 <sup>aA</sup>	136.0±108.3 <sup>aA</sup>	
Total	High	350.2±18.4	325.6±33.6°	31.1±7.5 <sup>a</sup>	304.5±99.8 <sup>a</sup>	
10141	Low	346.2±41.0	296.0±23.7 <sup>a</sup>	30.0±4.1 <sup>a</sup>	330.8±60.4 <sup>a</sup>	

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The equations for biomass estimates (AGB) were:  $R. mangle = 0.1282*DBH^{2.6}$ ;  $A. germinans = 0.14*DBH^{2.4}$ ; and Total =  $0.168*\rho*DBH^{2.47}$ , where  $\rho_{R. mangle} = 0.87$ ;  $\rho_{A. germinans} = 0.72$  (Howard et al., 2014).

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# 3.5 Drivers of greenhouse gas fluxes

In the rainy season,  $CO_2$  efflux was correlated with  $T_{air}$  (Pearson = 0.23, p = 0.03), RH (Pearson = -0.32, p < 0.00) and  $T_s$  (Pearson = 0.21, p = 0.04) only at the low topography. In the dry season  $CO_2$  flux was correlated with  $T_s$  (Pearson = 0.39, p < 0.00) at the low topography. The dry season was the period in which we found the greatest amount of significant correlations between CO2 efflux and soil chemical parameters, while the C:N ratio, OM, and Eh were correlated with CO<sub>2</sub> efflux in both seasons (Table 4). The negative correlation between T<sub>C</sub>, N<sub>T</sub>, C/N, and OM, along with the positive correlation of N<sub>mic</sub> with soil CO<sub>2</sub> flux, in the dry period, indicates that microbial activity is a decisive factor for CO<sub>2</sub> efflux (Table 4). Soil moisture in the Mojuim River mangrove forest negatively influenced CO<sub>2</sub> flux in both seasons (Table 4). However, soil moisture was not correlated with CH<sub>4</sub> flux. No significant correlations were found between CH<sub>4</sub> efflux and the chemical properties of the soil in the mangrove of the Mojuim River estuary (Table 4). However, more detailed studies on CH<sub>4</sub> efflux and on its relationship with methanotrophic bacteria and abiotic factors (mainly ammonia and sulfate) are needed due to the average flux of 4.70 mg C m<sup>-2</sup> h<sup>-1</sup> and the extreme monthly and seasonal variations.

Table 4. Correlation coefficient (Pearson) of CO<sub>2</sub> and CH<sub>4</sub> fluxes with chemical parameters of the soil in a mangrove area in the Mojuim River estuary.

Gas Flux	Season	$T_{\rm C}$	$T_{N}$	C <sub>mic</sub>	N <sub>mic</sub>	C/NI	OM	Sal	Eh	"II	Moisture
$(g m^{-2} d^{-1})$		$(g kg^{-1})$	$(g kg^{-1})$	(mg kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )	C/N	$(g kg^{-1})$	(ppt)	(mV)	pН	(%)
-	Dry	-0.68**	-0.59 <sup>*</sup>	0.18 <sup>NS</sup>	0.61**	-0.66**	-0.67**	-0.07 <sup>NS</sup>	0.51*	0.21 <sup>NS</sup>	-0.49*
$CO_2$	Rainy	-0.44 <sup>NS</sup>	-0.20 <sup>NS</sup>	-0.15 <sup>NS</sup>	$-0.32^{NS}$	-0.50*	-0.63**	-0.54*	0.53*	$0.47^{NS}$	-0.54*
	Annual	-0.50**	-0.35*	-0.18 <sup>NS</sup>	$0.00^{NS}$	-0.53**	-0.48**	-0.30 <sup>NS</sup>	0.39*	0.23 <sup>NS</sup>	-0.56**
CH <sub>4</sub>	Dry	$0.30^{NS}$	0.07 <sup>NS</sup>	-0.14 <sup>NS</sup>	-0.24 <sup>NS</sup>	0.34 <sup>NS</sup>	0.02 <sup>NS</sup>	-0.04 <sup>NS</sup>	-0.38 <sup>NS</sup>	0.26 <sup>NS</sup>	0.26 <sup>NS</sup>
	Rainy	$0.05^{NS}$	$-0.09^{NS}$	$0.44^{NS}$	-0.27 <sup>NS</sup>	$0.09^{NS}$	-0.11 <sup>NS</sup>	-0.04 <sup>NS</sup>	-0.13 <sup>NS</sup>	-0.07 <sup>NS</sup>	$0.04^{NS}$
	Annual	$0.04^{NS}$	-0.10 <sup>NS</sup>	-0.01 <sup>NS</sup>	-0.18 <sup>NS</sup>	$0.08^{\mathrm{NS}}$	-0.01 <sup>NS</sup>	-0.17 <sup>NS</sup>	-0.21 <sup>NS</sup>	-0.08 <sup>NS</sup>	$0.02^{NS}$

Total Carbon (T<sub>C</sub>; g kg<sup>-1</sup>); Total Nitrogen (T<sub>N</sub>; g kg<sup>-1</sup>); Microbial Carbon (Cmic, g kg<sup>-1</sup>); Microbial Nitrogen (N<sub>mic</sub>, g kg<sup>-1</sup>); Carbon and Nitrogen ratio (C/N); Organic Matter (OM; g kg<sup>-1</sup>); Salinity (Sal; ppt); Redox Potential (Eh; mV); Soil Moisture (Moisture, %).

NS= not significant; \* significant effects at p  $\leq$  0.05; \*\* significant effects at p  $\leq$  0.01

#### 4 Discussion

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#### 4.1 Carbon dioxide and methane flux

337 It is important to consider that the year under study was rainier in the dry season (2017) 338 and less rainy in the wet season (2018) when the climatological average is concerned 339 (1981-2010) (Figure 3). Perhaps this variation is related to the effects of global climate 340 changes. Under these conditions, negative and positive flows of the two greenhouse gases were found (negative values represent gas consumption) Under these conditions, 341 the CO<sub>2</sub> flux from the mangrove soil ranged from -5.06 to 68.96 g CO<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup> (mean 342  $6.66 \text{ g CO}_2 \text{ m}^{-2} \text{ d}^{-1}$ ), while the CH<sub>4</sub> flux ranged from -5.07 to 11.08 g CH<sub>4</sub> m<sup>-2</sup> d<sup>-1</sup> (mean 343 0.13 g CH<sub>4</sub> m<sup>-2</sup> d<sup>-1</sup>), resulting in a total carbon rate of 1.92 g C m<sup>-2</sup> d<sup>-1</sup> or 7.00 Mg C ha<sup>-1</sup> 344 345 y<sup>-1</sup> (Figure 2). The negative CO<sub>2</sub> flux is apparently a consequence of the increased CO<sub>2</sub> 346 solubility in tidal waters or of the increased sulfate reduction, as described in the 347 literature (Borges et al., 2018; Chowdhury et al., 2018; Nóbrega et al., 2016). 348 Fluctuations in redox potential altered the availability of the terminal electron acceptor 349 and donor, and the forces of recovery of their concentrations in the soil, such that a 350 disproportionate release of CO<sub>2</sub> can result from the alternative anaerobic degradation 351 processes such as sulfate and iron reduction (Chowdhury et al., 2018). The soil carbon 352 flux in the mangrove area in the Amazon region was within the range of findings for other tropical mangrove areas (2.57 to 11.00 g CO<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup>; Shiau and Chiu, 2020). 353 However, the mean flux of 6.2 mmol CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup> recorded in this Amazonian mangrove 354 was much higher than the mean efflux of 2.9 mmol CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup> recorded in 75 355 356 mangroves during low tide periods (Alongi, 2009). An emission of 0.010 Tg CH<sub>4</sub> y<sup>-1</sup>, 0.64 g CH<sub>4</sub> m<sup>-2</sup> d<sup>-1</sup> (Rosentreter et al., 2018a), or 26.7 357 mg CH<sub>4</sub> m<sup>-2</sup> h<sup>-1</sup> has been reported for tropical latitudes (0 and 5°). In our study, the 358 monthly average of CH<sub>4</sub> flux was higher at the low  $(7.3 \pm 8.0 \text{ mg CH}_4 \text{ m}^{-2} \text{ h}^{-1})$  than at the high topography  $(0.9 \pm 0.6 \text{ mg C m}^{-2} \text{ h}^{-1})$ , resulting in 0.13 g CH<sub>4</sub> m<sup>-2</sup> d<sup>-1</sup> or 0.48 Mg 360 CH<sub>4</sub> ha<sup>-1</sup> y<sup>-1</sup> (Figure 2). Therefore, the CH<sub>4</sub>-C fluxes from the mangrove soil in the 361 362 Mojuim River estuary were much lower than expected. It is known that there is a 363 microbial functional module for CH<sub>4</sub> production and consumption (Xu et al., 2015) and 364 diffusibility (Sihi et al., 2018), and considers three key mechanisms: aceticlastic 365 methanogenesis (acetate production), hydrogenotrophic methanogenesis (H<sub>2</sub> and CO<sub>2</sub> production), and aerobic methanotrophy (CH<sub>4</sub> oxidation and O<sub>2</sub> reduction). The average 366 emission from the soil of 8.4 mmol CH<sub>4</sub> m<sup>-2</sup> d<sup>-1</sup> was well below the fluxes recorded in 367

the Bay of Bengal, with 18.4 mmol CH<sub>4</sub> m<sup>-2</sup> d<sup>-1</sup> (Biswas et al., 2007). In the Amazonian mangrove studied the mean annual carbon equivalent efflux was 429.6 mg CO<sub>2-eq</sub> m<sup>-2</sup> h<sup>-1</sup>. This value is 0.00004% of the erosion losses of 103.5 Tg CO<sub>2-eq</sub> ha<sup>-1</sup> y<sup>-1</sup> projected for the next century in tropical mangrove forests (Adame et al., 2021). These higher CO<sub>2</sub> flux concomitantly with lower CH<sub>4</sub> flux in this Amazonian estuary are probably a consequence of changes in the rainfall pattern already underway, where the dry season was wetter and the rainy season drier when compared to the climatological normal.

### 4.2 Drivers of greenhouse gas fluxes

Mangrove areas are periodically flooded, with a larger flood volume during the syzygy tides, especially in the rainy season. The hydrological condition of the soil is determined by the microtopography and can regulate the respiration of microorganisms (aerobic or anaerobic), being a decisive factor in controlling the CO<sub>2</sub> efflux (Dai et al., 2012; Davidson et al., 2000; Ehrenfeld, 1995). In the two climatic periods of the year, the high topography produced more  $CO_2$  (7.869  $\pm$  1.873 g  $CO_2$  m<sup>-2</sup> d<sup>-1</sup>) than the low topography  $(5.212 \pm 1.225 \text{ g CO}_2 \text{ m}^{-2} \text{ d}^{-1})$  (Figure 2; SI 1). No significant influence on CO<sub>2</sub> flux was observed due to the low variation in high tide level throughout the year (0.19 m) (Figure 2), although it was numerically higher at the high topography. However, tidal height and the rainy season resulted in a higher CO<sub>2</sub> flux (rate high/low =1.7) at the high topography (7.858  $\pm$  0.039 g CO<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup>) than at the low topography (4.734  $\pm$  0.335 g CO<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup>) (Figure 2; SI 1). This result is because the root systems of most flood-tolerant plants remain active when flooded (Angelov et al., 1996). Still, the high topography has longer flood-free periods, which only happens when the tides are syzygy or when the rains are torrential. CO<sub>2</sub> efflux was higher in the high topography than in the low topography in the rainy 

season (when soils are more subject to inundation), i.e., 39.8% lower in the forest soil exposed to the atmosphere for less time. Measurements performed on 62 mangrove forest soils showed an average flux of 2.87 mmol CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup> when the soil was exposed to the atmosphere, while 75 results on flooded mangrove forest soils showed an average emission of 2.06 mmol CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup> (Alongi, 2007, 2009), i.e., 28.2% less than for the dry soil. This reflects the increased facility gases have for molecular diffusion than fluids, and the increased surface area available for aerobic respiration and chemical oxidation during air exposure (Chen et al., 2010). Some studies attribute this variation to the temperature of the soil when it is exposed to tropical air (Alongi, 2009), which

- 401 increases the export of dissolved inorganic carbon (Maher et al., 2018). However,
- 402 although despite the lack of significant variation in soil temperature between
- 403 topographies at each time of year (Figure 4b), there was a positive correlation (Pearson
- 404 = 0.15, p = 0.05) between CO<sub>2</sub> efflux and soil temperature at the low topography.
- 405 Some studies show that CH<sub>4</sub> efflux is a consequence of the seasonal temperature
- 406 variation in mangrove forest under temperate/monsoon climates (Chauhan et al., 2015;
- 407 Purvaja and Ramesh, 2001; Whalen, 2005). However, in your study CH<sub>4</sub> efflux was
- 408 correlated with Ta (Pearson = -0.33, p < 0.00) and RH (Pearson = 0.28, p = 0.01) only
- 409 in the dry season and at the low topography. The results show that the physical
- 410 parameters do not affect the fluxes in a standardized way, and their greater or lesser
- 411 influence depends on the topography and seasonality.
- 412 A compilation of several studies showed that the total CH<sub>4</sub> emissions from the soil in a
- 413 mangrove ecosystem range from 0 to 23.68 mg C m<sup>-2</sup> h<sup>-1</sup> (Shiau and Chiu, 2020), and
- our study showed a range of -0.01 to 31.88 mg C m<sup>-2</sup> h<sup>-1</sup> (mean of  $4.70 \pm 5.00$  mg C m<sup>-2</sup>
- 415  $h^{-1}$ ). The monthly CH<sub>4</sub> fluxes were generally higher at the low (0.232  $\pm$  0.256) than at
- 416 the high  $(0.026 \pm 0.018)$  topography, especially during the rainy season when the tides
- 417 were higher (Figure 2). Only in the dry season was there a significantly higher
- 418 production at the low than at the high topography (Figure 2; SI 1). The low topography
- produced 0.0249 g C m<sup>-2</sup> h<sup>-1</sup> more to the atmosphere in the rainy season than in the dry
- 420 season (Figure 2), and a similar seasonal pattern was recorded in other studies
- 421 (Cameron et al., 2021).
- The mangrove soil in the Mojuim River estuary is rich in silt and clay (Table 1), which
- 423 reduces sediment porosity and fosters the formation and maintenance of anoxic
- 424 conditions (Dutta et al., 2013). In addition, the lack of oxygen in the flooded mangrove
- 425 soil favors microbial processes such as denitrification, sulfate reduction,
- 426 methanogenesis, and redox reactions (Alongi and Christoffersen, 1992). A significant
- amount of CH<sub>4</sub> produced in wetlands is dissolved in the pore water due to high pressure,
- 428 causing supersaturation, which allows CH<sub>4</sub> to be released by diffusion from the
- sediment to the atmosphere and by boiling through the formation of bubbles.
- 430 Studies show that the CO<sub>2</sub> flux tends to be lower with high soil saturation (Chanda et
- al., 2014; Kristensen et al., 2008). A total of 395 Mg C ha<sup>-1</sup> was found at the soil surface
- 432 (0.15 m) in the mangrove of the Mojuim River estuary, which was slightly higher than
- 433 the 340 Mg C ha<sup>-1</sup> found in other mangroves in the Amazon (Kauffman et al., 2018),

- however being significantly 1.8 times greater at the low topography (Table 2). The finer
- soil texture at the low topography (Table 1) reduces groundwater drainage which
- facilitates the accumulation of C in the soil (Schmidt et al., 2011).

### 4.3 Mangrove biomass

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- 438 Only the species R. mangle and A. germinans were found in the floristic survey carried
- out, which is aligned with the results of other studies in the same region (Menezes et al.,
- 440 2008). Thus, the variations found in the flux between the topographies in the Mojuim
- River estuary are not related to the mangrove forest structure, because there was no
- difference in the aboveground biomass. Since there was no difference in the species
- composition, the belowground biomass is not expected to differ either (Table 3).
- Assuming that the amount of carbon stored is 0.42 of the total biomass (Sahu and
- Kathiresan, 2019), the mangrove forest biomass of the Mojuim River estuary stores
- 446 127.9 and 138.9 Mg C ha<sup>-1</sup> at the high and low topographies, respectively. This result is
- 447 well below the 507.8 Mg C ha<sup>-1</sup> estimated for Brazilian mangroves (Hamilton and
- 448 Friess, 2018), but are near the 103.7 Mg C ha<sup>-1</sup> estimated for a mangrove at Guará's
- 449 island (Salum et al., 2020), 108.4 Mg C ha<sup>-1</sup> for the Bragantina region (Gardunho,
- 450 2017), and 132.3 Mg C ha<sup>-1</sup> in French Guiana (Fromard et al., 1998). The biomass
- 451 found in the Mojuim estuary does not differ from the biomass found in other
- Amazonian mangroves, despite being much lower than that found in other Brazilian
- 453 mangroves. The estimated primary production for tropical mangrove forests is  $218 \pm 72$
- <sup>454</sup> Tg C y (Bouillon et al., 2008).

#### 4.4 Biogeochemical parameters

- 456 During the seasonal and annual periods, CH<sub>4</sub> efflux was not significantly correlated
- with chemical parameters (Table 5), which is similar to the observed in another study
- 458 (Chen et al., 2010). Flooded soils present reduced gas diffusion rates, which directly
- 459 affects the physiological state and activity of microbes, by limiting the supply of the
- dominant electron acceptors (e.g., oxygen), and gases (e.g., CH<sub>4</sub>) (Blagodatsky and
- Smith, 2012). The importance of soil moisture was evident in the richness and diversity
- of bacterial communities in a study that compared the different pore spaces filled with
- 463 water (Banerjee et al., 2016). Furthermore, sulfate reduction in flooded soils (another
- pathway of organic matter metabolism) is dependent on the redox potential of the soil.
- However, no sulfate reduction occurs when the redox potential has values are above -

466 150 mv (Connell and Patrick, 1968). In our study, Eh was above 36.0 mV indicating that sulfate reduction probably did not influence the OM metabolism.

On the other hand, increasing soil moisture provides the microorganisms with essential substrates such as ammonium, nitrate, and soluble organic carbon, and increases gas diffusion rates in the water (Blagodatsky and Smith, 2012). Biologically available nitrogen often limit marine productivity (Bertics et al., 2010), and thus can affect CO<sub>2</sub> fluxes to the atmosphere. However, a mangrove fertilization experiment showed that CH<sub>4</sub> emission rates were not affected by N addition (Kreuzwieser et al., 2003). A higher concentration of C<sub>mic</sub> and N<sub>mic</sub> in the dry period (Table 2), both in the high and low topographies, indicated that microorganisms are more active when the soil spends more time aerated in the dry period (Table 2), period when only the high tides produce anoxia in the mangrove soil mainly in the low topography. Under reduced oxygen conditions, in a laboratory incubated mangrove soil, the addition of nitrogen resulted in a significant increase in the microbial metabolic quotient, showing no concomitant change in microbial respiration, which was explained by a decrease in microbial biomass (Craig et al., 2021).

The high OM concentration at the two topographic heights (Table 2), at the two seasons studied, and the respective negative correlation with CO<sub>2</sub> flux (Table 5) confirm the importance of microbial activity in mangrove soils (Gao et al., 2020). Also, CH<sub>4</sub> produced in flooded soils can be converted mainly to CO<sub>2</sub> by the anaerobic oxidation of CH<sub>4</sub> (Boetius et al., 2000; Milucka et al., 2015; Xu et al., 2015) which may contribute to the higher CO<sub>2</sub> efflux in the Mojuim River estuary compared to other tropical mangroves (Rosentreter et al., 2018b). The belowground C stock is considered the largest C reservoir in a mangrove ecosystem, and it results from the low OM decomposition rate due to flooding (Marchand, 2017).

The higher water salinity influenced by the tidal movement in the dry season (Table 1) seems to result in a lower CH<sub>4</sub> flux at the low topography (Dutta et al., 2013; Lekphet et al., 2005; Shiau and Chiu, 2020). Sulfate (SO<sub>4</sub><sup>2-</sup>) in the brine affects the competition between SO<sub>4</sub><sup>2-</sup> reduction and methanogenic fermentation, as sulfate-reducing bacteria are more efficient at using hydrogen than methanotrophic bacteria (Abram and Nedwell, 1978; Kristjansson et al., 1982), a key factor fostering reduced CH<sub>4</sub> emissions. At high SO<sub>4</sub><sup>2-</sup> concentrations methanotrophic bacteria use CH<sub>4</sub> as an energy source and oxidize it to CO<sub>2</sub> (Coyne, 1999; Segarra et al., 2015), increasing the efflux of CO<sub>2</sub> and reduced

499 CH<sub>4</sub> (Megonigal and Schlesinger, 2002; Roslev and King, 1996). This may explain the 500 high CO<sub>2</sub> efflux found throughout the year at the high and, especially, at the low 501 topographies (Figure 3). 502 Studies in coastal ecosystems in Taiwan have reported that methanotrophic bacteria can 503 be sensitive to soil pH, and reported an optimal growth at pH ranging from 6.5 to 7.5 504 (Shiau et al., 2018). The higher soil acidity in the Mojuim River wetland (Table 1) may 505 be inhibiting the activity of methanogenic bacteria by increasing the population of 506 methanotrophic bacteria, which are efficient in CH<sub>4</sub> consumption (Chen et al., 2010; 507 Hegde et al., 2003; Shiau and Chiu, 2020). In addition, the pneumatophores present in 508 R. mangle increase soil aeration and reduce CH<sub>4</sub> emissions (Allen et al., 2011; He et al., 509 2019). Spatial differences (topography) in CH<sub>4</sub> emissions in the soil can be attributed to 510 substrate heterogeneity, salinity, and the abundance of methanogenic and 511 methanotrophic bacteria (Gao et al., 2020). The high Eh values found in both 512 topographies, mainly in the dry period (Table 1), hinder CH<sub>4</sub> emission. Soil Eh above -513 150 mV has been considered limiting for CH<sub>4</sub> production (Yang and Chang, 1998). 514 Increases in CH<sub>4</sub> efflux with reduced salinity were found as a consequence of intense oxidation or reduced competition from the more energetically efficient  $SO_4^{2-}$  and  $NO^{3-}$ 515 516 reducing bacteria when compared to the methanogenic bacteria (Biswas et al., 2007). This fact can be observed in the CH<sub>4</sub> efflux in the mangrove of the Mojuim River, 517 518 because there was an increased CH<sub>4</sub> production especially in the low topography in the 519 rainy season (Figure 3), when water salinity is reduced (Table 1) due to the increased

# 5 Conclusions

as previously reported (Purvaja and Ramesh, 2001)

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523 The most recent estimate between latitude 0° to 23.5° S shows an emission of 2.3 g CO<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup> (Rosentreter et al., 2018b). However, the efflux in the mangrove of the Mojuim 524 River estuary was 6.7 g CO<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup>. For the same latitudinal range, Rosentreter et al. 525 (2018c) estimated an emission of 0.64 g CH<sub>4</sub> m<sup>-2</sup> d<sup>-1</sup>, and we found an efflux of 0.13 g 526 CH<sub>4</sub> m<sup>-2</sup> d<sup>-1</sup>. Seasonality was important for CH<sub>4</sub> efflux but did not influence CO<sub>2</sub> efflux. 527 528 The differences in fluxes may be an effect of global climate changes on the terrestrial 529 biogeochemistry at the plant-soil-atmosphere interface, as indicated by the deviation in 530 precipitation values from the climatology normal, making it necessary to extend this 531 study for more years. Using the factor of 23 to convert the global warming potential of

precipitation. However, we did not find a correlation between CH<sub>4</sub> efflux and salinity,

- 532 CH<sub>4</sub> to CO<sub>2</sub> (IPCC, 2001), the CO<sub>2</sub> equivalent emission was 35.4 Mg CO<sub>2-eq</sub> ha<sup>-1</sup> yr<sup>-1</sup>.
- Over a 100-year time period, a radiative forcing due to the continuous emission of 0.05
- kg CH<sub>4</sub> m<sup>-2</sup> y<sup>-1</sup> found in this study, would be offset if CO<sub>2</sub> sequestration rates were 2.16
- kg  $CO_2$  m<sup>-2</sup> y<sup>-1</sup> (Neubauer and Megonigal, 2015).
- Microtopography should be considered when determining the efflux of CO<sub>2</sub> and CH<sub>4</sub> in
- mangrove forests in an Amazon estuary. The low topography in the mangrove forest of
- Mojuim River had a higher concentration of organic carbon in the soil. However, it did
- not produce a higher CO<sub>2</sub> efflux because it was negatively influenced by soil moisture,
- which was indifferent to CH<sub>4</sub> efflux. MO, C/N ratio, and Eh were critical in soil
- 541 microbial activity, which resulted in a variation in CO<sub>2</sub> flux during the year and
- seasonal periods. Thus, the physicochemical properties of the soil are important for CO<sub>2</sub>
- 543 flux, especially in the rainy season. Still, they did not influence CH<sub>4</sub> fluxes.
- Data availability: The data used in this article belong to the doctoral thesis of Saul
- 545 Castellón, within the Postgraduate Program in Environmental Sciences, at the Federal
- 546 University of Pará. Access to the data can be requested from Dr. Castellón
- 547 (saulmarz22@gmail.com), which holds the set of all data used in this paper.
- 548 Author contributions: SEMC and JHC designed the study and wrote the article with the
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