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

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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

scenario of global climate change. This study is the first to quantify <u>carbon dioxide</u> ( $CO_2$ ) and methane ( $CH_4$ ) and <u>carbon dioxide ( $CO_2$ )</u> emissions using a dynamic

16 chamber in a natural mangrove soil of the Amazon. The plots for the trace gases study

17 were allocated at contrasting topographic heights. The results showed that the mangrove

soil of the Amazon estuary is a source of CO<sub>2</sub> (6.66 g CO<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup>) and CH<sub>4</sub> (0.13 g CH<sub>4</sub>

- 19  $\int m^{-2} d^{-1}$  to the atmosphere. The CO<sub>2</sub> flux was higher in the high topography (7.858-86 g
- 20  $CO_2 \text{ m}^{-2} \text{ d}^{-1}$ ) than in the low topography (4.734 g  $CO_2 \text{ m}^{-2} \text{ d}^{-1}$ ) in the rainy season, and
- 21 CH<sub>4</sub> was higher in the low topography  $(0.128-13 \text{ g CH}_4 \text{ m}^{-2} \text{ d}^{-1})$  than in the high
- 22 topography (0.014 g CH<sub>4</sub> m<sup>-2</sup> d<sup>-1</sup>) in the dry season. However, in the dry period, the low
- topography soil produced more  $CH_4$ . Soil organic matter, carbon and nitrogen ratio (C/N), and redox potential influenced the annual and seasonal variation of  $CO_2$ emissions; however, they did not affect  $CH_4$  fluxes. The mangrove soil of the Amazon
- estuary produced 35.40 Mg  $CO_{2-eq}$  ha<sup>-1</sup> y<sup>-1</sup>. A total of 2.16 kg  $CO_2$  m<sup>-2</sup> y<sup>-1</sup> needs to be sequestered by the mangrove ecosystem -to counterbalance CH<sub>4</sub> emissions.

#### 28 1 Introduction

MThe mangrove areas are estimated to be the main contributors to greenhouse gas emissions in marine ecosystems (Allen et al., 2011; Chen et al., 2012). However, mangrove forests are highly productive due to a high nutrient turnover rate (Robertson et al., 1992) and have mechanisms that maximize carbon gain and minimize water loss through plant transpiration (Alongi and Mukhopadhyay, 2015). A study conducted in 25 mangrove forests (between 30° latitude and 73° longitude) revealed that these forests are the richest in carbon (C) storage in the tropics, containing on average 1,023 Mg C
ha<sup>-1</sup> of which 49 to 98% is present in the soil (Donato et al., 2011).

The estimated soil CO<sub>2</sub> outgassingflux, in tropical estuarine areas is 16.2 Tg C y<sup>-1</sup> 37 (Alongi, 2009). However, soil efflux measurements from tropical mangroves revealed 38 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., 39 2014; Shiau and Chiu, 2020). In situ CO<sub>2</sub> production is related to the water input of 40 41 terrestrial, riparian, and groundwater brought by rainfall (Rosentreter et al., 2018b). Due 42 to the periodic tidal movement, the mangrove ecosystem is daily flooded, leaving the 43 soil anoxic and consequently reduced, favoring methanogenesis (Dutta et al., 2013). 44 Thus, estuaries are considered hotspots for CH<sub>4</sub> production and emission (Bastviken et 45 al., 2011; Borges et al., 2015). The oOrganic 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  $CH_4$  formation. On the other hand, and 50 ecosystem withhigh salinity levels, above 18 ppt, may show result in an absence of CH4 51 emissions (Poffenbarger et al., 2011), since methane-CH<sub>4</sub> dissolved in pores is typically 52 oxidized anaerobically by sulfate (Chuang et al., 2016). Currently the uncertainty in 53 emitted CH<sub>4</sub> values in vegetated coastal wetlands is approximately 30% (EPA, 2017). Soil flux measurements from tropical mangroves revealed emissions rangeing from 0.3 54 to 4.4 mg  $CH_4$  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

movements, mangrove soils may become saturated and present a-reduced oxygen availability, or suffer total aeration caused by the ebb tide. Studies attribute soil carbon flux responses to moisture perturbations because of seasonality and flooding events (Banerjee et al., 2016), with fluxes being dependent on tidal extremes (high tide and low 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 accumulation of organic matter in mangrove forest soils (Friesen et al., 2018). Formatado: Subscrito

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  $CO_2$  and  $CH_4$  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.

## 74 2 Material and Methods

## 75 **2.1 Study site**

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 and belongs to a mangrove area of 2,177 km<sup>2</sup> in the state of Pará 80 81 (Souza Filho, 2005). The climate is type Am (tropical monsoon) according to the 82 Köppen classification (Peel et al., 2007). The climatological data were obtained from 83 the 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 rainy period (26 °C) and the maximum in the dry period (29 °C). The Mojuim estuary 88 89 has a macrotidal regime, with an average amplitude of 4.9 m during spring tide and 3.2 m during low tide (Rollnic et al., 2018). During the wet season the Mojuim River has a 90 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, whereas in-91 **During**\_the dry season, the maximum currents reach 1.9 m s<sup>-1</sup> at the flood and 1.67 m s<sup>-1</sup> 92 at the ebb tide (Rocha, 2015). The annual mean salinity is 26.95 PSU (Valentim et al., 93 94 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 (plot A1 and plot A2) topographies (plot A1 and plot
A2). Image Source: © Google Earth

100 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 101 102 al., 2001) associated with the formation of mangroves, dunes, and beaches (El-Robrini 103 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 104 105 reaching the estuary, the Mojuim River crosses an area of a dryland forest highly 106 fragmented by family farming, forming remnants of secondary forest (< 5.0 ha) of 107 various ages (Fernandes and Pimentel, 2019). The population economically exploited 108 the estuary, primarily by artisanal fishing, crab (Ucides cordatus L.) extraction, and 109 oyster farms.

110 The flora of the mangrove area of Macaca Island is little anthropized and comprises the

- 111 plant genera Rhizophora, Avicenia, Laguncularia, and Acrostichum (Ferreira, 2017;
- 112 França et al., 2016). The estuarine plains are influenced by macrotide dynamics and can

**[C1] Comentário:** I think this information is unnecessary, as you cannot see São João da Ponta in Figure 1.

113 be physiographically divided into four sectors <u>according to the different vegetation</u>

114 covers, associated with the landforms distribution, topographic gradient, tidal

115 inundation, and levels of anthropic transformation-(França et al., 2016). The Macaca

116 Island is ranked as being from the fourth sector, which implies having woods of adult

117 trees of the genus *Ryzophora* with an average height of 10 to 25 m, being is located at

an elevation of 0 to 5 m, and having silt-clay soil (França et al., 2016).

119 Four sampling plots were selected in the Macaca Island (Figure 1) on 19/05/2017, when

120 the moon was in the waning quarter phase: two plots where flooding occurs every day

121 (plots B1 and B2; Figure 1), called low topography (Top\_Low), and two plots where 122 flooding occurs only at high tides during the solstice and on the high tides of the rainy

122 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

124 (Top\_High).

## 125 2.2 Greenhouse gas flux measurements

126 In each plot, eight Polyvinyl Chloride rings with 0.20 m diameter and 0.12 m height 127 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 128 129 remained in place until the study was completed. Once a month, gas fluxes were the 130 Greenhouse gas flux was measured during periods of waning or crescent moon, as these 131 are the times when the soil in the low topography is more exposed. To avoid the 132 influence of mangrove roots on the gas fluxes, the rings were placed in locations 133 without any seedlings or aboveground mangrove roots. The CO<sub>2</sub> and CH<sub>4</sub> 134 concentrations (ppm) were measured using the dynamic chamber methodology 135 (Norman et al., 1997; Verchot et al., 2000), sequentially connected to a Los Gatos 136 Research portable gas analyzer (Mahesh et al., 2015). The device was calibrated 137 monthly with a high quality standard gas (500 ppm CO<sub>2</sub>; 5 ppm CH<sub>4</sub>). The rings were 138 sequentially closed for three minutes with a PVC cap, being connected to the analyzer 139 through two 12.0 m polyethylene hoses. The gas concentration was measured every two 140 seconds and automatically stored by the analyzer. CO<sub>2</sub> and CH<sub>4</sub> fluxes were calculated 141 from the linear regression of increasing/decreasing CO2 and CH4 concentrations within 142 the chamber, usually between one and three minutes after the ring cover was placed 143 (Frankignoulle, 1988; McEwing et al., 2015). The flux is considered zero when the 144 linear regression reaches an  $R^2 < 0.30$  (Sundqvist et al., 2014). However, in our analyses, most regressions reached  $R^2 > 0.70$ , and the regressions were weak and 145

146 considered zero in only 6% of the samples. At the end of each flux measurement, the

147 height of the ring above ground was measured at four equidistant points with a ruler.

148 The seasonal data were analyzed by comparing the average monthly fluxes in the wet

season and dry season separately.

#### 150 **2.3 Vegetation structure and biomass**

151 The floristic survey was conducted in October 2017 using circular 1,256.6 m<sup>2</sup> plots (Kauffman et al., 2013) divided into four 314.15 m<sup>2</sup> subplots, which is the equivalent to 152 153 0.38 ha (Figure 1), at the same topographies as the gas flux analysis (Figure 1). We 154 recorded the diameter above the aerial roots, the diameter of the stem, and total height 155 of all trees with DBH (diameter at breast height; m) greater than 0.05m. The allometric 156 equations (Howard et al., 2014) to calculate tree biomass (aboveground biomass; AGB) were:  $AGB = 0.1282 * DBH^{2.6}$  ( $R^2 = 0.92$ ) for *R. mangle*;  $AGB = 0.140 * DBH^{2.4}$  ( $R^2 = 0.140 * DBH^{2.4}$ ) 157 0.97) for A. germinans; and Total AGB = 0.168 \*  $\rho$  \* DBH<sup>2.47</sup> (R<sup>2</sup> = 0.99), where  $\rho_{R.}$ 158 159 mangle = 0.87;  $\rho_{A. \text{ germinans}} = 0.72$  ( $\rho = \text{wood density}$ ).

# 160 2.4 Soil sampling and environmental characterization

161 Four soil samples were collected with an auger at a depth of 0.10 m in all the studied 162 plots for gas flux measurements (Figure 1) in July 2017 (beginning of the dry season) 163 and January 2018 (beginning of the rainy season; Figure 1). Before the soil samples 164 were removed, pH and redox potential (Eh; mV) were measured with a Metrohm 744 165 equipment by inserting the platinum probe directly into the intact soil at a depth of 0.10 166 m (Bauza et al., 2002). The soil samples collected in the field were transported to the 167 laboratory (Chemical Analysis Laboratory of the Museu Paraense Emílio Goeldi) in 168 thermal boxes containing ice. The soil samples were analyzed on the day after collection 169 at the laboratory, and the samples were kept in a freezer. Salinity (Sal; ppt) was 170 measured with PCE-0100, and soil moisture (Sm; %) by the residual gravimetric 171 method (EMBRAPA, 1997).

172 Organic Matter (OM; g kg<sup>-1</sup>), Total Carbon ( $T_C$ ; g kg<sup>-1</sup>) and Total Nitrogen ( $T_N$ ; g kg<sup>-1</sup>) 173 were calculated by volumetry (oxidoreduction) using the Walkley-Black method 174 (Kalembasa and Jenkinson, 1973). Microbial carbon ( $C_{mic}$ ; mg kg<sup>-1</sup>) and microbial 175 nitrogen ( $N_{mic}$ ; mg kg<sup>-1</sup>) were determined through the 2.0 min of Irradiation-extraction 176 method of soil by microwave technique (Islam and Weil, 1998). Microwave heated soil 177 extraction proved to be a simple, fast, accurate, reliable, and safe method to measure 178 soil microbial biomass (Araujo, 2010; Ferreira et al., 1999; Monz et al., 1991). The C<sub>mic</sub> 179 was determined by dichromate oxidation (Kalembasa and Jenkinson, 1973; Vance et al., 180 1987). The N<sub>mic</sub> was analyzed following the method described by Brookes et al. (1985), 181 changing fumigation to irradiation, which uses the difference between the amount of T<sub>N</sub> 182 in irradiated and non-irradiated soil. We used the flux conversion factor of 0.33 183 (Sparling and West, 1988) and 0.54 (Almeida et al., 2019; Brookes et al., 1985), for 184 carbon and nitrogen, respectively. Particle size analysis was performed separately on 185 four soil samples collected at each flux plot, in the two seasons (October 2017 and 186 March 2018), according to EMBRAPA (1997).

At each gas flux measurement, environmental variables such as air temperature ( $T_{air}$ , °C), relative humidity (RH, %), and wind speed ( $W_s$ , 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 ( $T_s$ , °C) was measured with a portable digital thermometer (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 São Caetano das Odivelas (coordinates: -0.738333 latitude; -48.013056 longitude).

#### 194 2.5 Statistical analyses

195 On the Macaca Island, two treatments were allocated (low and high topography), with 196 two plots in either treatment. In each plot, eight chambers were randomly distributed, 197 which were considered sample repetitions. The normality of the data of  $FCH_4$  and  $_{7}$ 198 FCO<sub>2</sub> flux, and soil physicochemical parameters was evaluated using the Shapiro-Wilks 199 method. The soil CO<sub>2</sub> and CH<sub>4</sub> flux showed a non-normal distribution. Therefore, we 200 used the non-parametric ANOVA (Kruskal-Wallis, p < 0.05) to test the differences 201 between the two treatments among months and seasons. The physicochemical 202 parameters were normally distributed. Therefore, a parametric ANOVA was used to test 203 the statistical differences (p < 0.05) between the two treatments among months and 204 seasons. Pearson correlation coefficients were calculated to determine the relationships 205 between soil properties and gas fluxes in the months (dry and wet season) when the 206 chemical properties of the soil were analyzed at the same time as gas fluxes were 207 measured. Statistical analyses were performed with the free statistical software Infostat 208 2015®.

### 209 3 Results

#### 210 **3.1** Carbon dioxide and methane fluxes

211  $CO_2$  fluxes differed significantly between topographies only in January (H = 3.915; p = 0.048), July (H = 9.091; p = 0.003), and November (H = 11.294; p < 0.00001) (Figure 212 213 2; Supplementary Information, SI 1), with generally higher fluxes at the high 214 topography than at the low topography. At the high topography, CO<sub>2</sub> fluxes were significantly higher (H = 24.510; p = 0.011) in July compared to August and December, 215 216 March, October, and May, not differing from the other months of the year. Similarly, at 217 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 218 months. We found a mean monthly flux of  $\frac{327.9 \pm 780.0 - 7 \text{ mg}}{1000 \text{ mg}}$  CO<sub>2</sub> m<sup>-2</sup> hd<sup>-1</sup> (mean ± 219 standard error) and  $\frac{2175.2}{4} \pm \frac{510.0}{5}$  mg CO<sub>2</sub> m<sup>-2</sup> hd<sup>-1</sup> at the high and low 220 221 topographies, respectively.









- 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 = 0.407).
- Greenhouse gas fluxes (Figure 2) were only significantly different between 235 236 topographies in the dry season (Figure 3), period when  $CO_2$  fluxes were higher (H = 237 7.378; p = 0.006) at the high topography and CH<sub>4</sub> fluxes at the low topography (H = 238 8.229; p < 0.001). In the Macaca Island, the mean annual fluxes of CO<sub>2</sub> and CH<sub>4</sub> were  $6.659 \pm 0.419$  g CO<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup> and  $0.132 \pm 0.053$  g CH<sub>4</sub> m<sup>-2</sup> d<sup>-1</sup>, respectively. <u>During the</u> 239 study year, 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> 240  $d^{-1}$  (mean 6.66 g CO<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup>), while the CH<sub>4</sub> flux ranged from -5.07 to 11.08 g CH<sub>4</sub> m<sup>-2</sup> 241  $d^{-1}$  (mean 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 7.00 242 Mg C ha<sup>-1</sup> y<sup>-1</sup> (Figure 2). 243

# 244 3.2 Weather data

245 There was a marked seasonality during the study period (Figure 2), with 2,155.0 mm of 246 precipitation during the rainy period and 1,016.5 mm during the dry period. The highest 247 tides occurred in the period of greater precipitation (Figure 3) due to the rains. However, 248 the rainfall distribution was different from the climatological normal (Figure 3). The 249 precipitation in the rainy season was 553.2 mm below and in the dry season was 589.1 250 mm above the climatological normal. Thus, in the period studied, the dry season was 251 rainier and the rainy season drier than the climatological normal, which may be a 252 consequence of global climate change the La Niña event (Wang et al., 2019).

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255 Figure 3. Monthly climatological normal in the municipality of Soure (1981-2010, mm),

256 monthly precipitation (mm), and maximum tide height (m) from 2017 to 2018, in the 257 municipality of São Caetano de Odivelas (PA).

T<sub>air</sub> 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<sub>s</sub> 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<sub>s</sub> (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.



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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

269 rainy season (n = 16). The bars represent the standard error.

### 270 3.3 Soil characteristics

271 Silt concentration was higher at the low topography (LSD: 14.763; p= 0.007) and clay 272 concentration was higher at the high topography plots (LSD: 12.463; p = 0.005), in both 273 seasons studied (Table 1). Soil particle size analysis did not differ statistically (p > 0.05)274 between the two seasons (Table 1). Soil moisture did not vary significantly (p > 0.05)275 between topographies at each season, or between seasonal periods at the same 276 topography (Table 1). The pH varied statistically (LSD: 5.950; p= 0.006) only at the 277 low topography when the two seasons were compared, being more acidic in the dry 278 period (Table 1). The pH values were significantly (LSD: 0.559; p= 0.008) higher in the 279 dry season (Table 1). No variation in Eh was identified between topographies and 280 seasons (Table 1), although it was higher in the dry season than in the rainy season. 281 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
- $\label{eq:list} 284 \qquad (LSD: 3.003; \, p < 0.001) \ topographies \ (Table 1). \ (Table 1).$

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).

<u> </u>	т 1	Sand	Silt	Clay	Moisture		Eh	Sal
Season	Topograpny	(%)	(%)	(%)	(%)	рн	(mV)	(ppt)
	High	$12.1{\pm}1.4^{aA}$	41.8±3.3 <sup>bA</sup>	46.1±2.6 <sup>aA</sup>	73.1±6.6 <sup>aA</sup>	$5.5\pm0.2^{aA}$	190.25±45.53 <sup>aA</sup>	35.25±1.11 <sup>aA</sup>
Dry	Low	9.7±2.5 <sup>aA</sup>	$63.6{\pm}6.1^{aA}$	$26.6{\pm}5.2^{bA}$	$86.9\pm3.4^{aA}$ $5.3\pm0.3^{aA}$		106.38±53.76 <sup>aA</sup>	$30.13 \pm 1.16^{bA}$
	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>
	High	$12.1{\pm}1.4^{aA}$	41.8±3.3 <sup>bA</sup>	46.1±2.6 <sup>aA</sup>	88.9±3.5 <sup>aA</sup>	$4.9{\pm}0.4^{aA}$	$92.50{\pm}56.20^{aA}$	$7.50{\pm}0.78^{aB}$
Rainy	Low	$9.7{\pm}2.5^{aA}$	$63.6{\pm}6.1^{aA}$	$26.6{\pm}5.2^{bA}$	$88.6 \pm 3.7^{aA}$	$4.4{\pm}0.1^{aB}$	36.25±49.97 <sup>aA</sup>	$8.13{\pm}0.79^{aB}$
	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±0.2 <sup>B</sup>	64.38±37.04 <sup>A</sup>	7.81±0.54 <sup>B</sup>

290 The C<sub>mic</sub> did not differ between topographies in the two seasons (Table 2). However, T<sub>C</sub> 291 was significantly higher in the low topography in the dry season (LSD: 5.589; p < 292 0.000) and in the rainy season (LSD: 5.777; p = 0.024). In addition,  $C_{mic}$  was higher in 293 the dry season in both the high (LSD: 11.325; p < 0.010) and low (LSD: 9.345; p < 0.010) 294 0.000) topographies (Table 2). N<sub>mic</sub> did not vary between topographies seasonally. 295 However,  $N_{mic}$  in the high (LSD: 9.059; p = 0.013) and low topographies (LSD: 4.447; 296 p = 0.001) was higher during the dry season (Table 2). The C/N ratio (Table 2) was 297 higher in the low than in the high topography in both the dry (LSD: 3.142; p < 0.000) 298 and rainy seasons (LSD: 3.675; p = 0.033). However, only in the low topography was 299 the C/N ratio higher (LSD: 1.863; p < 0.000) in the dry season than in the rainy season 300 (Table 2). Soil OM was higher at the low topography in the rainy (LSD: 9.950; p = 301 0.024) and in the dry seasons (LSD: 9.630; p < 0.000). However, only in the lowland 302 topography was the OM concentration higher in the dry season than in the rainy season 303 (Table 2).

Season	Topography	$\mathbf{C}_{\mathrm{mic}}$	$\mathbf{N}_{mic}$	T <sub>C</sub>	$T_N$	C/N	OM
		$mg kg^{-1}$	$mg kg^{-1}$	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±4.20 <sup>aA</sup>	14.12±2.23 <sup>bA</sup>	1.43±0.06 <sup>aA</sup>	9.60±1.20 <sup>bA</sup>	24.35±3.84 <sup>bA</sup>
Dry	Low	$26.34{\pm}4.23^{aA}$	10.34±2.05 <sup>aA</sup>	26.44±1.35 <sup>aA</sup>	1.56±0.04 <sup>aA</sup>	$16.98{\pm}0.84^{aA}$	45.59±2.32 <sup>aA</sup>
	Mean	24.23±3.29 <sup>A</sup>	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 <sup>A</sup>
	High	7.40±0.79 <sup>aB</sup>	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	$5.95{\pm}1.06^{aB}$	$1.23{\pm}0.28^{aB}$	$18.27{\pm}1.06^{aB}$	1.46±0.06 <sup>aA</sup>	$12.47 \pm 0.22^{aB}$	$31.51{\pm}1.83^{aB}$
	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 <sup>A</sup>	10.44±0.98 <sup>A</sup>	25.63±2.71 <sup>B</sup>

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 (±standard error).

306 Lower case letters compare topographies at each season, and upper-case letters compare the topography between seasons.

## 308 **3.4 Vegetation structure and biomass**

309 Only the species R. mangle and A. germinans were found in the floristic survey carried

310 out. The DBH did not vary significantly between the topographies for either species

311 (Table 3). However, R. mangle had a higher DBH than A. germinaris at both high

312 (LSD: 139.304; p = 0.037) and low topographies (LSD: 131.307; p = 0.001). The basal

313 area (BA) and AGB did not show significant variation (Table 3). A total aboveground

314 biomass of  $322.1 \pm 49.6 \text{ Mg ha}^{-1}$  was estimated.

316	Table 3: Summed Diameter at Breast Heig	ht (DBH; cm), Basal Are	a (BA; $m^2$ ha <sup>-1</sup> ) and Aboves	ground Biomass (AGB;	Mg ha <sup>-1</sup> ) at high and low

317 topographies in the mangrove forest of the Mojuim River estuary. Numbers represent the mean ± standard error of the mean. Lower case letters

318	compare topographic height for each	species, and upper-case letters	compare species at each topograph	ic height, using Tukey's test ( $p < 0.05$	5).

		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±2.0 <sup>aA</sup>	219.3±25.7 <sup>aA</sup>	
mangle	Low	310.4±37.6	$283.5 \pm 45.0^{aA}$	$24.2 \pm 4.3^{aA}$	$338.7{\pm}62.9^{aA}$	
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±29.3 <sup>aB</sup>	$11.8 \pm 8.8^{aA}$	136.0±108.3 <sup>aA</sup>	
T-4-1	High	350.2±18.4	325.6±33.6 <sup>a</sup>	31.1±7.5 <sup>a</sup>	304.5±99.8 <sup>a</sup>	
1 otal	Low	346.2±41.0	296.0±23.7 <sup>a</sup>	30.0±4.1 <sup>a</sup>	$330.8 \pm 60.4^{a}$	

319 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  $\rho_{R. mangle} = 0.87$ ;  $\rho_{A. germinans} = 0.14*DBH^{2.47}$ ; and Total =  $0.168*\rho*DBH^{2.47}$ , where  $\rho_{R. mangle} = 0.87$ ;  $\rho_{A. germinans} = 0.14*DBH^{2.47}$ ; and Total =  $0.168*\rho*DBH^{2.47}$ , where  $\rho_{R. mangle} = 0.87$ ;  $\rho_{A. germinans} = 0.14*DBH^{2.47}$ ; and Total =  $0.168*\rho*DBH^{2.47}$ , where  $\rho_{R. mangle} = 0.87$ ;  $\rho_{A. germinans} = 0.14*DBH^{2.47}$ ; and Total =  $0.168*\rho*DBH^{2.47}$ .

320 = 0.72 (Howard et al., 2014).

321

### 322 **3.5 Drivers of greenhouse gas fluxes**

323 In the rainy season, CO<sub>2</sub> efflux was correlated with  $T_{air}$  (Pearson = 0.23, p = 0.03), RH 324 (Pearson = -0.32, p < 0.00) and T<sub>s</sub> (Pearson = 0.21, p = 0.04) only at the low 325 topography. In the dry season CO<sub>2</sub> flux was correlated with  $T_s$  (Pearson = 0.39, p < 326 0.00) at the low topography. The dry season was the period in which we found the 327 greatest amount of significant correlations between CO2 efflux and soil chemical 328 parameters, while the C:N ratio, OM, and Eh were correlated with CO<sub>2</sub> efflux in both 329 seasons (Table 4). The negative correlation between T<sub>C</sub>, N<sub>T</sub>, C/N, and OM, along with 330 the positive correlation of  $N_{mic}$  with soil  $CO_2$  flux, in the dry period, indicates that 331 microbial activity is a decisive factor for CO<sub>2</sub> efflux (Table 4). Soil moisture in the 332 Mojuim River mangrove forest negatively influenced CO<sub>2</sub> flux in both seasons (Table 333 4). However, soil moisture was not correlated with CH4 flux. No significant correlations 334 were found between CH<sub>4</sub> efflux and the chemical properties of the soil in the mangrove 335 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 336 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 337 338 extreme monthly and seasonal variations.

Table 4. Correlation coefficient (Pearson) of  $CO_2$  and  $CH_4$  fluxes with chemical parameters of the soil in a mangrove area in the Mojuim River estuary.

Gas Flux	Season	T <sub>C</sub>	$T_{\rm N}$	C <sub>mic</sub>	N <sub>mic</sub>	C/N	ОМ	Sal	Eh	aII	Moisture
$(g m^{-2} d^{-1})$	I	(g kg <sup>-1</sup> )	(g kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )	C/N	(g kg <sup>-1</sup> )	(ppt)	(mV)	рн	(%)
	Dry	-0.68**	-0.59*	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 <sub>2</sub>	Rainy	-0.44 <sup>NS</sup>	-0.20 <sup>NS</sup>	-0.15 <sup>NS</sup>	-0.32 <sup>NS</sup>	-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 <sup>NS</sup>	0.07 <sup>NS</sup>	-0.14 <sup>NS</sup>	-0.24 <sup>NS</sup>	$0.34^{NS}$	$0.02^{NS}$	-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 <sup>NS</sup>	$0.44^{NS}$	-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>	$0.08^{NS}$	-0.01 <sup>NS</sup>	-0.17 <sup>NS</sup>	-0.21 <sup>NS</sup>	-0.08 <sup>NS</sup>	$0.02^{NS}$

342 Total Carbon ( $T_C$ ; g kg<sup>-1</sup>); Total Nitrogen ( $T_N$ ; g kg<sup>-1</sup>); Microbial Carbon (Cmic, g kg<sup>-1</sup>); Microbial Nitrogen ( $N_{mic}$ , g kg<sup>-1</sup>); Carbon and Nitrogen

343 ratio (C/N); Organic Matter (OM; g kg<sup>-1</sup>); Salinity (Sal; ppt); Redox Potential (Eh; mV); Soil Moisture (Moisture, %).

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

#### 346 4 Discussion

#### 347 **4.1** Carbon dioxide and methane flux

348 It is important to consider that the year under study was rainier in the dry season (2017) 349 and less rainy in the wet season (2018) when the climatological average is concerned 350 (1981-2010) (Figure 3). Perhaps this variation is related to the effects of global climate changes. Under these conditions, negative and positive flows fluxes of the two 351 352 greenhouse gases were found (negative values represent gas consumption). Under these 353 conditions, 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>  $d^+$  (mean 6.66 g CO<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup>), while the CH<sub>4</sub> flux ranged from 5.07 to 11.08 g CH<sub>4</sub> m<sup>-2</sup> 354 d<sup>+</sup>-(mean 0.13 g CH<sub>4</sub>·m<sup>-2</sup>·d<sup>+</sup>), resulting in a total carbon rate of 1.92 g C m<sup>-2</sup>·d<sup>+</sup> or 7.00 355 Mg C ha<sup>-1</sup> y<sup>-1</sup> (Figure 2). The negative CO<sub>2</sub> flux is apparently a consequence of the 356 increased CO2 solubility in tidal waters or of the increased sulfate reduction, as 357 358 described in the literature (Borges et al., 2018; Chowdhury et al., 2018; Nóbrega et al., 359 2016). Fluctuations in redox potential altered the availability of the terminal electron 360 acceptor and donor, and the forces of recovery of their concentrations in the soil, such 361 that a disproportionate release of CO2 can result from the alternative anaerobic 362 degradation processes such as sulfate and iron reduction (Chowdhury et al., 2018). The 363 soil carbon flux in the mangrove area in the Amazon region was within the range of findings for other tropical mangrove areas (2.57-6 to 11.00 g CO<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup>; Shiau and 364 Chiu, 2020). However, the mean flux of 6.2 mmol  $CO_2$  m<sup>-2</sup> h<sup>-1</sup> recorded in this 365 Amazonian mangrove was much higher than the mean efflux of 2.9 mmol  $CO_2 \text{ m}^{-2} \text{ h}^{-1}$ 366 367 recorded in 75 mangroves during low tide periods (Alongi, 2009).

An emission of  $0.01\theta$  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 26.7 368 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 369 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 370 the high topography (0.9  $\pm$  0.6 mg C m<sup>-2</sup> h<sup>-1</sup>), resulting in 0.1<sup>3</sup> g CH<sub>4</sub> m<sup>-2</sup> d<sup>-1</sup> or 0.48-5 371 Mg 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 372 373 Mojuim River estuary were much lower than expected. It is known that there is a 374 microbial functional module for CH<sub>4</sub> production and consumption (Xu et al., 2015) and 375 diffusibility of CH<sub>4</sub> (Sihi et al., 2018), and this module considers three key mechanisms: 376 aceticlastic 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). 377 The average emission from the soil of 8.4 mmol  $CH_4 \text{ m}^{-2} \text{ d}^{-1}$  was well below the fluxes 378

recorded in the Bay of Bengal, with 18.4 mmol  $CH_4 \text{ m}^{-2} \text{ d}^{-1}$  (Biswas et al., 2007). In the 379 380 Amazonian mangrove studied the mean annual carbon equivalent efflux was 429.6 mg CO<sub>2-ea</sub> m<sup>-2</sup> h<sup>-1</sup>. This value is insignificant compared to the projected is 0.00004% of the 381 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 382 383 mangrove forests (Adame et al., 2021). These higher CO<sub>2</sub> flux concomitantly with 384 lower CH<sub>4</sub> flux in this Amazonian estuary are probably a consequence of changes in the 385 rainfall pattern already underway, where the dry season was wetter and the rainy season drier when compared to the climatological normal. The most recent estimate between 386 latitude 0° to 23.5° S shows an emission of 2.3 g  $CO_2$  m<sup>-2</sup> d<sup>-1</sup> (Rosentreter et al., 2018b). 387 However, the efflux in the mangrove of the Mojuim River estuary was 6.7 g  $CO_2$  m<sup>-2</sup> d<sup>-1</sup> 388 <sup>1</sup>. For the same latitudinal range, Rosentreter et al. (2018c) estimated an emission of 389  $0.64 \text{ g CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ , and we found an efflux of  $0.13 \text{ g CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ . 390

## 391 4.2 Drivers of greenhouse gas fluxes

392 Mangrove areas are periodically flooded, with a larger flood volume during the syzygy 393 tides, especially in the rainy season. The hydrological condition of the soil is determined 394 by the microtopography and can regulate the respiration of microorganisms (aerobic or 395 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 396 topography produced more  $CO_2$  (7.869 ± 1.873 g  $CO_2$  m<sup>-2</sup> d<sup>-1</sup>) than the low topography 397  $(5.212 \pm 1.225 \text{ g CO}_2 \text{ m}^2 \text{ d}^4)$  (Figure 2; SI 1). No significant influence on CO<sub>2</sub> flux was 398 399 observed due to the low variation in high tide level throughout the year (0.19 m) (Figure 400 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 401 topography (7.858-86  $\pm$  0.039-04 g CO<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup>) than at the low topography (4.734  $\pm$ 402 0.335-34 g CO<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup>) (Figure 2; SI 1). This result is because may be due to the root 403 404 systems of most flood-tolerant plants remaining active when flooded (Angelov et al., 405 1996). Still, the high topography has longer flood-free periods, which only happens 406 when the tides are syzygy or when the rains are torrential.

407  $CO_2$  efflux was higher in the high topography than in the low topography in the rainy 408 season (when soils are more subject to inundation), i.e., 39.8% lower in the forest soil 409 exposed to the atmosphere for less time. Measurements performed on 62 mangrove 410 forest soils showed an average flux of 2.87 mmol  $CO_2$  m<sup>-2</sup> h<sup>-1</sup> when the soil was 411 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 412 413 for the dry soil. This reflects the increased facility gases have for molecular diffusion 414 than fluids, and the increased surface area available for aerobic respiration and chemical 415 oxidation during air exposure (Chen et al., 2010). Some studies attribute this variation 416 to the temperature of the soil when it is exposed to tropical air (Alongi, 2009), which 417 increases the export of dissolved inorganic carbon (Maher et al., 2018). However, 418 although despite the lack of significant variation in soil temperature between 419 topographies at each time of year (Figure 4b), there was a positive correlation (Pearson 420 = 0.15, p = 0.05) between CO<sub>2</sub> efflux and soil temperature at the low topography.

Some studies show that  $CH_4$  efflux is a consequence of the seasonal temperature variation in mangrove forest under temperate/monsoon climates (Chauhan et al., 2015; Purvaja and Ramesh, 2001; Whalen, 2005). However, in your study  $CH_4$  efflux was correlated with Ta (Pearson = -0.33, p < 0.00) and RH (Pearson = 0.28, p = 0.01) only in the dry season and at the low topography. The results show that the physical parameters do not affect the fluxes in a standardized way, and their greater or lesser influence depends on the topography and seasonality.

428 A compilation of several studies showed that the total  $CH_4$  emissions from the soil in a mangrove ecosystem range from 0 to 23.68 mg C  $m^{-2} h^{-1}$  (Shiau and Chiu, 2020), and 429 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> 430 h<sup>-1</sup>). The monthly CH<sub>4</sub> fluxes were generally higher at the low (0.232  $\pm$  0.256 g CH<sub>4</sub> m<sup>-2</sup> 431  $d^{-1}$ ) than at the high (0.026 ± 0.018 g CH<sub>4</sub> m<sup>-2</sup> d<sup>-1</sup>) topography, especially during the 432 rainy season when the tides were higher (Figure 2). Only in the dry season was there a 433 434 significantly higher 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 435 436 season than in the dry season (Figure 2), and a similar seasonal pattern was recorded in 437 other studies (Cameron et al., 2021).

The mangrove soil in the Mojuim River estuary is rich in silt and clay (Table 1), which reduces sediment porosity and fosters the formation and maintenance of anoxic conditions (Dutta et al., 2013). In addition, the lack of oxygen in the flooded mangrove soil favors microbial processes such as denitrification, sulfate reduction, methanogenesis, and redox reactions (Alongi and Christoffersen, 1992). A significant amount of  $CH_4$  produced in wetlands is dissolved in the pore water due to high pressure,

- 444 causing supersaturation, which allows  $CH_4$  to be released by diffusion from the 445 sediment to the atmosphere and by boiling through the formation of bubbles.
- 446 Studies show that the  $CO_2$  flux tends to be lower with high soil saturation (Chanda et 447 al., 2014; Kristensen et al., 2008). A total of 395 Mg C ha<sup>-1</sup> was found at the soil surface 448 (0.15 m) in the mangrove of the Mojuim River estuary, which was slightly higher than 449 the 340 Mg C ha<sup>-1</sup> found in other mangroves in the Amazon (Kauffman et al., 2018), 450 however being significantly 1.8 times greater at the low topography (Table 2). The finer 451 soil texture at the low topography (Table 1) reduces groundwater drainage which 452 facilitates the accumulation of C in the soil (Schmidt et al., 2011).

#### 453 4.3 Mangrove biomass

- 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., 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).
- 460 Assuming that the amount of carbon stored is 0.42.0% of the total biomass (Sahu and 461 Kathiresan, 2019), the mangrove forest biomass of the Mojuim River estuary stores 127.9 and 138.9 Mg C ha<sup>-1</sup> at the high and low topographies, respectively. This result is 462 lower than well below the 507.8 Mg C ha<sup>-1</sup> estimated for Brazilian mangroves 463 (Hamilton and Friess, 2018), but are near the 103.7 Mg C ha<sup>-1</sup> estimated for a mangrove 464 at Guará's island (Salum et al., 2020), 108.4 Mg C ha<sup>-1</sup> for the Bragantina region 465 (Gardunho, 2017), and 132.3 Mg C ha<sup>-1</sup> in French Guiana (Fromard et al., 1998). Thus, 466 467 Tthe biomass found in the Mojuim estuary does not differ from the biomass found in 468 other Amazonian mangroves, despite being much lower than that found in other 469 Brazilian mangroves. The estimated primary production for tropical mangrove forests is  $218 \pm 72$  Tg C y<sup>-1</sup> (Bouillon et al., 2008). 470

## 471 4.4 Biogeochemical parameters

472 During the seasonal and annual periods,  $CH_4$  efflux was not significantly correlated 473 with chemical parameters (Table 5), which is similar to the observed in another study 474 (Chen et al., 2010). Flooded soils present reduced gas diffusion rates, which directly 475 affects the physiological state and activity of microbes, by limiting the supply of the 476 dominant electron acceptors (e.g., oxygen), and gases (e.g., CH<sub>4</sub>) (Blagodatsky and 477 Smith, 2012). The importance of soil can be reflected in bacterial moisture was evident 478 in the richness and diversity of bacterial communities in a study that compared theo 479 different pore spaces filled with water (Banerjee et al., 2016). Furthermore, sulfate 480 reduction in flooded soils (another pathway of organic matter metabolism) is dependent 481 on the redox potential of the soil. However, no sulfate reduction occurs when the redox 482 potential has values are above 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 483

484 metabolism.

485 On the other hand, increasing soil moisture provides the microorganisms with essential 486 substrates such as ammonium, nitrate, and soluble organic carbon, and increases gas 487 diffusion rates in the water (Blagodatsky and Smith, 2012). Biologically available 488 nitrogen often limit marine productivity (Bertics et al., 2010), and thus can affect CO<sub>2</sub> 489 fluxes to the atmosphere. However, a mangrove fertilization experiment showed that 490 CH<sub>4</sub> emission rates were not affected by N addition (Kreuzwieser et al., 2003). A higher 491 concentration of C<sub>mic</sub> and N<sub>mic</sub> in the dry period (Table 2), both in the high and low 492 topographies, indicated that microorganisms are more active when the soil spends more 493 time aerated in the dry period (Table 2), period-time when only the high tides produce 494 anoxia in the mangrove soil mainly in the low topography. Under reduced oxygen 495 conditions, in a laboratory incubated mangrove soil, the addition of nitrogen resulted in 496 a significant increase in the microbial metabolic quotient, showing no concomitant 497 change in microbial respiration, which was explained by a decrease in microbial 498 biomass (Craig et al., 2021).

The high OM concentration at the two topographic heights locations (Table 2), at the 499 500 two seasons studied, and the respective negative correlation with  $CO_2$  flux (Table 5) 501 confirm the importance of microbial activity in mangrove soils (Gao et al., 2020). Also, 502  $CH_4$  produced in flooded soils can be converted mainly to  $CO_2$  by the anaerobic 503 oxidation of CH<sub>4</sub> (Boetius et al., 2000; Milucka et al., 2015; Xu et al., 2015) which may 504 contribute to the higher CO<sub>2</sub> efflux in the Mojuim River estuary compared to other 505 tropical mangroves (Rosentreter et al., 2018b). The belowground C stock is considered 506 the largest C reservoir in a mangrove ecosystem, and it results from the low OM 507 decomposition rate due to flooding (Marchand, 2017).

Código de campo alterado

508 The higher water salinity influenced by the tidal movement in the dry season (Table 1) 509 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-High (SO<sub>4</sub><sup>2-</sup> concentration in the marine 510 511 sediments inhibits methane formation due to competition between  $SO_4^{2-}$  reduction and 512 methanogenic fermentation, as) in the brine affects the competition between  $SO_4^2$ 513 reduction and methanogenic fermentation, as sulfate-reducing bacteria are more 514 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> 515 516 concentrations methanotrophic bacteria use CH4 as an energy source and oxidize it to 517 CO<sub>2</sub> (Coyne, 1999; Segarra et al., 2015), increasing the efflux of CO<sub>2</sub> and reduced CH<sub>4</sub> 518 (Megonigal and Schlesinger, 2002; Roslev and King, 1996). This may explain the high 519 CO<sub>2</sub> and low CH<sub>4</sub> efflux found throughout the year at the high and, especially, at the 520 low topographies (Figure 3).

521 Studies in coastal ecosystems in Taiwan have reported that methanotrophic bacteria can 522 be sensitive to soil pH, and reported an optimal growth at pH ranging from 6.5 to 7.5 523 (Shiau et al., 2018). The higher soil acidity in the Mojuim River wetland (Table 1) may 524 be inhibiting the activity of methanogenic bacteria by increasing the population of 525 methanotrophic bacteria, which are efficient in CH<sub>4</sub> consumption (Chen et al., 2010; 526 Hegde et al., 2003; Shiau and Chiu, 2020). In addition, the pneumatophores present in 527 *R. mangle* increase soil aeration and reduce CH<sub>4</sub> emissions (Allen et al., 2011; He et al., 528 2019). Spatial differences (topography) in  $CH_4$  emissions in the soil can be attributed to 529 substrate heterogeneity, salinity, and the abundance of methanogenic and 530 methanotrophic bacteria (Gao et al., 2020). The high Eh values found in both 531 topographies, mainly in the dry period (Table 1), hinder CH4 emission. Soil Eh above-532 150 mV has been considered limiting for CH<sub>4</sub> production (Yang and Chang, 1998). 533 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 SO42- and NO3-534 535 reducing bacteria when compared to the methanogenic bacteria (Biswas et al., 2007). 536 This fact can be observed in the CH<sub>4</sub> efflux in the mangrove of the Mojuim River, 537 because there was an increased  $CH_4$  production especially in the low topography in the 538 rainy season (Figure 3), when water salinity is reduced (Table 1) due to the increased 539 precipitation. However, we did not find a correlation between CH<sub>4</sub> efflux and salinity, 540 as previously reported (Purvaja and Ramesh, 2001)

### 541 5 Conclusions

542 most recent estimate between latitude 0° to 23.5° S shows an emission of 2.3 The m<sup>-2</sup>-d<sup>-4</sup> (Rosentreter et al., 2018b). However, the efflux in the mangrove of the Mojuim 543 River estuary was 6.7 g CO<sub>2</sub> m<sup>2</sup> d<sup>4</sup>. For the same latitudinal range, Rosentreter et al. 544 (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 545 CH4-m<sup>-2</sup>-d<sup>-4</sup>. Seasonality was important for CH4 efflux but did not influence CO2-efflux. 546 547 Seasonality was important for CH<sub>4</sub> efflux but did not influence CO<sub>2</sub> efflux. The 548 differences in fluxes may be an effect of global climate changes on the terrestrial 549 biogeochemistry at the plant-soil-atmosphere interface, as indicated by the deviation in 550 precipitation values from the climatology normal, making it necessary to extend this 551 study for more years. Using the factor of 23 to convert the global warming potential of 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>. 552 Over a 100-year time period, a radiative forcing due to the continuous emission of 0.05 553 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 2.16 554 kg CO<sub>2</sub> m<sup>-2</sup> y<sup>-1</sup> (Neubauer and Megonigal, 2015). 555

556 Microtopography should be considered when determining the efflux of  $CO_2$  and  $CH_4$  in 557 mangrove forests in an Amazon estuary. The low topography in the mangrove forest of 558 Mojuim River had a higher concentration of organic carbon in the soil. However, it did 559 not produce a higher CO<sub>2</sub> efflux because it was negatively influenced by soil moisture, 560 which was indifferent to CH<sub>4</sub> efflux. MO, C/N ratio, and Eh were critical in soil 561 microbial activity, which resulted in a variation in  $CO_2$  flux during the year and 562 seasonal periods. Thus, the physicochemical properties of the soil are important for CO<sub>2</sub> 563 flux, especially in the rainy season. Still, they did not influence CH<sub>4</sub> fluxes.

564 *Data availability*: The data used in this article belong to the doctoral thesis of Saul 565 Castellón, within the Postgraduate Program in Environmental Sciences, at the Federal 566 University of Pará. Access to the data can be requested from Dr. Castellón

567 (saulmarz22@gmail.com), which holds the set of all data used in this paper.

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