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1 **Title**

2 Contrasting organic matter composition in pristine and eutrophicated mangroves  
3 revealed by fatty acids and stable isotopes (Rio de Janeiro, Brazil)

4

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21

22

23 **Abstract**

24 Mangrove sediments have a high capacity of carbon storage, as the result of larger  
25 organic matter (OM) inputs from mangrove trees (litter fall and fine roots production)  
26 than OM microbial degradation and export to coastal waters. Mangrove sediments also  
27 act as traps for suspended matter and particulate OM (POM) from surrounding water  
28 masses. Fatty acids (FAs) markers,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures were used here to  
29 characterize the OM composition in three mangroves located in three coastal  
30 embayments of the Rio de Janeiro state (Brazil) with increasing urbanization from a  
31 pristine mangrove M1 to a moderately impacted mangrove M2 and a highly impacted  
32 mangrove M3. In these mangroves, the  $\delta^{15}\text{N}$  signature of tree leaves and sediments  
33 increases with anthropogenic influence, consistent with a large-scale eutrophication  
34 gradient along the three regions. At mangrove M1, predominant OM inputs from  
35 mangrove trees are highlighted by high proportions of long-chain fatty acids,  
36 particularly in the inland station, where high organic carbon concentrations ( $126\pm 108$   
37  $\text{mg}\cdot\text{g}^{-1}$ ) indicate limited sedimentation of mineral particles and high carbon storage  
38 capacities. The sedimentary OM of M3 mangrove was more labile as confirmed by the  
39 higher proportions of algal fatty acids, enriched  $\delta^{13}\text{C}$  signature and the C/N ratio of 1.6  
40 times lower ( $p < 0.001$ ) than in the pristine mangrove M1. At the M2 mangrove site,  
41 high contribution of bacterial FAs (around 20%) to sedimentary OM and high proportion  
42 of poorly biodegradable saturated fatty acids suggest that bacteria degrade algal labile  
43 OM in surface sediments but do not mineralize the most refractory fraction of OM. At  
44 the eutrophic M3 site, our findings suggest that deposition of labile POM induced an  
45 increase of fungal biomass on the sediment, apparently enhancing the microbial loop,  
46 and potentially leading to mineralization of refractory OM and carbon losses through a  
47 priming effect.

48 **Keywords**

49 Blue carbon; Organic matter biodegradability; Cultural eutrophication; Microalgal  
50 blooms; Fungi; Bacteria.

## 51 **1. Introduction**

52 Mangrove forests are coastal ecosystems characterized by the presence of woody  
53 plants tolerant to extreme environmental conditions such as high salinity and sediment  
54 anoxia. These forest systems are present in tropical and subtropical areas, and  
55 colonize a worldwide surface area between 137 600 km<sup>2</sup> (Bunting et al., 2018) and 157  
56 000 km<sup>2</sup> (FAO, 2007). Brazil is the country with the 3<sup>rd</sup> largest mangrove cover on the  
57 globe, which representing 8.5% of the total surface area of mangroves worldwide  
58 (Webber, 2016).

59 Mangrove forests have an average net primary production (NPP) between 1360 ± 450  
60 g C m<sup>-2</sup> year<sup>-1</sup> (Bouillon et al., 2008a) and 1522 g C m<sup>-2</sup> year<sup>-1</sup> (Alongi, 2014), that is, 4  
61 to 7 times higher than the NPP by coastal marine phytoplankton (Cloern et al., 2014).  
62 An important fraction of the OM produced by mangrove NPP is transferred to their  
63 sediments as litter fall and fine roots production, fuelling microbial respiration and CO<sub>2</sub>  
64 and CH<sub>4</sub> emission from the sediments to waters and the atmosphere (Alongi, 2020;  
65 Maher et al., 2018). However, anoxic conditions in the sediments slow down the  
66 degradation rates of OM and favour its preservation and burial over the long term. In  
67 addition, due to tidal inundation, exchange of organic and inorganic C occurs between  
68 mangrove sediments and the surrounding coastal waters (David et al., 2018; Maher et  
69 al., 2018; Santos et al., 2019). Recent estimates indicate that C accumulation in  
70 mangrove sediments may reach 41 Mt annually (Wang et al., 2021), representing a  
71 long-term C burial with high impact on global carbon budget and global warming  
72 mitigation (Kristensen et al., 2008a). Mangroves account for 15% of the "blue carbon"  
73 actually buried among all coastal marine ecosystems (Anand et al., 2020).

74 The composition of mangrove sedimentary organic matter (SOM) is strongly influenced  
75 by local sources of OM (Bouillon et al., 2003). It is generally assumed that litterfall and

76 roots from mangrove trees are the most abundant sources of SOM (Alongi, 2014),  
77 largely predominant in pristine mangroves (Sanders et al., 2014). However,  
78 microphytobenthos and phytoplankton as well as their detritus in the particulate organic  
79 matter (POM) may be an additional source of SOM produced on the mangrove surface  
80 sediment and/or brought from the surrounding coastal waters and deposited by the  
81 tide (David et al., 2018). Increasing human densities along coastal zones and the  
82 intensification of agricultural activities are increasing runoff of OM and nutrient to the  
83 coastal zone (Kitsiou & Karydis, 2011; Sanders et al., 2014). This leads in highly  
84 anthropized coastal areas to a proliferation of phytoplankton, microphytobenthos and  
85 macroalgae that can enter the mangrove surface sediment (Pérez et al., 2018a). A  
86 third source is the direct discharge of untreated sewage which is also common in  
87 anthropized watersheds (Kitsiou & Karydis, 2011).

88 At low tide, direct exposure of highly surface sediments to the atmosphere leads to the  
89 oxygenation of these sediments contributing to intense microbial remineralization of  
90 SOM in surface sediments (Sun et al., 2002). Tides also drive mangrove POM exports  
91 to adjacent creeks (Meziane & Tsuchiya, 2002). In mangrove sediments, deeper layers  
92 are typically depleted in oxygen and SOM is degraded by anaerobic processes (e.g.  
93 reductions of nitrate, manganese, iron or sulfides, and methanogenesis), which are  
94 less efficient than aerobic remineralization (Sun et al., 1993). Bioturbating organisms  
95 (e.g. crabs) and the presence of roots contribute to increased sediment oxidation and  
96 subsequent remineralization of SOM (Kristensen & Alongi, 2006; Zhu et al., 2018;  
97 Sarker et al., 2021). Indeed, mangrove sediments emit to the atmosphere at low tide  
98 and export with tidal pumping, significant amounts of CO<sub>2</sub> and CH<sub>4</sub> coming from the  
99 mineralisation of SOM (Kristensen et al., 2008b; Pongparn et al., 2009; Nóbrega et  
100 al., 2016).

101 In addition to the quantity of OM deposited, the quality of SOM is a crucial factor that  
102 control remineralization or preservation. Labile compounds (e.g. algal or urban  
103 domestic OM) are more easily degraded than refractory compounds (e.g. lignin,  
104 cellulose from the mangrove forest) (Fontaine et al., 2003; Guenet et al., 2010).  
105 Previous evidence has indicated that the production of labile OM by microalgal blooms  
106 can also potentially generate an intensification of the refractory OM remineralization  
107 (Gontikaki et al., 2015), through a phenomenon called the priming effect (Bianchi,  
108 2011; Gontikaki et al., 2015). Further research is needed to better understand the  
109 changes of the OM quality under increasing eutrophic condition over time and  
110 particularly the role of microorganisms in sediment OM degradation (Bianchi, 2011;  
111 Bouillon et al., 2008a; Lovelock et al., 2017; Queiroz et al., 2020).

112 Fatty acid and stable isotope composition of organic substrates are useful to identify  
113 different POM and SOM sources and are also proxies of the eutrophication process in  
114 a wide variety of ecosystems (Bergamino et al., 2014). FAs contribute to between 0.1  
115 and 5% in coastal marine SOM (Canuel, 2001), between 10 and 30% in POM  
116 (Wakeham et al., 1997) and between 5 and 25% in DOM (Nebbioso & Piccolo, 2013).  
117 Some FAs are synthesized by specific groups of organisms and others are synthesized  
118 in most organisms and in specific proportions (Dalsgaard et al., 2003; Kaneda, 1991).  
119 FAs are intensively produced and degraded by organisms by heterotrophic micro and  
120 macrofauna, especially the polyunsaturated FAs (Wakeham et al., 1997; Camacho-  
121 Ibar et al., 2003). FAs are also powerful taxonomic indicators (from the species to  
122 class) of 1) the living biomass in algal blooms, 2) sewage OM degradability and 3)  
123 bacterial and fungal components within sediments and biofilms in the ecosystem  
124 (Meziane & Tsuchiya, 2002; Xu & Jaffé, 2007; Koppryo et al., 2018). Consequently,  
125 FAs are an efficient tool to trace the more labile part of SOM and POM, especially

126 when combined with bulk isotope C and N signature. Moreover, FAs can provide semi-  
127 quantitative information on various sources to the OM pool.

128 Despite research attesting to coastal eutrophication around the world (Breitburg et al.,  
129 2018), little is known on its impact on SOM composition in mangrove sediments. The  
130 coast of Rio de Janeiro state (Brazil) shows contrasting conserved and highly  
131 eutrophicated areas due to the discharge of untreated urban sewage (Cotovicz et al.,  
132 2018; Ribeiro & Kjerfve, 2002). The aim of this study is to characterize the source and  
133 fate of OM in three mangroves within the state of Rio de Janeiro (Brazil) along a  
134 eutrophication gradient. For these purposes, the present study uses fatty acids (FAs)  
135 and the isotopic signatures of carbon and nitrogen ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) to assess the origin  
136 and transfers of OM in mangrove sediments located along a gradient of urbanization  
137 and eutrophication.

138

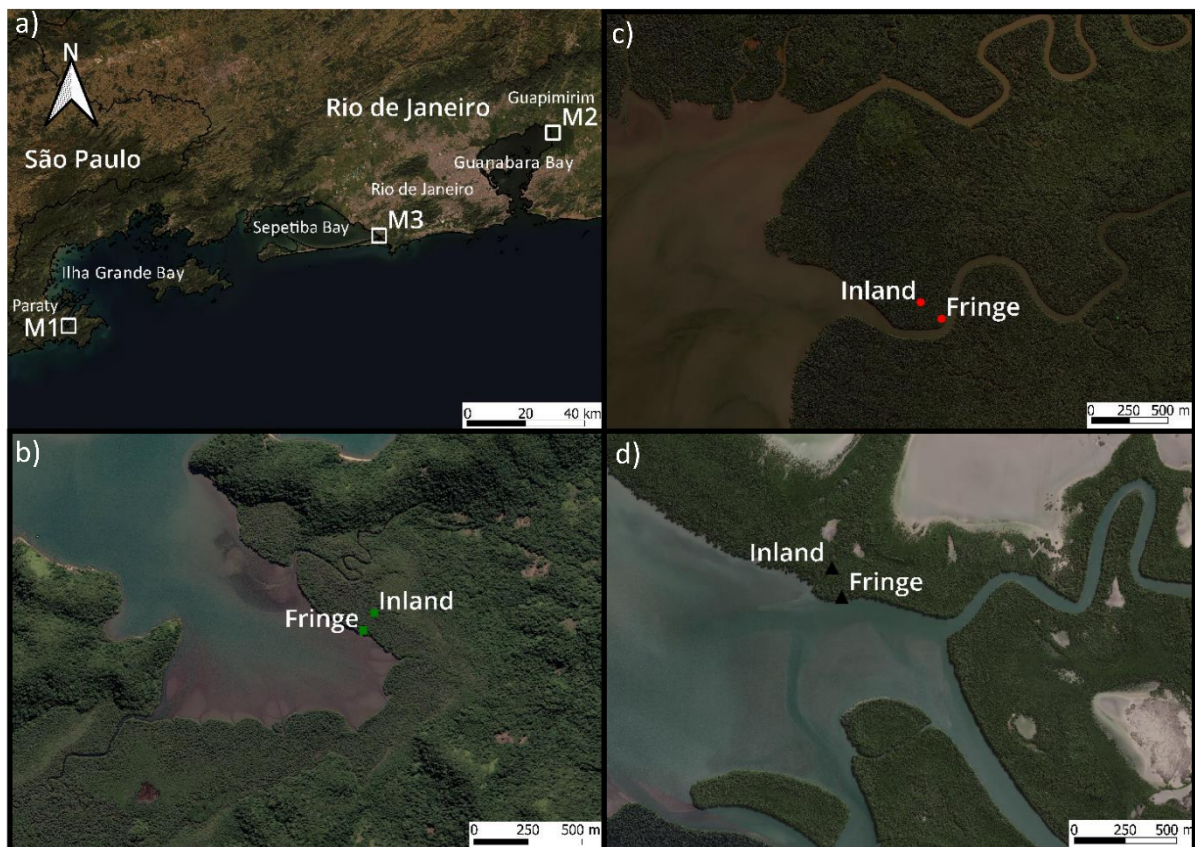
## 139 **2. Materials and Methods**

### 140 2.1 Study sites

141 The three studied mangroves in the State of Rio de Janeiro (Brazil) (Fig. 1) were  
142 chosen because of their different levels of urban influence. Indeed, in the State of Rio  
143 de Janeiro the small increase in wastewater treatment combined with the very large  
144 increase in population leads to a wastewater discharge proportional to the watershed  
145 population (Abril et al., 2022), thus highly polluted rivers discharge organic matter,  
146 nitrogen and phosphorus especially in the bays of Guanabara and Sepetiba (Fistarol  
147 et al., 2015; Rodrigues et al., 2009). In contrast, the southwestern region of the state  
148 is not densely populated and the bay of Ilha Grande remains relatively pristine (Fig.  
149 1A). The most pristine studied mangrove M1 is located at the back end of an 8km-long



150 elongated embayment called *Saco de Mamangua* with a very small watershed  
151 containing a limited population (Table A1), and classified as an environmental reserve.  
152 Although the entire drainage basin of Guanabara Bay is one of most urbanized in  
153 Brazil, with an estimated population ~17 million compared to ~1.8 million in Sepetiba  
154 Bay, the immediate watershed of the M2 mangrove in the region called *Guapi Mirim* is  
155 larger but less populated than that of the M3 mangrove in the area called *Guaratiba*  
156 (Table A1; Barroso et al. revised). Accordingly to the microtopography data of Barroso  
157 et al. (revised), the estimated inundation times of these mangroves were relatively  
158 similar. Finally, the  $\delta^{15}\text{N}$  values of sediments and mangrove leaves attest to the  
159 differences in eutrophication between the three sites (Table A1). The plant community  
160 composition of each site is displayed in table A1.



161  
162 *Figure 1 : Location of the tree studied mangroves at Rio de Janeiro (a), southeastern Brazil. (b) M1*  
163 *in Ilha Grande Bay, (c) M2 in Guanabara Bay and M3 (d) in Sepetiba Bay. (2 column)*

## 164 2.2 Sampling

165 Samples from each of the study sites were collected during the rainy season between  
166 October 11 and December 7, 2018. At each site, three zones were sampled: water  
167 column (adjacent creek or channel), zone F corresponding to the mangrove fringe and  
168 zone I corresponding to most inland location of the mangrove forest. The fringe  
169 corresponds to the nearest part (about 20m) to the coast and the inland zone  
170 corresponds to the part a furthest within the forest (between 100 and 200 meters).

171 Sediment samples were collected at low tide at the three study sites. Four 9cm-long  
172 cores were taken at each of the 6 zones. Three sediment depths were sectioned: 0-2  
173 cm (P0), 3-4 cm (P3) and 7-9 cm (P9). In order to characterize potential OM sources,  
174 fresh leaves of *A.shaueriana*, *R.mangle* et *L.racemosa* were taken directly from the  
175 trees. Samples from the water column were taken as 5 replicates. Water and material  
176 collected with a 73  $\mu\text{m}$  mesh plankton net were filtered on GF/F filters (0.7  $\mu\text{m}$ ). For  
177 M3, because suspended material was very concentrated, the material from the  
178 plankton nets was not filtered on GF/F filters but separated by decantation. For M2,  
179 the water column samples were taken in two distinct areas: in the proper Guanabara  
180 Bay (2B) at about 5km from the mangrove, and in the river channel (2C) during a rising  
181 tide. All samples were frozen the day of sampling and were freeze-dried within three  
182 days of collection.

## 183 2.3. Laboratory analysis

### 184 2.3.1. Fatty Acids

185 Prior to extraction, an internal standard (Tricosanoic acid: 23:0) was added in each  
186 sample. Lipids were extracted according to the protocol of Bligh and Dyer as modified  
187 by Meziane et al. (2007). Samples are extracted by sonication (20 minutes) a first time

188 in a mixture of chloroform, water and methanol (1:1:2; v:v:v). 1 mL chloroform and 1  
189 mL water are added and the samples are vortexed and centrifuged 5 min at 3000 rpm.  
190 The lipids are retained in the chloroform phase, which is collected. 2 mL chloroform is  
191 added to the samples before a second extraction with the sonicator (20 min). The  
192 samples are centrifuged again, then the chloroform is collected and the total 4 mL is  
193 evaporated under nitrogen (N<sub>2</sub>) flow. Lipids are saponified by adding a  
194 methanol:sodium hydroxide (2N) mixture (2:1; v:v). Samples are put at 90°C for 1h30  
195 then 500 µl of chloridric acid (37%) and 1.5 ml of CHCl<sub>3</sub> are added. The tubes are then  
196 vortexed and centrifuged (3000 rpm). The chloroform phase is collected and  
197 evaporated under nitrogen flow. Samples are methylated to form fatty acid methyl ester  
198 (FAME) with 1 mL boron trifluoride and then placed in a dry bath for 10 min at 90°C. 1  
199 mL H<sub>2</sub>O and 1mL CHCl<sub>3</sub> are added. They are vortexed and centrifuged and then part  
200 of the surface water is pipetted and discarded in order to rinse the solution well and  
201 purify the final fatty acid extract. 1 mL H<sub>2</sub>O and 1 mL CHCl<sub>3</sub> are added again and the  
202 tubes are vortexed and centrifuged for 5 min (3000 rpm). All the chloroform containing  
203 FAME is removed and placed in 2 mL vials. The vials are stored in the freezer at -  
204 20°C. FAME are quantified by gas chromatography (Varian 3800-GC) with an ionizing  
205 flame detector. The oven temperature is maintained at 60°C for 1 min, then it increases  
206 to 150° (40°/min) for 3 min and then to 240° (3°/min) for 25 min. Fatty acids are  
207 identified with a mass spectrometer (Varian 220-MS) and the comparison of fatty acid  
208 retention times with the retention times of a commercial standard (Supelco® 37). We  
209 report the values as % of total FA.

### 210 2.3.2. Stable isotopes

211 For  $\delta^{13}\text{C}$  analysis, sediment and filter samples were acidified in order to eliminate  
212 carbonates. For sediment samples, 10% HCl was directly added to the samples during

213 24 hours. Filter samples were fumigated by adding chlorhydric acid (37%). The filters  
214 were exposed to the fumes generated by the acid for 6 hours.

215 Acidification can distort the values of  $\delta^{15}\text{N}$ , therefore the measurements of  $\delta^{15}\text{N}$  were  
216 performed separately for filters and sediments of which the 10% HCl was not applied.  
217 About 15 mg of sediments and suspended material scratched from the filters were  
218 weighted and stored in tin capsules.

219 The samples were analyzed at the University of California at the Davis Stable Isotopes  
220 Facility (Department of Plant Sciences, University of California, Davis, California) with  
221 an Elementar Vario EL Cube or Micro Cube Elemental analyzer (Elementar Analysen  
222 systeme GmbH, Hanau, Germany) combined with a PDZ Europa 20-20 isotope ratio  
223 mass spectrometer (Sercon Ltd., Cheshire, UK). Stable isotope results are reported in  
224 parts per thousand (‰), using the standard delta notation ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) relative to  
225 international standards: VPDB (Vienna PeeDee Belemnite) for carbon and  
226 atmospheric air for nitrogen.

#### 227 2.4. Statistical analysis

228 Due to the too small number of replicates (4 replicates), statistical analyses were  
229 performed using non-parametric tests. ANOSIMs (Clarke, 1993) with Bray-Curtis  
230 distance were done to analyze the effect of depth, sampling site and sampling zone  
231 within mangrove on fatty acids profiles (significance level  $p < 0.05$ ). To characterize the  
232 effect of depth, sampling site and sampling zone within mangrove in individual fatty  
233 acid distributions, pairwise Wilcoxon tests (Bauer, 1972) were performed (significance  
234 level  $p < 0.05$ ).

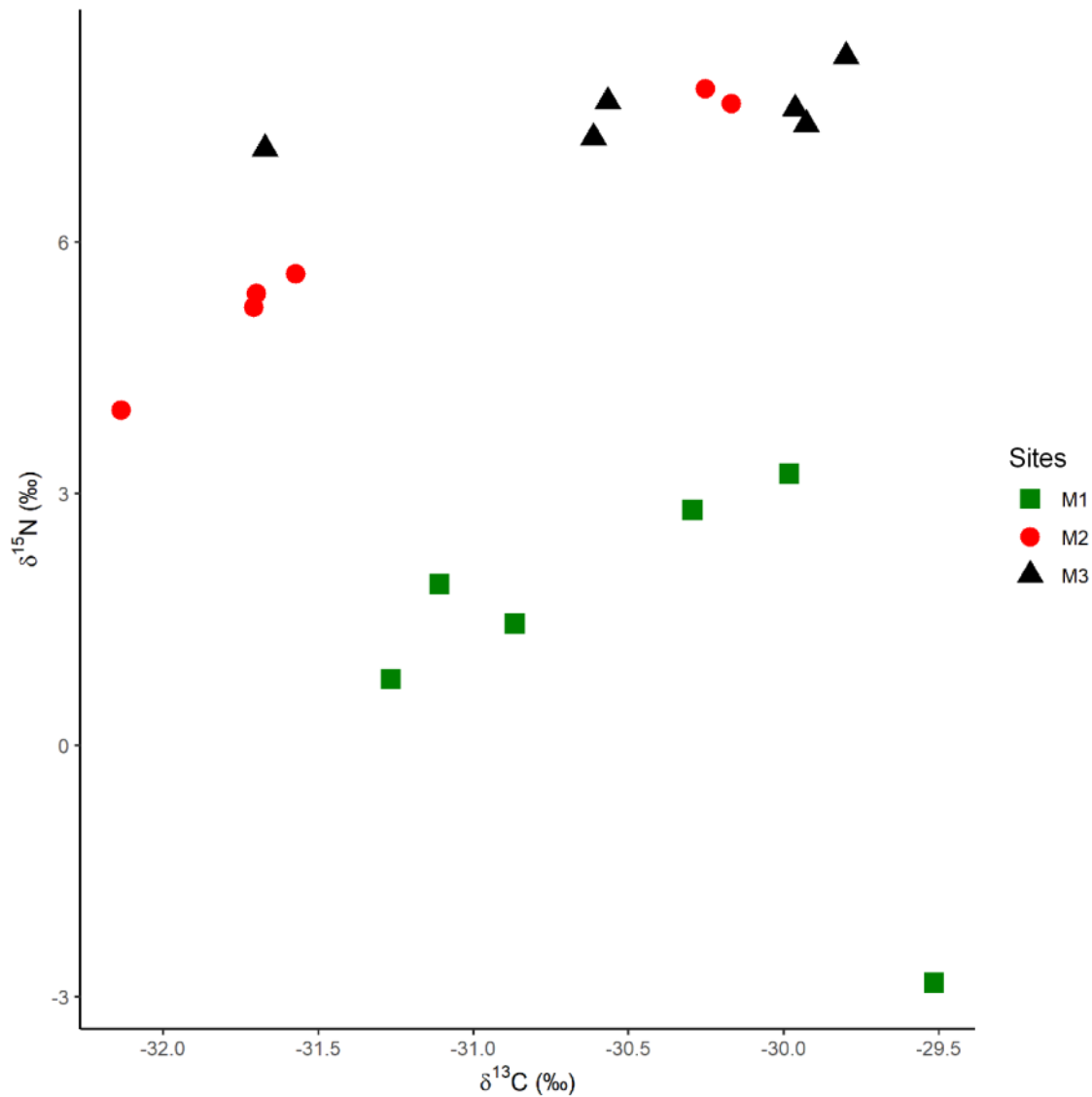
235 Data were analyzed with R software (version 4.0.5). Wilcoxon-Mann-Whitney  
236 comparisons were performed with the "stat" package. ANOSIMs analysis were  
237 performed with the package "vegan" (Oksanen et al., 2015).

### 238 **3. Results**

#### 239 3.1. Isotopic composition and C/N ratio

##### 240 3.1.1. Leaves

241 The  $\delta^{13}\text{C}$  signature of mangrove leaves was relatively similar between the three sites  
242 (mean  $\pm$  SD:  $29.9 \pm 1.4\text{‰}$ ; Fig. 2). Conversely,  $\delta^{15}\text{N}$  was higher in leaves from M3  
243 ( $7.3 \pm 0.4\text{‰}$ ) than in leaves of M2 ( $5.6 \pm 1.4\text{‰}$ ;  $p < 0.01$ ) and higher in leaves of M2 than  
244 in those of M1 ( $1.1 \pm 1.6\text{‰}$ ;  $p < 0.001$ ).



246

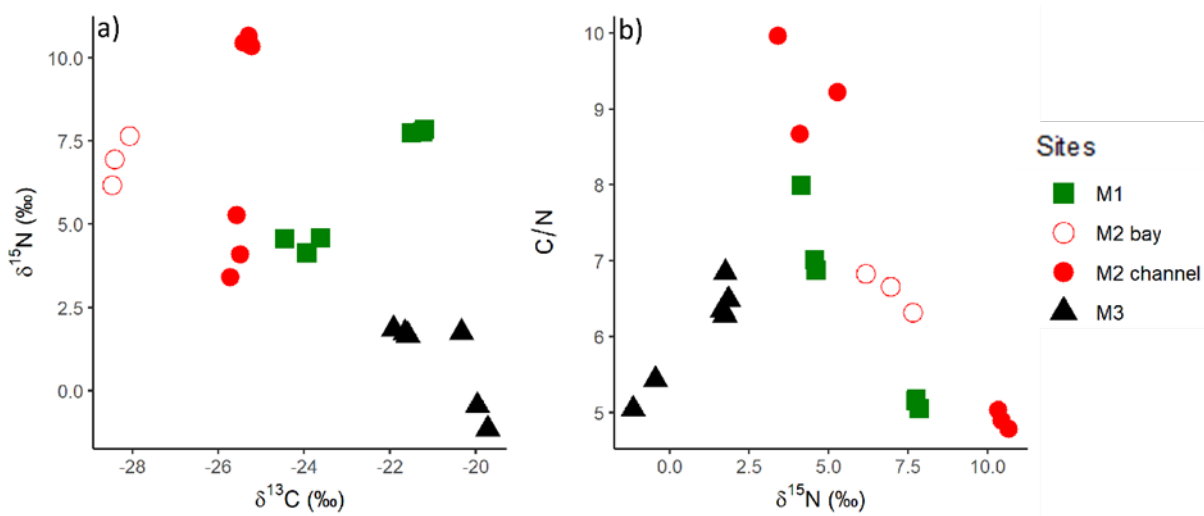
247 *Figure 2: Plot of (a)  $\delta^{13}\text{C}$  vs  $\delta^{15}\text{N}$  values of mangroves leaves in the three mangroves sites: M1 in*  
 248 *Ilha Grande Bay, M2 in Guanabara Bay and M3 in Sepetiba Bay. (1 Column)*

249

### 250 3.1.2. Particulate organic matter (POM)

251 The characterization of isotopic signatures and C/N ratio of POM were performed from  
 252 both plankton nets and GF/F filters for each zone. At the site M2, two areas were  
 253 sampled: the channel and the bay (Fig. 3).

254 The  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  signatures and C/N ratios of POM are presented in Figure 3. The  $\delta^{13}\text{C}$   
 255 values in M2 reached  $-28.3\pm 0.2\text{‰}$ . The  $\delta^{13}\text{C}$  values were significantly depleted at M2  
 256 channel ( $-25.5\pm 0.2\text{‰}$ ) than in M1 and M3 ( $-22.7\pm 1.5\text{‰}$  and  $-20.9\pm 1.0\text{‰}$ ;  $p < 0.01$ ). The  
 257  $\delta^{13}\text{C}$  signature of POM was similar at M1 and M3 ( $p > 0.05$ ). The  $\delta^{15}\text{N}$  of POM was  
 258 higher at M1 and M2 ( $6.1\pm 1.9\text{‰}$  and  $7.2\pm 2.8\text{‰}$ ) compared with M3 ( $0.9\pm 1.3\text{‰}$ ;  $p <$   
 259  $0.01$  &  $p < 0.001$ ). The C/N ratio values in the POM was  $6.4\pm 1.5$  on average and similar  
 260 between sites.

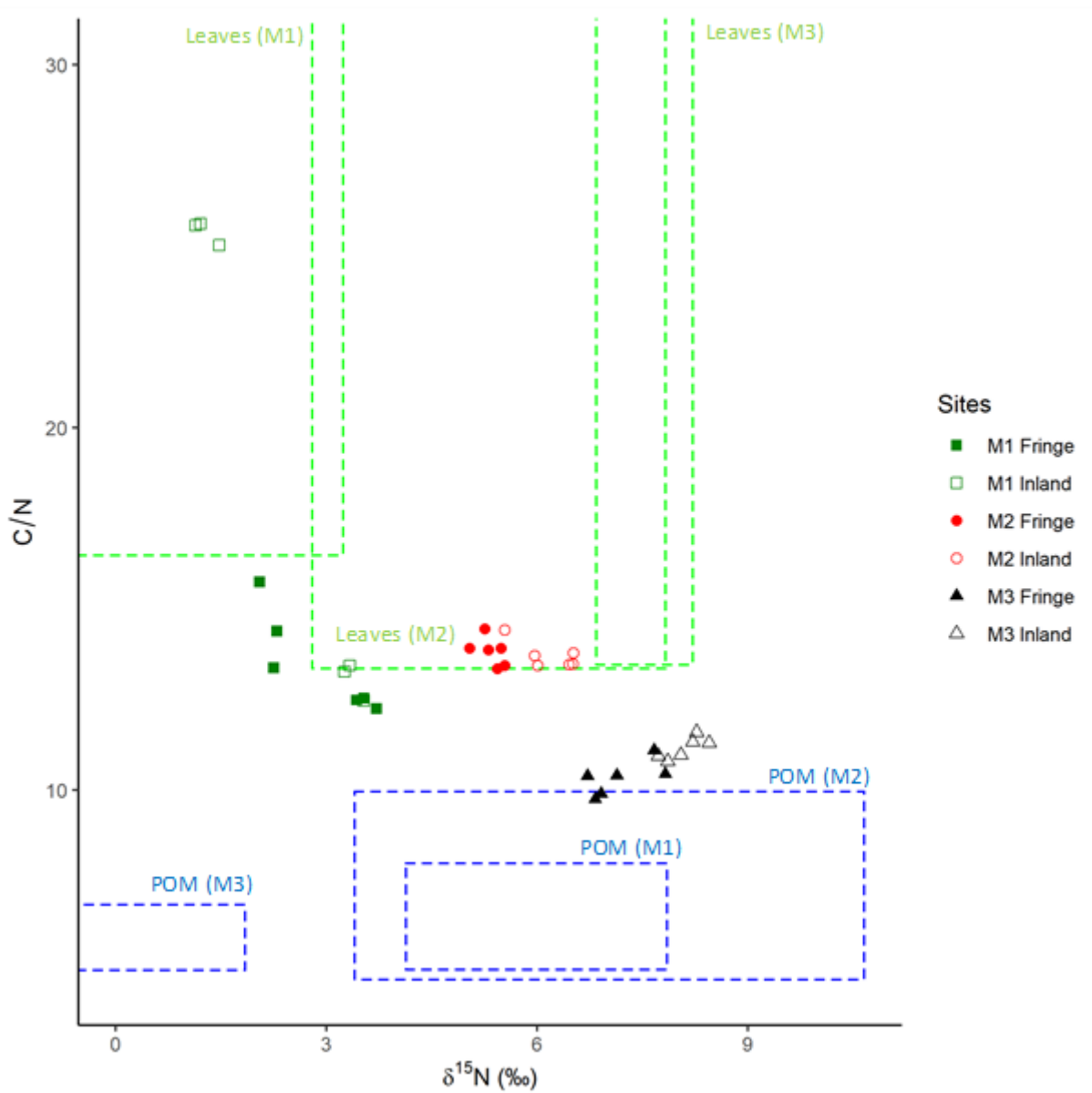


261  
 262 *Figure 3: Plot of (a)  $\delta^{13}\text{C}$  vs  $\delta^{15}\text{N}$  and (b)  $\delta^{15}\text{N}$  vs C/N values of particulate organic matter in the*  
 263 *three mangroves sites: M1 in Ilha Grande Bay, M2 in Guanabara Bay and M3 in Sepetiba Bay. (2*  
 264 *column)*

### 265 3.1.3. Sediments

266 There was no difference ( $p > 0.05$ ) at M1 in  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and C/N values between F and  
 267 I zone sediments (Fig. 4&5). At M2 I was significantly enriched in  $\delta^{15}\text{N}$  compared to  
 268 F (I:  $6.2\pm 0.4\text{‰}$ ; F:  $5.3\pm 0.2\text{‰}$ ;  $p < 0.01$ ), while  $\delta^{13}\text{C}$  and C/N were similar ( $p < 0.05$ )  
 269 between the two zones (Fig. 4&5). At M3 the I zone was depleted in  $\delta^{13}\text{C}$  ( $-25.1\pm 0.1\text{‰}$ ;  
 270  $-23.8\pm 0.4\text{‰}$ ;  $p < 0.01$ ), enriched in  $\delta^{15}\text{N}$  ( $8.1\pm 0.3\text{‰}$ ;  $7.2\pm 0.5\text{‰}$ ;  $p < 0.01$ ) and had a  
 271 higher C/N ratio ( $11.2\pm 0.3$ ;  $10.3\pm 0.5$ ;  $p < 0.01$ ) compared to the F sediments. No

272 differences in carbon or nitrogen isotopic signature or C/N ratio were observed  
273 between surface and subsurface (3 and 9 cm) sediments at all sites.

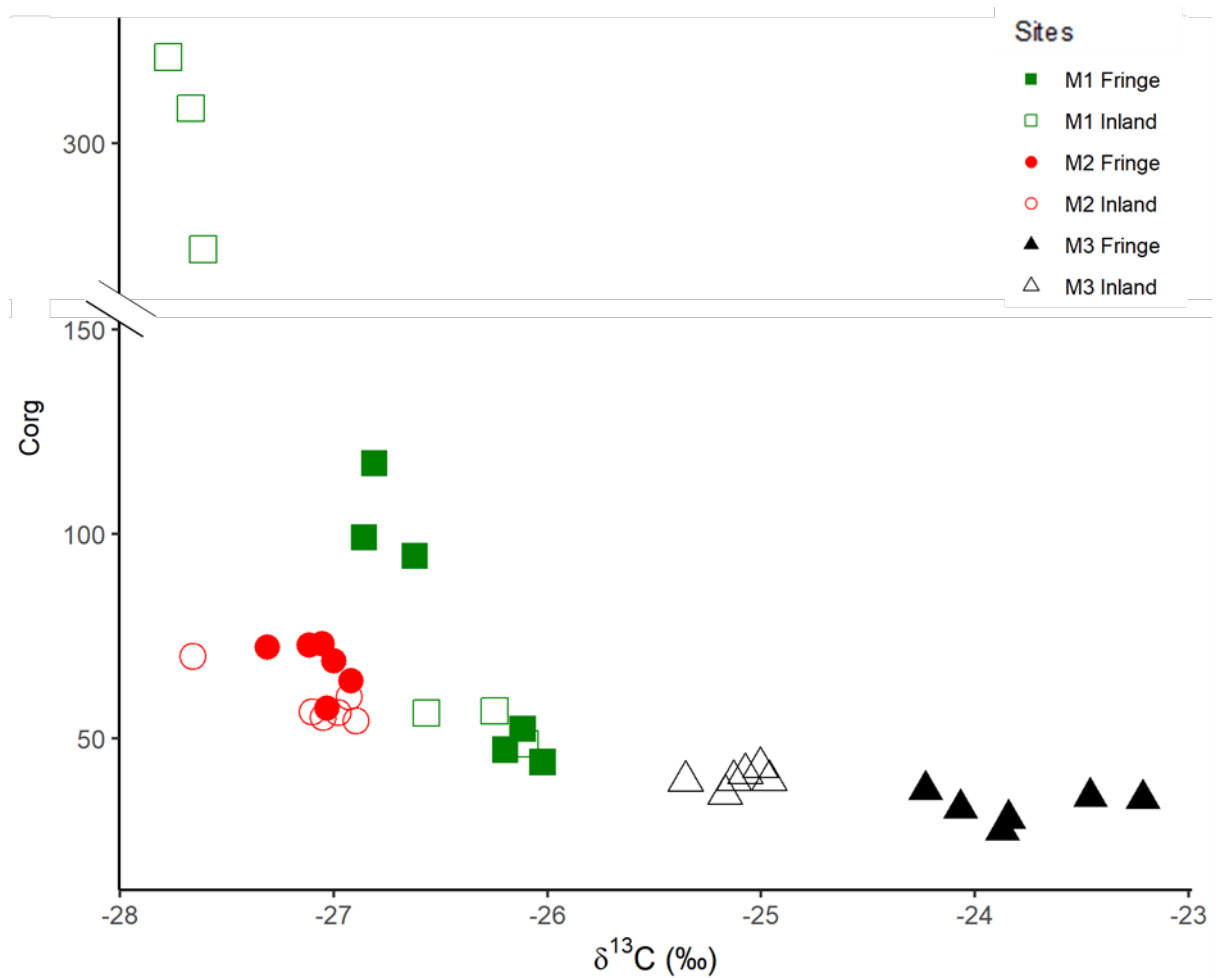


274  
275 *Figure 4: Plot of  $\delta^{15}\text{N}$  vs C/N in sediments mangroves of the three mangroves sites -: M1 in Ilha*  
276 *Grande Bay, M2 in Guanabara Bay and M3 in Sepetiba Bay. The envelopes of leaves are*  
277 *represented by green boxes and those of particulate organic matter in water are represented by*  
278 *blue boxes. (1 column)*

279  
280 Considering surface and subsurface sediments together as a pool, the  $\delta^{13}\text{C}$  was  
281 significantly depleted at M2 ( $-27.1\text{‰} \pm 0.2\text{‰}$ ) than at the other two sites (M1 :  $-26.8 \pm$



282 0.6‰,  $p < 0.05$ ; M3 :  $-24.4‰ \pm 0.7‰$ ,  $p < 0.001$ ). Similarly,  $\delta^{13}\text{C}$  was depleted at M1 (-  
283  $26.8‰ \pm 0.6‰$ ) compared to M3 ( $-24.4‰ \pm 0.7‰$ ;  $p < 0.001$ ). M1 has the lowest  $\delta^{15}\text{N}$ ,  
284 followed by M2 and then M3 ( $2.6 \pm 1.0‰ > 5.7 \pm 0.5‰ > 7.6 \pm 0.6‰$ ,  $p < 0.001$ ). The C/N  
285 ratio showed no significant difference between M1 and M2, but was significantly higher  
286 in subsurface sediments of I site of M1 mangrove (Fig. 4;  $p < 0.001$ ). The C/N values  
287 of surface sediments at the F and the I zones in M1 were comparable to those at M2.  
288 M1 and M2 had higher C/N values ( $16.3 \pm 5.5$  and  $13.8 \pm 0.4$  respectively) than M3  
289 ( $10.7 \pm 0.6$ ;  $p < 0.001$ ). M1 and M2 had higher sedimentary organic carbon ( $\text{C}_{\text{org}}$ )  
290 concentrations than M3 ( $126.6 \pm 108.3 \text{ mg.g}^{-1}$ ,  $54.2 \pm 7.6 \text{ mg.g}^{-1}$  and  $36.5 \pm 4.6 \text{ mg.g}^{-1}$ ;  $p$   
291  $< 0.001$ ). The high values and standard deviation of OC content in M1 sediments was  
292 due to the very high organic carbon concentrations in the subsurface sediment of zone  
293 I (Fig. 5).



294

295 *Figure 5: Plot of  $\delta^{13}\text{C}$  vs. organic carbon in sediments mangroves of the three mangroves sites :*  
 296 *M1 in Ilha Grande Bay, M2 in Guanabara Bay and M3 in Sepetiba Bay. (1 column)*

297

### 298 3.2. Fatty acids composition

#### 299 3.2.1. Mangrove leaves

300 Long chain fatty acids ( $\geq 24$  carbon) reached an average of 6.6% (Table 1). Among  
 301 PUFAs, 18:3 $\omega$ 3 was the dominant fatty acid followed by 18:2 $\omega$ 6. The monounsaturated  
 302 fatty acids (MUFAs) signature of mangrove leaves was almost entirely caused by the  
 303 presence of 18:1 $\omega$ 9.

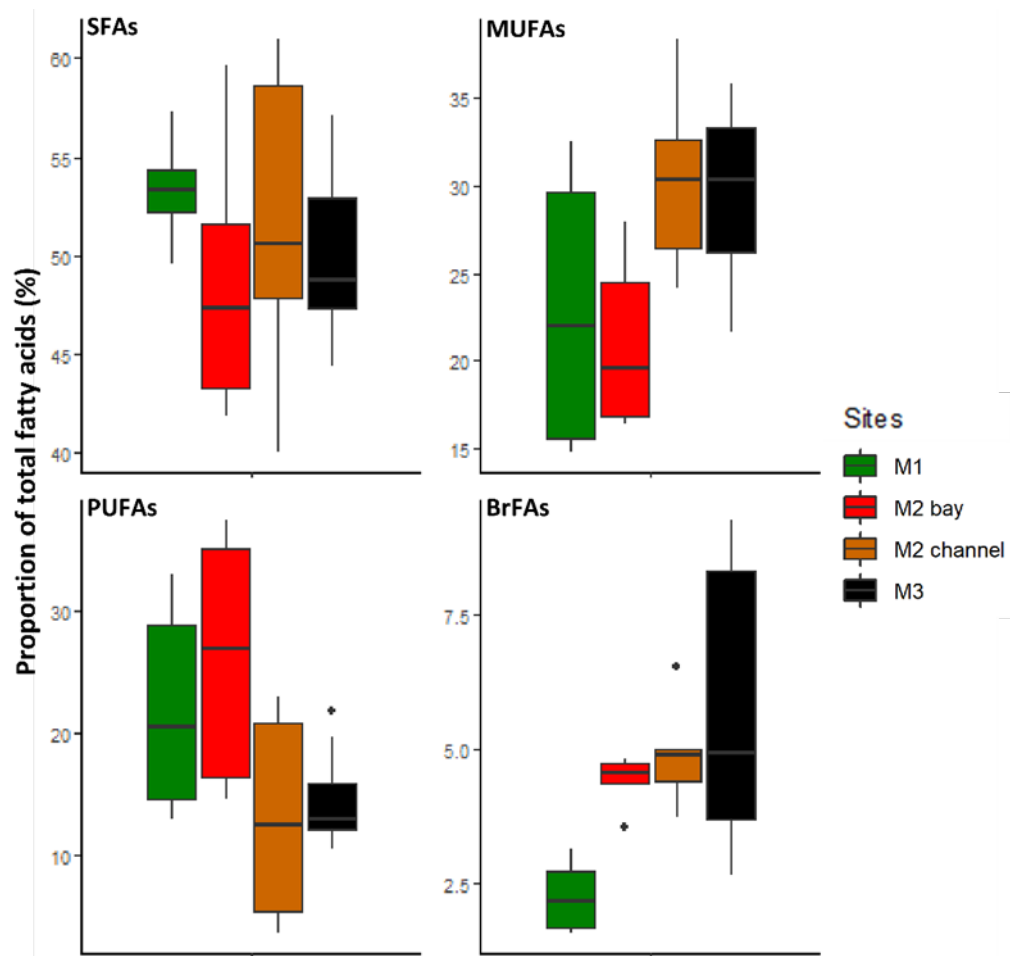
304 *Table 1 : Mean ( $\pm$  sd) of selected fatty acid composition of leaves in three sampling locations (M1*  
 305 *in Ilha Grande Bay, M2 in Guanabara Bay and M3 in Sepetiba Bay). BD: Below Detection (1*  
 306 *column)*

	Site M1		Site M2		Site M3	
	Fringe (n = 4)	Inland (n = 4)	Fringe (n = 4)	Inland (n = 4)	Fringe (n = 4)	Inland (n = 4)
<b>16 :0</b>	23.3 $\pm$ 2.5	31.2 $\pm$ 2.6	24.6 $\pm$ 1.5	22.9 $\pm$ 3.6	25.2 $\pm$ 6.8	31.9 $\pm$ 5.1
<b><math>\Sigma</math>LCFAs</b>	2.6 $\pm$ 2.8	4.5 $\pm$ 2.9	3.1 $\pm$ 2.6	2.8 $\pm$ 1.5	0.4 $\pm$ 0.3	9.0 $\pm$ 11.3
<b>Other SFAs</b>	3.2 $\pm$ 0.8	1.7 $\pm$ 0.6	6.3 $\pm$ 1.5	7.4 $\pm$ 2.3	4.4 $\pm$ 1.2	2.2 $\pm$ 1.0
<b><math>\Sigma</math>SFAs</b>	29.3 $\pm$ 5.1	41.3 $\pm$ 4.5	30.0 $\pm$ 1.7	28.8 $\pm$ 3.1	29.6 $\pm$ 7.6	44.7 $\pm$ 7.4
<b>18:1<math>\omega</math>9</b>	13.3 $\pm$ 1.1	9.2 $\pm$ 0.4	11.3 $\pm$ 1.3	12.7 $\pm$ 2.0	13.8 $\pm$ 2.4	12.9 $\pm$ 2.5
<b>Other MUFAs</b>	6.0 $\pm$ 2.6	10.1 $\pm$ 3.5	5.4 $\pm$ 2.3	5.9 $\pm$ 1.5	4.4 $\pm$ 0.9	12.8 $\pm$ 8.9
<b><math>\Sigma</math>MUFAs</b>	16.5 $\pm$ 1.1	10.9 $\pm$ 0.3	17.6 $\pm$ 1.3	20.2 $\pm$ 3.5	18.3 $\pm$ 3.0	15.1 $\pm$ 2.0
<b>18:2<math>\omega</math>6</b>	11.2 $\pm$ 1.9	12.0 $\pm$ 4.0	13.3 $\pm$ 2.0	13.6 $\pm$ 3.3	13.5 $\pm$ 2.9	15.0 $\pm$ 2.8
<b>18:3<math>\omega</math>3</b>	31.7 $\pm$ 12.9	34.3 $\pm$ 7.6	37.0 $\pm$ 1.3	35.7 $\pm$ 3.6	30.0 $\pm$ 8.4	23.7 $\pm$ 6.4
<b><math>\Sigma</math>PUFAs</b>	43.0 $\pm$ 12.2	46.9 $\pm$ 4.6	50.4 $\pm$ 1.7	49.3 $\pm$ 2.8	43.5 $\pm$ 6.8	38.9 $\pm$ 9.1
<b><math>\Sigma</math>BrFAs</b>	11.3 $\pm$ 13.5	1.5 $\pm$ 0.2	2.0 $\pm$ 0.3	1.6 $\pm$ 0.6	8.5 $\pm$ 11.1	1.5 $\pm$ 0.4

307

### 308 3.2.2. Particulate organic matter (POM)

309 The bay of M2 POM had significantly lower MUFA proportion than channel of M2 and  
 310 M3 (Table 2;  $p < 0.05$ ). At all sites, 16:1 $\omega$ 7 was the dominant fatty acid among MUFAs.  
 311 PUFAs at M3 and M2 (bay) was significantly lower than at M1 and M2 (channel) ( $p <$   
 312  $0.05$ ; Table 2; Fig. 6). 20:5 $\omega$ 3 and 22:6 $\omega$ 3 were the dominant PUFAs at all 4 sites  
 313 (Table 2). However, we observed a predominance of 20:5 $\omega$ 3 over 22:6 $\omega$ 3 at M3 ( $p <$   
 314  $0.001$ ). BrFAs contributed twofold more to the POM fatty acid pool at M3 and M2 than  
 315 at M1 (Table 2;  $p < 0.001$ ).



316

317 *Figure 6 : Contribution (% of total fatty acids) of SFAs, MUFAs, PUFAs and BrFAs to the total fatty*  
 318 *acid pool in four particulate organic matter sampling locations (Ilha Grande Bay (M1), Guanabara*  
 319 *Bay (M2 bay) and channel (M2 channel) and Sepetiba Bay (M3)). Median values (horizontal solid*  
 320 *line inside the box), 25th and 75th percentiles (lower and upper ends of the boxes), 95% confidence*  
 321 *intervals (whiskers) and outliers (circles) are shown. (1 column)*

322 Table 2: Mean ( $\pm$  sd) proportion (% of total fatty acids) of selected fatty acid composition of  
 323 particulate organic matter in four sampling locations (M1 in Ilha Grande Bay, M2 in Guanabara Bay  
 324 and M3 in Sepetiba Bay). BD: Below Detection (**1 column**)

	Site M1	Site M2 Channel	Site M2 Bay	Site M3
<b>14:0</b>	11.6 $\pm$ 1.5	9.8 $\pm$ 2.3	5.6 $\pm$ 0.7	18.3 $\pm$ 6.9
<b>16:0</b>	29.2 $\pm$ 1.9	29.0 $\pm$ 3.3	27.2 $\pm$ 3.2	22.9 $\pm$ 2.1
<b>18:0</b>	8.1 $\pm$ 1.3	6.7 $\pm$ 3.1	14.4 $\pm$ 8.6	4.8 $\pm$ 3.9
$\Sigma$ Other SFAs	4.2 $\pm$ 0.2	3.0 $\pm$ 0.5	4.6 $\pm$ 1.0	3.8 $\pm$ 1.0
$\Sigma$ SFAs	53.0 $\pm$ 2.5	48.5 $\pm$ 6.9	51.8 $\pm$ 8.1	49.9 $\pm$ 4.2
<b>16:1<math>\omega</math>7</b>	7.2 $\pm$ 1.0	12.5 $\pm$ 2.8	7.5 $\pm$ 2.8	17.9 $\pm$ 2.8
<b>18:1<math>\omega</math>9</b>	10.4 $\pm$ 7.9	3.8 $\pm$ 1.9	13.4 $\pm$ 6.4	5.7 $\pm$ 4.0
$\Sigma$ Other MUFAs	4.2 $\pm$ 1.5	4.6 $\pm$ 0.6	9.4 $\pm$ 3.6	6.9 $\pm$ 3.3
$\Sigma$ MUFAs	21.9 $\pm$ 8.1	20.9 $\pm$ 5.0	30.3 $\pm$ 5.2	29.7 $\pm$ 4.8
<b>20:5<math>\omega</math>3</b>	6.3 $\pm$ 3.6	9.1 $\pm$ 5.2	3.3 $\pm$ 2.7	6.2 $\pm$ 3.2
<b>22:6<math>\omega</math>3</b>	7.5 $\pm$ 4.9	7.9 $\pm$ 6.1	3.7 $\pm$ 3.0	1.5 $\pm$ 1.0
$\Sigma$ Other PUFAs	9.1 $\pm$ 0.7	9.1 $\pm$ 1.2	6.1 $\pm$ 3.1	6.9 $\pm$ 1.8
$\Sigma$ PUFAs	23.0 $\pm$ 8.1	26.1 $\pm$ 10.7	13.1 $\pm$ 8.7	14.5 $\pm$ 4.1
$\Sigma$ BrFAs	2.1 $\pm$ 0.6	4.4 $\pm$ 0.5	4.9 $\pm$ 0.9	5.8 $\pm$ 2.7

325

### 326 3.2.3. Sediments

#### 327 3.2.3.1. Variations with depth at each site

328 At M1, the percentages of LCFAs that were higher at 3-9 cm sediments of I zone than  
 329 in F zone ( $p < 0.01$ ). The fatty acid 16:1 $\omega$ 7 was significantly less abundant at the P3  
 330 and P9 depths at I zone of M1 than in the F zone and surface sediments at I zone ( $p$   
 331  $< 0.001$ ). The BrFAs group showed the same trend with a lower percentage for P3 and  
 332 P9 depth at the I zone of the M1 compared to the other samples ( $p < 0.05$ ). PUFAs  
 333 were the least abundant group at M1 and showed no significant difference between  
 334 the different depths of the F and I zones ( $p > 0.05$ ).

335 The FA composition of the mangrove I and F zones of M2 were not significantly  
 336 different (ANOSIM,  $R = 0.186$ ,  $p > 0.05$ ). Thus, for M2 data from I and F zones were  
 337 pooled and only tested according to depth. There was a significant difference in term  
 338 of FA composition between the three depths ( $R=0.62$ ;  $p=0.001$ ; Table 3). LCFAs

339 contributed twice ( $p < 0.05$ ) as much to the fatty acid pool at P9 depth as they did at P0  
340 and P3 depths. The fatty acid 16:1 $\omega$ 7 was more abundant at the surface than below.  
341 Conversely, 18:1 $\omega$ 9 was lower in surface sediments than at subsurface. The group of  
342 BrFAs was similar between the three sampled depths. The group of PUFAs tend to  
343 decreased from P0, to P9 (no significant difference).

344 At M3, the FA 16:1 $\omega$ 7 was more abundant in surface than in subsurface sediments at  
345 both zones F and I ( $p < 0.05$ ; Table 3). FA 18:1 $\omega$ 9 showed the opposite trend ( $p <$   
346  $0.05$ ). BrFAs were more abundant in I sediment profiles than in F ( $p < 0.05$ ). BrFAs  
347 were also more abundant in subsurface sediments (P3 and P9 depths) than in surface  
348 sediments ( $p < 0.05$ ). At M3, PUFAs did not differ significantly between I and F but  
349 were more abundant at the surface than at depth in the I ( $p < 0.05$ ) and more abundant  
350 at P0 and P3 than at P9 in the F ( $p < 0.05$ ).

351 Table 3: Mean ( $\pm$  sd) proportion (% of total fatty acids) of selected fatty acid composition of sediments of inland and fringe mangrove at 0 cm, 3 cm and  
 352 9cm in three sampling locations (M1 in Ilha Grande Bay, M2 in Guanabara Bay and M3 in Sepetiba Bay). BD: Below Detection (**2 column**)

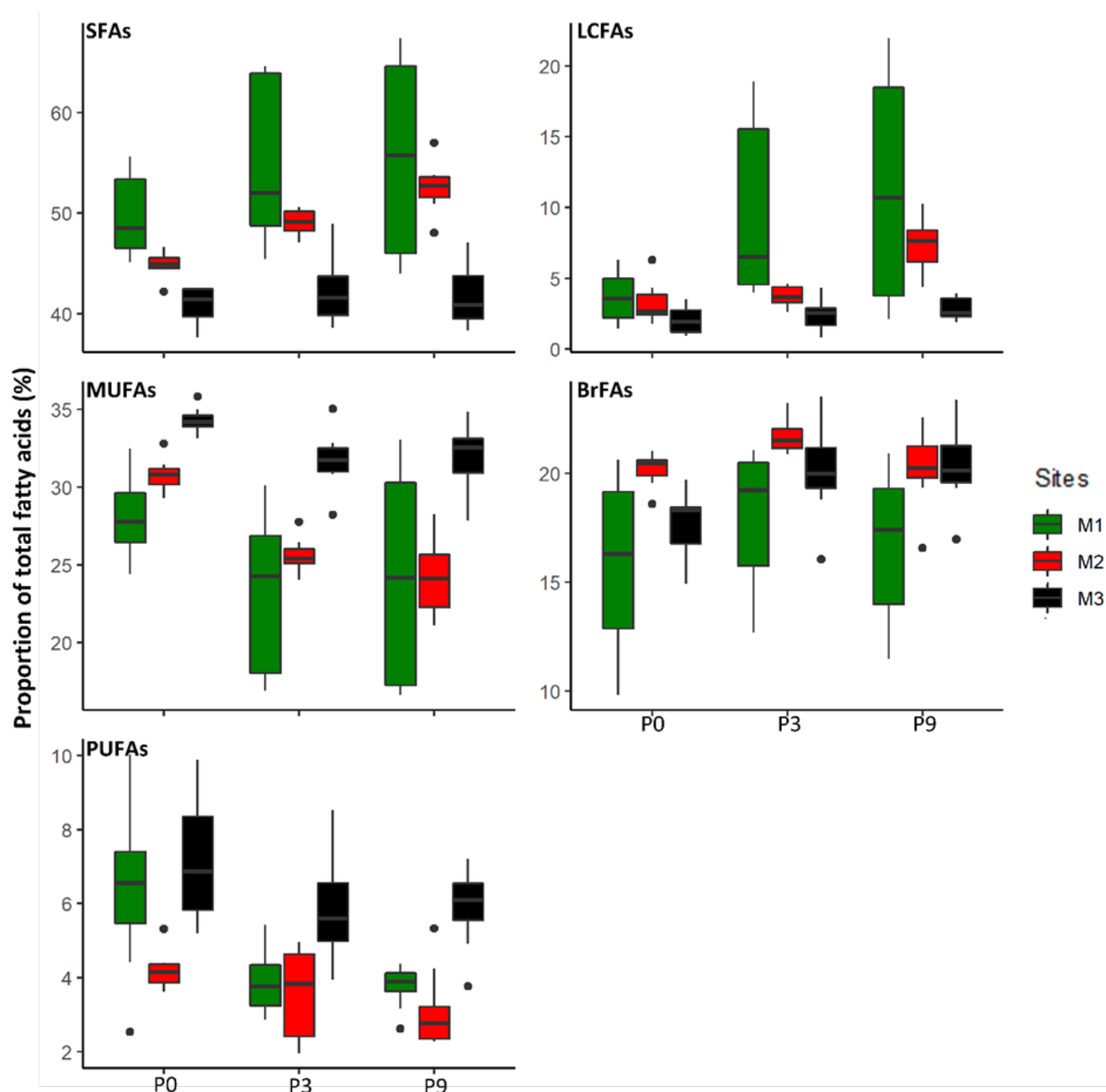
Depth	Site M1						Site M2						Site M3					
	Fringe			Inland			Fringe			Inland			Fringe			Inland		
	0 cm	3 cm	9 cm	0 cm	3 cm	9 cm	0 cm	3 cm	9 cm	0 cm	3 cm	9 cm	0 cm	3 cm	9 cm	0 cm	3 cm	9 cm
16:0	20.9 $\pm$ 1.8	22.1 $\pm$ 0.2	20.1 $\pm$ 0.6	20.7 $\pm$ 1.4	23.9 $\pm$ 1.8	23.1 $\pm$ 3.0	20.4 $\pm$ 0.7	22.6 $\pm$ 1.5	21.2 $\pm$ 0.4	22.4 $\pm$ 0.9	22.4 $\pm$ 0.6	22.4 $\pm$ 1.1	22.0 $\pm$ 0.7	21.1 $\pm$ 1.5	22.1 $\pm$ 1.7	21.1 $\pm$ 0.8	21.3 $\pm$ 0.4	20.8 $\pm$ 0.6
$\Sigma$ LCFAs	5.1 $\pm$ 0.9	4.5 $\pm$ 0.4	3.9 $\pm$ 1.8	3.4 $\pm$ 1.5	15.0 $\pm$ 5.1	18.9 $\pm$ 3.1	4.4 $\pm$ 1.9	3.7 $\pm$ 0.6	8.8 $\pm$ 1.3	2.5 $\pm$ 0.7	3.8 $\pm$ 1.1	5.9 $\pm$ 1.5	2.0 $\pm$ 1.2	2.9 $\pm$ 1.1	3.1 $\pm$ 0.6	2.1 $\pm$ 1.0	1.8 $\pm$ 1.1	2.6 $\pm$ 0.9
$\Sigma$ SFAs	47.3 $\pm$ 2.3	48.0 $\pm$ 1.8	45.8 $\pm$ 1.8	47.3 $\pm$ 1.2	61.8 $\pm$ 4.9	65.1 $\pm$ 1.6	44.6 $\pm$ 2.2	48.9 $\pm$ 1.1	54.4 $\pm$ 1.7	45.0 $\pm$ 0.6	49.3 $\pm$ 1.9	50.7 $\pm$ 1.9	42.5 $\pm$ 0.1	44.5 $\pm$ 3.2	44.5 $\pm$ 2.3	39.1 $\pm$ 1.4	39.4 $\pm$ 0.7	39.2 $\pm$ 0.6
16:1 $\omega$ 7	9.3 $\pm$ 0.7	7.3 $\pm$ 1.6	8.4 $\pm$ 0.7	6.0 $\pm$ 1.2	2.9 $\pm$ 0.8	2.1 $\pm$ 0.6	9.0 $\pm$ 1.9	5.2 $\pm$ 1.2	4.0 $\pm$ 0.9	8.4 $\pm$ 0.4	6.6 $\pm$ 1.1	5.7 $\pm$ 1.4	9.4 $\pm$ 1.9	4.3 $\pm$ 1.5	3.2 $\pm$ 0.6	6.8 $\pm$ 2.4	4.3 $\pm$ 0.8	4.3 $\pm$ 2.6
16:1 $\omega$ 5	1.3 $\pm$ 0.3	1.2 $\pm$ 0.3	1.6 $\pm$ 0.1	1.2 $\pm$ 0.2	0.7 $\pm$ 0.3	0.6 $\pm$ 0.2	2.0 $\pm$ 0.1	1.5 $\pm$ 0.3	1.2 $\pm$ 0.3	2.0 $\pm$ 0.2	1.6 $\pm$ 0.3	1.6 $\pm$ 0.2	2.2 $\pm$ 0.3	2.4 $\pm$ 0.3	2.6 $\pm$ 0.9	3.8 $\pm$ 0.7	4.1 $\pm$ 1.0	3.2 $\pm$ 0.2
18:1 $\omega$ 9	5.2 $\pm$ 0.3	6.7 $\pm$ 1.4	6.7 $\pm$ 0.7	8.2 $\pm$ 2.4	6.5 $\pm$ 1.0	6.7 $\pm$ 1.9	5.9 $\pm$ 0.2	8.9 $\pm$ 1.4	7.9 $\pm$ 1.1	7.3 $\pm$ 0.3	7.5 $\pm$ 0.3	8.3 $\pm$ 1.1	7.4 $\pm$ 0.3	10.3 $\pm$ 2.5	11.1 $\pm$ 0.7	8.2 $\pm$ 1.4	9.6 $\pm$ 0.6	10.9 $\pm$ 0.8
18:1 $\omega$ 7	8.2 $\pm$ 1.6	7.9 $\pm$ 1.3	8.1 $\pm$ 0.2	7.1 $\pm$ 0.5	3.5 $\pm$ 0.4	2.7 $\pm$ 0.7	8.3 $\pm$ 0.4	4.7 $\pm$ 0.7	3.9 $\pm$ 0.9	7.1 $\pm$ 0.3	5.6 $\pm$ 0.5	4.8 $\pm$ 1.1	7.4 $\pm$ 0.9	4.8 $\pm$ 1.3	4.4 $\pm$ 0.3	6.6 $\pm$ 0.3	5.8 $\pm$ 0.7	5.1 $\pm$ 0.4
19:1 $\omega$ 9	1.5 $\pm$ 0.2	1.8 $\pm$ 0.3	1.0 $\pm$ 0.1	1.0 $\pm$ 0.2	2.7 $\pm$ 0.4	2.5 $\pm$ 0.4	1.8 $\pm$ 0.1	2.5 $\pm$ 0.3	2.6 $\pm$ 0.5	1.7 $\pm$ 0.2	2.1 $\pm$ 0.1	2.3 $\pm$ 0.2	2.2 $\pm$ 0.8	4.3 $\pm$ 1.1	5.4 $\pm$ 0.6	4.8 $\pm$ 0.9	6.3 $\pm$ 0.5	6.7 $\pm$ 0.6
$\Sigma$ Other MUFAs	4.3 $\pm$ 0.8	2.8 $\pm$ 0.4	5.1 $\pm$ 0.9	4.7 $\pm$ 1.0	2.5 $\pm$ 0.9	2.7 $\pm$ 0.4	3.8 $\pm$ 0.3	2.3 $\pm$ 0.4	2.5 $\pm$ 0.3	4.3 $\pm$ 0.4	2.8 $\pm$ 1.1	3.4 $\pm$ 0.5	5.5 $\pm$ 0.8	4.4 $\pm$ 1.0	3.8 $\pm$ 0.6	4.4 $\pm$ 0.4	3.2 $\pm$ 0.3	3.1 $\pm$ 0.4
$\Sigma$ MUFAs	29.8 $\pm$ 2.9	27.7 $\pm$ 2.1	31.0 $\pm$ 1.5	28.2 $\pm$ 1.8	18.9 $\pm$ 2.8	17.3 $\pm$ 0.8	30.8 $\pm$ 1.8	25.2 $\pm$ 1.0	22.2 $\pm$ 1.0	30.8 $\pm$ 0.5	26.2 $\pm$ 1.3	26.1 $\pm$ 1.5	34.1 $\pm$ 0.8	30.5 $\pm$ 1.5	30.6 $\pm$ 2.2	34.5 $\pm$ 1.0	33.3 $\pm$ 1.5	33.3 $\pm$ 1.1
18:2 $\omega$ 6	1.3 $\pm$ 0.2	2.0 $\pm$ 1.1	1.0 $\pm$ 0.2	1.7 $\pm$ 0.7	3.0 $\pm$ 0.9	2.9 $\pm$ 0.8	1.2 $\pm$ 0.1	3.1 $\pm$ 1.0	2.3 $\pm$ 1.3	1.3 $\pm$ 0.1	1.1 $\pm$ 0.2	1.7 $\pm$ 0.8	1.6 $\pm$ 0.4	2.9 $\pm$ 0.9	3.0 $\pm$ 1.0	2.4 $\pm$ 1.4	2.4 $\pm$ 0.6	3.4 $\pm$ 0.6
20:4 $\omega$ 6	1.4 $\pm$ 0.3	0.6 $\pm$ 0.2	0.8 $\pm$ 0.2	0.9 $\pm$ 0.5	0.4 $\pm$ 0.2	BD	0.7 $\pm$ 0.1	0.5 $\pm$ 0.4	BD	0.7 $\pm$ 0.1	0.4 $\pm$ 0.1	0.3 $\pm$ 0.1	1.7 $\pm$ 1.1	1.5 $\pm$ 1.5	1.1 $\pm$ 0.3	1.8 $\pm$ 0.5	1.4 $\pm$ 0.4	1.0 $\pm$ 0.2
20:5 $\omega$ 3	1.6 $\pm$ 0.7	0.3 $\pm$ 0.1	0.7 $\pm$ 0.1	0.7 $\pm$ 0.3	BD	BD	0.7 $\pm$ 0.1	BD	BD	0.9 $\pm$ 0.2	BD	BD	1.5 $\pm$ 0.4	0.6 $\pm$ 0.6	BD	1.2 $\pm$ 0.6	BD	BD
$\Sigma$ Other PUFAs	2.0 $\pm$ 0.5	0.8 $\pm$ 0.1	1.2 $\pm$ 0.3	1.5 $\pm$ 0.4	0.6 $\pm$ 0.4	0.5 $\pm$ 0.4	1.4 $\pm$ 0.3	1.0 $\pm$ 0.2	0.8 $\pm$ 0.2	1.6 $\pm$ 0.3	0.6 $\pm$ 0.2	1.0 $\pm$ 0.2	1.9 $\pm$ 0.5	1.4 $\pm$ 0.4	1.4 $\pm$ 0.3	2.2 $\pm$ 0.3	1.2 $\pm$ 0.6	1.7 $\pm$ 0.3
$\Sigma$ PUFAs	6.3 $\pm$ 1.3	3.8 $\pm$ 0.8	3.7 $\pm$ 0.3	4.8 $\pm$ 1.8	4.0 $\pm$ 1.1	3.8 $\pm$ 0.8	4.0 $\pm$ 0.4	4.5 $\pm$ 0.5	3.3 $\pm$ 1.4	4.4 $\pm$ 0.6	2.3 $\pm$ 0.3	2.9 $\pm$ 0.9	6.8 $\pm$ 1.9	6.3 $\pm$ 1.7	5.7 $\pm$ 1.4	7.6 $\pm$ 1.7	5.3 $\pm$ 1.2	6.2 $\pm$ 1.0
$\Sigma$ BrFAs	16.5 $\pm$ 1.8	20.6 $\pm$ 0.4	19.5 $\pm$ 1.1	19.7 $\pm$ 0.7	15.4 $\pm$ 2.4	13.7 $\pm$ 2.3	20.7 $\pm$ 0.3	21.3 $\pm$ 0.6	20.1 $\pm$ 2.6	19.7 $\pm$ 0.8	22.2 $\pm$ 0.9	20.3 $\pm$ 0.8	16.7 $\pm$ 1.4	18.7 $\pm$ 1.8	19.3 $\pm$ 1.7	18.7 $\pm$ 0.7	22.0 $\pm$ 1.4	21.3 $\pm$ 1.6

353

### 354 3.2.3.2. Inter-sites comparison

355 At surface sediments, there was a significant difference in FA composition between  
356 the three mangrove sites (ANOSIM,  $R = 0.475$ ;  $p < 0.001$ ). LCFAs showed a higher  
357 contribution at M1 compared to M2 ( $p < 0.01$ ) and at M2 compared to M3 ( $p < 0.01$ ;  
358 Fig. 7). Among MUFAs, 16:1 $\omega$ 7 and 18:1 $\omega$ 9 had the largest contributions but did not  
359 show significant differences in surface sediments of the three sites ( $p > 0.05$ ; Table 3).  
360 BrFAs were significantly more abundant in surface sediment from M2 than from M1  
361 and M3 ( $p < 0.01$ ; Fig. 7). PUFAs contribute more to the TFA pool in the surface  
362 sediments of M1 and M3 than in M2 ( $p < 0.01$ , Fig. 7). In the surface sediments of M3  
363 there was a higher contribution of 20:5 $\omega$ 3 compared to M2 ( $p < 0.01$ ; Table 3).





364  
 365 *Figure 7: Contribution (% of total fatty acids) of SFAs, LCFAs, MUFAs, BrFAs and PUFAs to the*  
 366 *total fatty acid pool in the sediments of the three mangroves (M1 in Ilha Grande Bay, M2 in*  
 367 *Guanabara Bay and M3 in Sepetiba Bay.). Median values (horizontal solid line inside the box), 25th*  
 368 *and 75th percentiles (lower and upper ends of the boxes), 95% confidence intervals (whiskers) and*  
 369 *outliers (circles) are shown. (1 column)*

370 At three centimetres depth (P3), FA composition of the sediment were different  
 371 between the three sites (ANOSIM,  $R = 0.476$ ;  $p < 0.001$ ). The LCFAs were higher in M1  
 372 than in M2 ( $p < 0.01$ ) and in M2 than in M3 ( $p < 0.05$ ; Fig. 7). The most contributing

373 MUFAs were 16:1 $\omega$ 7, 18:1 $\omega$ 9, and 18:1 $\omega$ 7 (Table 3). BrFAs showed significant  
374 difference only between M1 and M2 ( $p < 0.01$ ; Fig. 7). PUFAs were less abundant in  
375 M1 and M2 than in M3 ( $p < 0.01$  and  $p < 0.05$  respectively; Fig. 7). Strong standard  
376 deviations were observed at M1 (both zones) for P3 sediments in contrast to the other  
377 two sites. These large standard deviations were caused by the strong difference in  
378 profiles between the I and the F zones of M1 (Fig. 7).

379 In sediments at P9 depth, the FA profiles were significantly different between the three  
380 sites (ANOSIM,  $R = 0.639$ ,  $p < 0.001$ ). LFCAs were particularly abundant in P9  
381 sediments at M1 and M2 but not at M3. MUFAs were less abundant in the sediment  
382 FA profiles of M1 and M2 compared to M3 ( $p < 0.05$  and  $< 0.001$  respectively; Fig. 7).  
383 The 16:1 $\omega$ 5 ( $p < 0.001$ ) was more abundant at M3 than at M1 and M2. BrFAs were  
384 lower at M1 compared to M2 and M3 (Fig. 7). PUFAs were less abundant in the P9  
385 sediments of M1 and M2 than in those of M3 ( $p < 0.01$ ; Fig. 7). Among PUFAs 18:2 $\omega$ 6  
386 was more abundant in the P9 sediments of M3 than those of M1 and M2 ( $p < 0.05$ ).

## 387 **4. Discussion**

### 388 **4.1. Eutrophication gradient in the coast of Rio de Janeiro**

389 Stable isotopes and fatty acids analysed in POM, SOM and mangrove leaves clearly  
390 indicate the existence of gradient of eutrophication in the three investigated mangroves  
391 in the state of Rio de Janeiro (Brazil). This gradient from M1 to M3 sites is consistent  
392 with urban occupation in the region (Table A1), as also analysed in Barroso et al. (in  
393 revision).

394 The high proportions of BrFA found at M3 highlight a strong bacterial (Table A2)  
395 contribution in the POM. Previous studies have shown that in the presence of  
396 wastewater, heterotrophic bacteria generate a strong recycling of nitrogen which leads  
397 to the excretion of ammonium with a depleted  $\delta^{15}\text{N}$  signature in the water column that  
398 can be further incorporated in the growth of marine phytoplankton (Ke et al., 2019).  
399 The high contribution of microalgal material in M3 mangroves POM is demonstrated  
400 by a high ratio of 16:1 $\omega$ 7/16:0 (Mortillaro et al., 2012; Napolitano et al., 1997) together  
401 with an enriched  $\delta^{13}\text{C}$  signature (Fig. 3). At site M3, these high microalgal and bacterial  
402 contributions in POM, lead to a  $\delta^{15}\text{N}$  signature near 0, therefore disconnected from  
403 SOM which is characterized by enriched  $\delta^{15}\text{N}$  signature of about 8 (sewage  
404 characteristic) (Fig. 3 & 4). Isotopic fractionation during nitrogen uptake (Liu et al.,  
405 2013) and the preferential use of  $^{14}\text{N}$ -rich ammonium (generated by bacterial recycling)  
406 as a nitrogen source by algae (Liu et al., 2013) could explain the  $\delta^{15}\text{N}$ -depleted isotopic  
407 signatures of the POM at M3. Our results reveal a greater influence of untreated  
408 sewage discharge to the M3 mangrove sediments compared to M1 and M2 consistent  
409 with Barroso et al. (in revision) based on the ratio of stanol biomarkers  
410 (coprostanol/(coprostanol+colestanol)). Urban sewage loads, enriched with nutrients,

411 received by M3 mangroves could also trigger the growth of marine microalgae (Unger  
412 et al., 2013) and generate important changes in the algal community (Smith, 2003). In  
413 the POM of the water column of M3, the relative abundance of 20:5 $\omega$ 3 was 3 times  
414 higher than that of 22:6 $\omega$ 3 (Table 3 and A2). This suggests that diatoms dominate  
415 dinoflagellates within the planktonic biomass unlike in the waters of the M1 and M2  
416 sites where the proportions of these two PUFAs were comparable (Antonio & Richoux,  
417 2016; Bergamino et al., 2014). In general, it is accepted that eutrophication favours  
418 dinoflagellates over diatoms (Dale, 2001). However, in eutrophic waters surrounding  
419 mangroves, a dominance of diatoms over dinoflagellates was also observed  
420 (Choudhury et al., 2015). In particular, high nitrogen concentrations are known to  
421 promote planktonic diatom growth in mangroves waters (Hilaluddin et al., 2020). In the  
422 waters of the Bay of Sepetiba, Rodrigues et al. (2009) showed a N/P ratio of 20 during  
423 the wet season as well as high concentrations of silica. In general, diatoms are  
424 favoured over dinoflagellates in coastal waters where nitrogen and silica are not limiting  
425 (Svensen et al., 2007). Thus, the nature of eutrophication-related inputs as well as the  
426 recycling of nitrogen by heterotrophs bacteria could explain the difference in the  
427 phytoplankton derived fatty acids in the M3 mangrove compared to the other two sites.

428 In addition, the  $\delta^{15}\text{N}$  signatures of sediments and leaves at the studied sites are  
429 showing a clear gradient of eutrophication (Fig. 2, Fig. 4). Because mangrove trees  
430 incorporate anthropogenic nitrogen present in the sediment porewaters, their  $\delta^{15}\text{N}$   
431 signatures can be used to identify a gradient of eutrophication locally or at a larger  
432 scale (Dugdale & Wilkerson, 1986; Gritcan et al., 2016). Sedimentary  $\delta^{15}\text{N}$  signatures  
433 are also sensitive to anthropogenic nitrogen inputs (Pérez et al., 2020). Accordingly,  
434 the mangrove surface sediments at M3 are the most eutrophic, followed by those of  
435 mangrove sediments at M2 and with M1 showing the most pristine conditions (Fig. 2

436 and Fig. 4). Barroso et al. (revised) also found at the same three study sites an increase  
437 in sedimentary inorganic phosphorus content from site M1 to M3. Several studies have  
438 described eutrophication in waters of Guanabara and Sepetiba Bays, where high  
439 concentrations of chlorophyll, inorganic nitrogen and phosphorus are found (Fistarol et  
440 al., 2015; Rodrigues et al., 2009). The eutrophication induced by domestic wastewater  
441 leads to an accumulation of exchangeable inorganic phosphorus in mangrove  
442 sediments (Barcellos et al., 2019). Although Guanabara Bay is more populated (Table  
443 A1) and eutrophic than Sepetiba Bay, because of local settings of the sampled  
444 mangroves, the M3 mangrove sampled in Sepetiba appeared more impacted by inputs  
445 of labile SOM from sewage and phytoplankton than the M2 mangrove in Guanabara  
446 Bay.

447 The C/N values in sediments in the inland of M1 mangrove (mean value: 19.3) are in  
448 the range of those reported in mangroves sediments under micro- to meso-tidal  
449 influence (i.e. Bouillon et al., 2003). These high values indicate a predominant input  
450 of mangrove tree material to the sediments. Indeed, sediments of inland M1 are  
451 composed of almost pure litter and *R. mangle* fine roots. In contrast, the fringe  
452 sediments at M1 and sediments of both fringe and inland zones at M2 mangrove have  
453 lower C/N ratios, in the range of those documented for mangroves influenced by tidal  
454 deposition and river inputs (Bouillon et al., 2003). The C/N values at M3 are even lower,  
455 in the range of values described by Leng & Lewis (2017) for riverine POC and by  
456 Jennerjahn and Ittekkot (1997) for sediments from another mangrove of the state of  
457 Rio de Janeiro influenced by a river (Paraiba do sul). This could be due to a larger  
458 influence of sewage inputs or marine phytoplankton in sediments of M3 than in the  
459 mangroves of the two other sites. This is also corroborated by the significantly depleted  
460  $\delta^{13}\text{C}$  signatures of superficial sediments in inland mangrove ( $-25,12 \pm 0,14$ ) compared

461 to the coastal fringe mangrove ( $-23,78 \pm 0,38$ ; more influenced by algal contributions)  
462 could be caused by a greater influence of urban wastewater at the inland zone, and/or  
463 of marine phytoplankton at the fringe zone.

#### 464 **4.2. Organic matter sources and exchange of OM between waters and** 465 **mangroves sediments**

466 At the most pristine mangrove location (M1), concentrations of  $C_{org}$  at the inland site  
467 were 3 times higher in subsurface sediments (3 and 9 cm depths) than at surface  
468 sediments (Fig. 5). At that site, we observed the presence of a dense rhizosphere of  
469 *R.mangle* in subsurface, in particular fine roots with very little silt. Root contribution  
470 was characterized by more than 3-fold higher proportions of LCFAs and 18:2 $\omega$ 6 (FAs  
471 characteristics of mangroves: Meziane et al., 2007; Table A2) and enriched  $\delta^{13}C$   
472 values in subsurface sediments compared to the surface ( $-26.3 \pm 0.2\text{‰}$  >  $-27.7 \pm 0.1\text{‰}$ ).  
473 The presence of *R.mangle* fine roots explains the higher  $C_{org}$  concentrations in these  
474 sediments at 3 and 9cm depth than in the surface sediments. Concerning the fringe  
475 M1 site, the sediment organic carbon concentration was between 3 and 7 times lower  
476 and the proportions of LCFAs and 18:2 $\omega$ 6 were 3 to 5-fold lower than at the inland site  
477 (both surface and subsurface; Fig. 5). This was apparently due to lower root density of  
478 *A. shaueriana* at the fringe compare to *R. mangle* at the inland site, as well as a larger  
479 contribution of deposited clays and silts at the fringe. This corroborates the study of  
480 Xiong et al. (2018) which shows a lower contribution of mangrove vegetation in  
481 mangroves near the sea than in those located inland. The sediments of fringe M1  
482 mangrove have  $\delta^{13}C$ -enriched signature compared to the inland subsurface sediments  
483 which reveals a greater influence of marine phytoplankton in the OM. The POM at M1  
484 was characterized by a high relative contribution of 18:1 $\omega$ 9, abundant in mangrove leaf,

485 and  $\delta^{13}\text{C}$  values close to those of mangrove leaves. This suggests an effective tidal  
486 export of mangrove-derived POM at site M1.

487 Concentrations of  $C_{\text{org}}$  in subsurface sediments at the inland site of M1 mangrove were  
488 4 to 6 times higher than M2 sediments, and between 7 and 10 times higher than in M3  
489 sediments. These lower  $C_{\text{org}}$  concentrations in the two more eutrophic mangroves  
490 when compared to the more pristine one can certainly be caused by the watersheds  
491 that convey more mineral matter *via* a higher river flow, which leads to a lower OM  
492 contribution to the sediments. In addition, the organic matter in a pristine mangrove in  
493 Ilha Grande (about 40 km of our M1 mangrove) was composed almost exclusively of  
494 mangrove material, with insignificant inputs of marine-derived organic matter (Sanders  
495 et al. 2008) similarly to our M1 site. This is consistent with the high contribution of  
496 LCFAs and 18:2 $\omega$ 6, the  $\delta^{13}\text{C}$  values in our samples, as well as with lignin and sterol  
497 biomarkers which revealed that mangrove's subsurface sediment at M1 and M2 are  
498 richer in plant material than at M3 (Barroso et al., revised). In the most eutrophic M3  
499 mangroves, the additional OM supply was due to a contribution of phytoplankton,  
500 benthic algae, and urban sources (Pérez et al., 2020; Silva-Filho et al., 2011). Finally,  
501 river inputs rich in mineral sediments has a strong influence on  $C_{\text{org}}$  concentration by  
502 diluting it.

503 At high tide, as for other estuaries and bays (Bouillon et al., 2008b), the water columns  
504 of the three studied bays contain some POM resuspended from the mangrove forest.  
505 The presence of 18:2 $\omega$ 6 and 18:3 $\omega$ 3 FAs (David et al. 2019) in the POM samples in  
506 the creeks and channels at all sites indicates an export of mangrove organic carbon to  
507 waters (Table 2 and A2). At M2, the higher contribution of OM produced in the  
508 mangrove forest and/or of terrestrial origin is also reflected by the signature of  $\delta^{13}\text{C}$  of  
509 the water column POM which is more depleted than the two other sites (M1 and M2).

510 At M1 (fringe) and M2, the  $\delta^{13}\text{C}$  values (respectively 26.7‰ and 27.1‰ on average)  
511 are typical of tidal sediments influenced by inputs from mangroves leaves (Kennedy et  
512 al., 2004; Pessenda et al., 2012) and therefore emphasize the significant contribution  
513 of mangroves carbon to the POM. The difference in  $\delta^{13}\text{C}$  signature between mangrove  
514 leaves and sediments at M1 (fringe) and M2 (fringe and inland) mangroves could be  
515 the result of a mixing with marine OM but also of diagenesis of litter compounds which  
516 leads to the enrichment in  $^{13}\text{C}$  of the sedimentary OM (Kennedy et al., 2004). At M3  
517 site, the low  $\delta^{13}\text{C}$  and C/N values in sediment (Fig. 4 & 5) corroborate the significant  
518 contribution of marine phytoplankton and/or microphytobenthic OM sources.

#### 519 **4.3. Impact of eutrophication on organic carbon degradation in mangrove** 520 **sediments**

521 At M1, the sedimentary OM appears relatively refractory with a high proportion of  
522 LCFAs and a low proportion of algal FAs, which are respectively the most refractory  
523 and the most labile fatty acids (Camacho-Ibar et al., 2003). Conversely, in mangroves  
524 of the more eutrophic bays (M2 and M3) the relative contributions of algal FAs as well  
525 as the enriched  $^{13}\text{C}$  signature suggest that SOM is more labile than at M1. Moreover,  
526 C/N values of sediments and water at M2 (~13 and 6 respectively) and M3 (~10 and 6  
527 respectively) indicate a higher microbial contribution than at M1 in view of their low  
528 values in bacteria (C/N ~ 5; Leng & Lewis (2017)) and  
529 phytoplankton/microphytobenthos (C/N ~ 6-7; Leng & Lewis (2017)) compared to  
530 mangrove leaves (C/N~22; present study). The C/N consistently decreased from site  
531 M1 to M2 and M3 and was 1.2 and 1.6 times lower at M2 and M3 respectively than at  
532 M1 (Fig. 4) showing that when eutrophication occurs, nitrogen-rich compounds  
533 become more abundant in SOM, relative to carbon-rich compounds. Also, along the  
534 eutrophication gradient of our three study sites, FAs,  $^{13}\text{C}$  signatures and C/N ratios



535 consistently reveal a larger contribution in the SOM of algal and microbial material,  
536 which are more degradable, than the part derived from litters and roots in the mangrove  
537 ecosystem. Thus, in our study, the C/N decreases with lability which is consistent with  
538 the finding of Pérez et al. (2018b) in another mangrove of Guanabara Bay. This is also  
539 consistent with Jennerjahn & Ittekkot (1997 and 1999) who show that the C/N ratio is  
540 low in fresh planktonic biomass but high in plant material or material that has  
541 undergone degradation because N-rich compounds such as amino acids are  
542 preferentially degraded. However, the variability of the lability of N-rich compounds  
543 showed by Nordhaus et al. (2011 and 2017) on total hydrolysable amino acids (THAA)  
544 reveal that low C/N ratio is not necessarily an indicator of higher lability of OM. Indeed,  
545 Nordhaus et al. (2017) found that during a litterbag experiments in an Indonesian  
546 mangrove, THAA concentration was negatively correlated with the C/N but that the  
547 basic THAA (more reactive than other amino acids groups) were preferentially  
548 degraded over other amino acids and that their concentration was not correlated with  
549 C/N ratio. Nordhaus et al. (2011) also found that crabs of various species feed  
550 preferentially on mangrove leaves with high N bioavailability (shown by THAA-N% of  
551 N) or high reactivity index (aromatic THAA/non-protein THAA) and that the food  
552 preferences of these crabs is not correlated with C/N composition. Various parameters  
553 can also influence the lability of SOM in a eutrophic mangrove such as, effluent type  
554 (Queiroz et al., 2020), distance from the pollution source (Queiroz et al., 2020),  
555 planktonic primary production (Cotovicz et al., 2018; Sanders et al., 2014) or  
556 heterotrophic activity (Davis et al., 2003).

557 The M2 mangrove was found to be moderately impacted by eutrophication. However,  
558 in both zones of M2 mangrove, SOM exhibits higher bacterial contribution than M1 and  
559 M3 as evidenced by the higher proportions of BrFAs (Mfilinge et al., 2003). The

560 proliferation of these bacteria in the SOM at M2 is enhanced by the presence of labile  
561 algal OM in sediments, as indicated by the high 16:1 $\omega$ 7/16:0 ratio, acting as a substrate  
562 for their growth (Bouillon et al., 2004). These FAs data suggest a higher activity of  
563 bacteria at M2 mangrove than at M1 mangrove, consistent with higher CO<sub>2</sub> emissions  
564 to the atmosphere reported by Barroso et al. (revised). At the same time, M1 and M2  
565 sediments have comparable contribution of LCFAs which indicate they have a similar  
566 contribution of refractory OM. At M2, our data suggest that bacteria degrade algal labile  
567 OM in surface sediments but do not mineralize the more refractory fraction of OM. This  
568 corroborates studies that have shown that eutrophication enhances OM sedimentation  
569 (Sanders et al. 2014) and at the same time enhances OM remineralization (Barroso et  
570 al. revised; Chen et al., 2010; Jessen, 2016; Martin et al., 2020).

571 The M3 was the most eutrophised site, particularly because of the sewage effluent  
572 inputs. High bacterial biomass in the POM is evidenced by the high proportion of BrFAs  
573 at M3 (Table 2), which may be attributed to sewage organic matter degraded by  
574 bacteria and fungi (Blaszczyk & Krzysko-Lupicka, 2013; Kaneda, 1991). Relative  
575 contributions of fungal markers (16:1 $\omega$ 5 and 18:1 $\omega$ 9; Chen et al., 2001; Meziane et al.,  
576 2006; Ngosong et al., 2012; Table A2) in the POM were not different at M1, M2 and  
577 M3 sites. In contrast, in the surface and subsurface sediments, these fungal FAs were  
578 1.2 to 2 times more abundant at site M3 than at sites M1 and M2 (Table 3). The fact  
579 that at M3, high contribution of fungal biomarkers was measured in the SOM, but not  
580 in the POM suggests that fungal growth occurred in the sediment. Fungi degrade  
581 complex and refractory compounds into more labile OM and thereby make OM  
582 bioavailable to bacteria (Chung & Suberkropp, 2008; Ferreira et al., 2006). The higher  
583 CO<sub>2</sub> fluxes at M3 than at M1 and M2 (Barroso et al., revised) can therefore be caused  
584 by the more efficient degradation of OM through the microbial loop stimulated by the

585 higher fungal contribution through the inputs of labile organic matter (Fenchel, 2008).  
586 In addition, the increase of fungal biomass at subsurface sediments could lead to a  
587 stronger degradation of low-quality OM at depth (LCFAs), contribute to increase the  
588 CO<sub>2</sub> effluxes. Finally, even though C<sub>org</sub> concentration is lower at M3 than at M1 and M2  
589 mainly due to the higher inputs of mineral material, the enhanced microbial loop could  
590 also to a lesser extent explain this measure. Our result reveal that coastal  
591 eutrophication results in a mixing of refractory mangrove derived SOM with labile OM  
592 from algal and sewage sources and this mixing enhances the fungal and bacterial  
593 growth and CO<sub>2</sub> fluxes, potentially through a priming effect particularly at the M3 site.

## 594 **5. Conclusion**

595 This study provides combined data of fatty acid markers along with  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$   
596 signatures that characterizes the source of OM in three mangroves within the state of  
597 Rio de Janeiro. These mangroves are located along a eutrophication gradient as  
598 assessed by  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  signatures and C/N ratio in sediments, and particulate organic  
599 matter. The organic matter at the more impacted site M3 sediments was found to be  
600 supplied with greater algal or microphytobenthic biomasses than in the lesser impacted  
601 site, M1 and M2, in which mangrove production is found to be the main organic input.  
602 The SOM at M3 was more labile as indicated by the high contribution of PUFAs.  
603 Concomitantly to the algal biomass increase, biomasses of fungi were higher within  
604 the M3 sediments than at pristine M1 which suggests an enhancement of the  
605 microbial loop. In the highly impacted M3 mangrove sediments, refractory LCFAs, were  
606 in lower proportion than in the sediments of pristine site. Although the lower  
607 concentration of C<sub>org</sub> at M3 than at M2 and M1 may be preliminary due to a larger input  
608 of mineral particles by rivers, it could also be promoted by a priming effect that  
609 potentially decomposes the refractory OM from the mangrove vegetation. In view of

610 the preliminary data presented here, a better understanding of the biogeochemical  
611 mechanisms involved in this important global storage of carbon could be gained by  
612 implementing experiments under controlled conditions in which known amounts of  
613 several OM sources can be added.

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