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1 **Fatty acids, C and N dynamics and stable isotope ratios during experimental**
2 **degradation of shrimp pond effluents in mangrove water**

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21 Abstract

22 Intensive shrimp farming generates high loads of wastewaters that are released
23 along tropical coastlines with potential impacts on the ecosystems. In this study, we
24 used an experimental approach to analyze the behavior of shrimp pond effluents
25 released in the Can Gio mangrove waterways (Southern Vietnam). We incubated shrimp
26 pond effluents (EF), river water (RV), and a mixture of both (MI; 90% RV + 10% EF) in a
27 dark room and measured fatty acid (FA) compositions, C and N concentrations and
28 stable isotopes ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of suspended particulate matter during 16 days.
29 Fatty acid concentrations rapidly decreased in EF with a 50% loss of FA during the first
30 24h of the experiment and a 75% loss after 4 days of incubation. Proportions of the FA
31 18:1 ω 7 increased in MI during incubation, suggesting that this FA may be used as a
32 tracer of anthropogenic substances release in marine environments.

33

34 *Keywords*

35 Lipid; stable isotopes; aquaculture; decomposition; denitrification, suspended particulate
36 matter (SPM); mangrove

37

38

39 *Abbreviations*

40 BrFA: branched fatty acid

41 DIN: dissolved inorganic nitrogen

42 EF: shrimp pond effluents

43 FA: fatty acid

44 MI: 10% shrimp pond effluents + 90% river water

45 OM: organic matter

46 POC: particulate organic carbon

47 PUFA: polyunsaturated fatty acid

48 RV: river water

49 SPM: suspended particulate matter

50 TFA: total fatty acids

51

52

53 1. Introduction

54 Shrimp farming has strongly grown in the last decades. Global shrimp production
55 was 1.1 billion tons in 2000, 3.4 billion tons in 2008 and reached 4.9 billion tons in 2015
56 (FAO, 2016). Although shrimp farming generates important social and economic
57 benefits (Primavera, 1997), its huge development is strongly criticized for its negative
58 environmental impacts (Anh et al., 2010). Intensive shrimp farming is characterized by
59 high shrimp density, high amount of inputs (food pellets, fertilizers and antibiotics) and
60 high water renewal in ponds (15% every two weeks in Vietnam; Anh et al. 2010). This
61 leads to high effluent releases (including dissolved nutrients, toxic compounds,
62 microorganisms, shrimp excrements, unconsumed food pellets, etc.) associated with
63 highly degraded water quality close to shrimp farming areas (Molnar et al., 2013; Páez-
64 Osuna, 2001; Páez-Osuna and Ruiz-Fernández, 2005) that may stimulate algal blooms
65 and create anoxic water conditions (Kemp et al., 2009; Naylor et al., 1998; Smith et al.,
66 1999).

67 Despite potential impacts of shrimp pond effluent discharges in estuarine waters
68 are recognized, their effect is mostly investigated regarding nutrients (Páez-Osuna and
69 Ruiz-Fernández, 2005; Trott and Alongi, 2000) and other parameters are poorly
70 documented, as for example the nutritional value of impacted water suspended
71 particulate matter (SPM). In New Caledonia, Aschenbroich et al. (2015) have shown that
72 the nutritional quality of mangrove surface sediments was affected by shrimp pond
73 effluents releases (i. e. modified fatty acid composition). Indeed, sediments integrate

74 chronic disturbances on a relatively long-time basis (Molnar et al., 2013), while SPM
75 naturally varies at short spatial scales in mangrove estuaries (Trott and Alongi, 2000),
76 making difficult to differentiate changes induced by environmental parameters (e.g.
77 season, salinity, SPM concentration, daylight presence) from those due to shrimp pond
78 effluent releases (David et al. 2019).

79 Lipids have been successfully employed to detail the nutritional composition of
80 organic matter (OM) in marine ecosystems (Canuel, 2001; David et al., 2019; Meziane
81 and Tsuchiya, 2000). They are good indicators of microalgae and bacteria relative
82 abundances and allow to evaluate the physiological state of phytoplankton cells (Pan et
83 al., 2017, 2014). In complement to fatty acids, stable isotopes may inform on the
84 processes occurring during OM decomposition (Caraco et al., 1998; De Brabandere et
85 al., 2002; Pan et al., 2014; 2017). Finally, nitrogen is a major compound of OM that is
86 newly made available for primary producers after OM has been degraded, thus
87 releasing ammonia in the surrounding waters which can fuel nitrification and
88 eutrophication.

89 A few studies have been examining the degradation of lipids from urban sewages
90 in natural environments (Matsumoto, 1983; Marty et al., 1996) but none has yet
91 investigated their decomposition in shrimp farm effluents, although the latter are known
92 to cause serious threats to coastal ecosystems in Southeast Asia (e.g. increasing
93 nitrogen influx and change in C/N balance; Lee, 2016). The Can Gio mangrove
94 biosphere reserve is located at the downstream end of Ho Chi Minh City (Southern
95 Vietnam) and covers an area of 719.6 km² (Tuan and Kuenzer, 2012). At the north-east
96 border of the mangrove forest, a transition area between the forested core and the

97 Southern limit of Ho Chi Minh City covers about 300 km² where shrimp farming is the
98 main economic activity, releasing wastewaters in the nearby waterways, with potential
99 impacts (e.g. high nutrient loading, spread of diseases, antibiotics accumulation in
100 sediments) on the ecosystem (Le and Muneke 2004, Anh et al., 2010). The release of
101 shrimp ponds effluents is not controlled in this region. Effluents are not being treated
102 and directly released on defined schedules in the nearby rivers throughout the year (Anh
103 et al., 2010), thus rapidly reaching the 600m wide and 10-20m deep main estuary
104 located a few hundred meters downstream and further flooding the mangrove forest (a
105 map of the Can Gio mangrove biosphere reserve can be found in David et al. 2019).

106 In the present study, we used an experimental approach based on the one
107 employed by Marty et al. (1996) to examine changes in SPM nutritional quality during
108 decomposition of shrimp pond effluents released in a mangrove river in the Can Gio
109 district. We aimed at characterizing the initial FA composition of shrimp pond effluents
110 and then follow the dynamics of FA once their release in the environment has been
111 simulated. We also measured variations of carbon and nitrogen forms and their isotopic
112 ratios as markers of degradation processes and their kinetics. We expected to measure
113 elevated phytoplanktonic abundances in shrimp ponds, as they generally exhibit high
114 primary production (Burford et al. 2003), which may induce a strong bacterial
115 development during effluents decomposition in mangrove waters. We sampled water
116 from shrimp ponds and a nearby mangrove river to study the degradation of lipids in
117 SPM in the absence of light (thus imitating natural conditions in the deep and turbid
118 estuary). Pure shrimp pond effluents, pure river water and a combination of both were
119 incubated in controlled conditions during 16 days. We hypothesized that (1) specific

120 bacterial strains (e.g. denitrifying ones) and/or degradation pathways were favored in a
121 mix of shrimp pond effluents with river water compared to both waters taken in isolation
122 (2) specific fatty acids could be used as tracers of shrimp pond effluents release in
123 natural ecosystems.

124

125 2. Materials and methods

126 2.1. Water collection

127 Water samples were collected in shrimp ponds and a nearby mangrove river of
128 the Can Gio mangrove Biosphere Reserve in February 2017. Surface estuarine water
129 (river water; RV) was collected using a wired bucket from the middle of the Rung Sat
130 bridge (10° 35' 2.94'' N; 106° 49' 32.68'' E) at the beginning of ebb tide, while a
131 composite sample of shrimp pond effluents (EF) was made using water from three
132 different intensive shrimp ponds (40-60 shrimps/m²) located close to the river water
133 sampling point.

134

135 2.2. Experimental design

136 Water samples were brought in white plastic jerrycans at ambient temperature to
137 the CARE (Centre Asiatique de Recherche sur l'Eau) facilities in Ho Chi Minh City in
138 February 2017. Jerrycans containing the same water source (EF or RV) were mixed
139 together before being disseminated in 14L plastic buckets. These buckets were meant
140 to test three conditions: (1) pure shrimp pond effluents (EF), (2) pure river water (RV)
141 and, (3) a combination of both (mixed water; MI: 90% RV + 10% EF). Each experimental

142 condition was tested in 4 replicates (12 buckets). The amount of wastewaters released
143 from shrimp ponds to the mangrove river is unknown but probably much below 10% of
144 the discharge of this large estuary (about $300 \text{ m}^3 \text{ s}^{-1}$ in February, the month we
145 conducted our experiment, Nguyen et al., 2019). Proportion of RV and EF in the MI
146 condition (90/10) was chosen in a way to place shrimp pond effluents in the
147 environmental conditions found in river waters, without excessively diluting the FA
148 signal. In addition, previous studies (unpublished data) revealed concentrations of FA
149 roughly 10 times higher in shrimp pond effluents compared to river water. Fatty acids
150 originating from EF and RV are thus expected to be added in similar quantities in the MI
151 condition. All buckets were incubated in a dark room at 30°C and continuously
152 homogenized with 200 L h^{-1} aquarium pumps during 16 days without aeration. Dark
153 conditions are meant to simulate the high turbidity of mangrove waters. The estimated
154 light penetration in the estuary is actually lower than 1m and considering the estuary
155 depth (10-20 m) most of SPM is transported in the dark (David et al. 2019) although
156 suspended particles may reach the photic zone from time to time due to elevated water
157 turbulence.

158 Water samples were taken at short intervals at the beginning of the experiment
159 and more spaced thereafter (Matsumoto, 1983; Marty et al., 1996). Sampling were
160 performed at T0, and after 8 h, 16 h, 24 h, 2 days, 3 days, 4 days, 6 days, 8 days, 12
161 days and 16 days of incubation (T8, T16, T24, D2, D4, D6, D8, D12 and D16). Two
162 water subsamples were collected at each sampling time from the 12 buckets and filtered
163 for analysis of fatty acids and stable isotopes of SPM. Filtrations were performed using
164 pre-combusted and pre-weighed (4h at 450°C) $0.7 \mu\text{m}$ Whatman® GF/F glass microfiber

165 filters and filtered water was kept for dissolved nutrients analysis. All filters were kept at -
166 25°C and analyzed within two months.

167

168 2.3. Sample processing

169 Salinity, dissolved oxygen and pH were measured at the beginning of the
170 experiment (T0) using a multi-parameter probe (WTW 3420®). Dissolved inorganic
171 nitrogen forms (NH_4^+ , NO_2^- and NO_3^-) were measured at T0, T8, T24, D2, D4, D8 and
172 D16 only, using a Hach-Lang kit photolab DR 1900 employing standard colorimetric
173 methods (APHA, 1995; Nguyen et al., 2019). Reproducibility for replicated
174 measurements was higher than 5%.

175 Lipids were extracted following a slightly modified method of (Bligh and Dyer,
176 1959) as described in Meziane et al. (2006). An internal standard (23:0) was added to
177 the samples prior to extraction. Lipids were first extracted using a
178 methanol:water:chloroform mixture (2:1:2 v:v:v). Chloroform, containing lipid extracts,
179 was isolated and evaporated under nitrogen (N_2) flux. Dried lipid extracts were then
180 saponified with a solution of methanol and sodium hydroxide (NaOH, 2N) (2:1, v:v) at
181 90°C for 1h30 min. Subsequently, methylation was performed, transforming all fatty acid
182 esters into fatty acid methyl esters using methanolic boron trifluoride ($\text{BF}_3\text{---CH}_3\text{OH}$).
183 Fatty acids were separated by gas chromatography (Varian 3800), using an Agilent® VF-
184 WAXms capillary column 30m long, 0.25mm inner diameter and with 0.25 μm film
185 thickness, and quantified using a flame ionization detector. Identification of fatty acids
186 was performed using gas chromatography-mass spectrometry (Varian 450-GC; Varian
187 220-MS) and comparison of GC retention times with commercial standards (Supelco®).

188 The internal standard (23:0) was used to determine the concentration of each fatty acid
189 (C_{FA}) in μg FA per mg SPM.

190 Stable isotopes of carbon and nitrogen were measured at T0, T24, D4 and D16.
191 SPM samples were fumigated for 4h using 35% HCl to remove all carbonates. Samples
192 were prepared in tin capsules at the Muséum National d'Histoire Naturelle (MNHN-Paris)
193 and analyzed at the University of California Davis Stable Isotope Facility (Department of
194 Plant Sciences, UC Davis, Davis, California), using a Vario EL Cube elemental analyzer
195 (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to a PDZ Europa 20–
196 20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, U.K.). During analysis,
197 laboratory standards (bovine liver, nylon 6, enriched alanine and glutamic acid)
198 previously calibrated against international standard reference materials were
199 interspersed with samples. Carbon and nitrogen stable isotope results were reported in
200 parts per thousand (‰), using standard delta notation ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and are relative to
201 V-PDB (Vienna PeeDee Belemnite) and atmospheric air, respectively. The C/N ratio
202 was calculated as the molar ratio of particulate organic carbon (POC) vs. particulate
203 organic nitrogen provided by the isotope mass ratio spectrometer.

204

205 2. 4. Data analysis

206 Degradation rate constants of fatty acids were calculated using an exponential decay
207 model on the form $C_{FA\text{final}} = C_{FA\text{initial}} \times e^{-kt}$, where C_{FA} is the final or initial concentration of
208 a given FA or group of FA in the treatment, k is the degradation rate constant in days^{-1}
209 and t is the incubation time in days. Data treatment and graphical representations were
210 performed using R 3.3.2.

211

212 3. Results

213 3.1. Water parameters

214 Salinity was lower in effluents (10.1 ± 0.0) compared to river water (13.2 ± 0.0)
215 and pH was higher in EF (8.35 ± 0.01) compared to RV (7.45 ± 0.01) (Table 1). As
216 intended, the mixed condition almost kept the conditions displayed by RV (salinity = 12.8
217 ± 0.0 and pH = 7.53 ± 0.00). Suspended particulate matter concentration was about half
218 as much in EF ($91.2 \pm 10.3 \text{ mg L}^{-1}$) compared to RV ($204.6 \pm 4.3 \text{ mg L}^{-1}$) and C/N ratio
219 was lower in EF (7.0 ± 0.0) compared to RV (9.7 ± 1.4 ; Table 1). Shrimp pond effluents
220 were enriched in both dissolved inorganic nitrogen (DIN) and total nitrogen compared to
221 RV (NH_4^+ , NO_2^- and NO_3^- were summed to get free of the nitrogen cycle steps that are
222 not meant to be discussed in this paper; Fig. 1). Concentrations of DIN slightly increased
223 in EF at the beginning of the experiment (from $1.26 \pm 0.04 \text{ mmol L}^{-1}$ at T0 to 1.76 ± 0.19
224 mmol L^{-1} at D2) and then dropped from $1.78 \pm 0.14 \text{ mmol L}^{-1}$ at D8 to $0.23 \pm 0.04 \text{ mmol}$
225 L^{-1} at D16 (Fig. 1a). Total nitrogen decreased during the experiment, reaching about one
226 third of its initial value at the end of the experiment ($2.95 \pm 0.05 \text{ mmol L}^{-1}$ at T0 vs. 1.01
227 $\pm 0.05 \text{ mmol L}^{-1}$ at D16), while it increased during the same period in MI and RV (from
228 $0.77 \pm 0.01 \text{ mmol L}^{-1}$ to $1.03 \pm 0.02 \text{ mmol L}^{-1}$ in MI and from $0.60 \pm 0.02 \text{ mmol L}^{-1}$ to 1.42
229 $\pm 0.11 \text{ mmol L}^{-1}$ in RV; Fig. 1b).

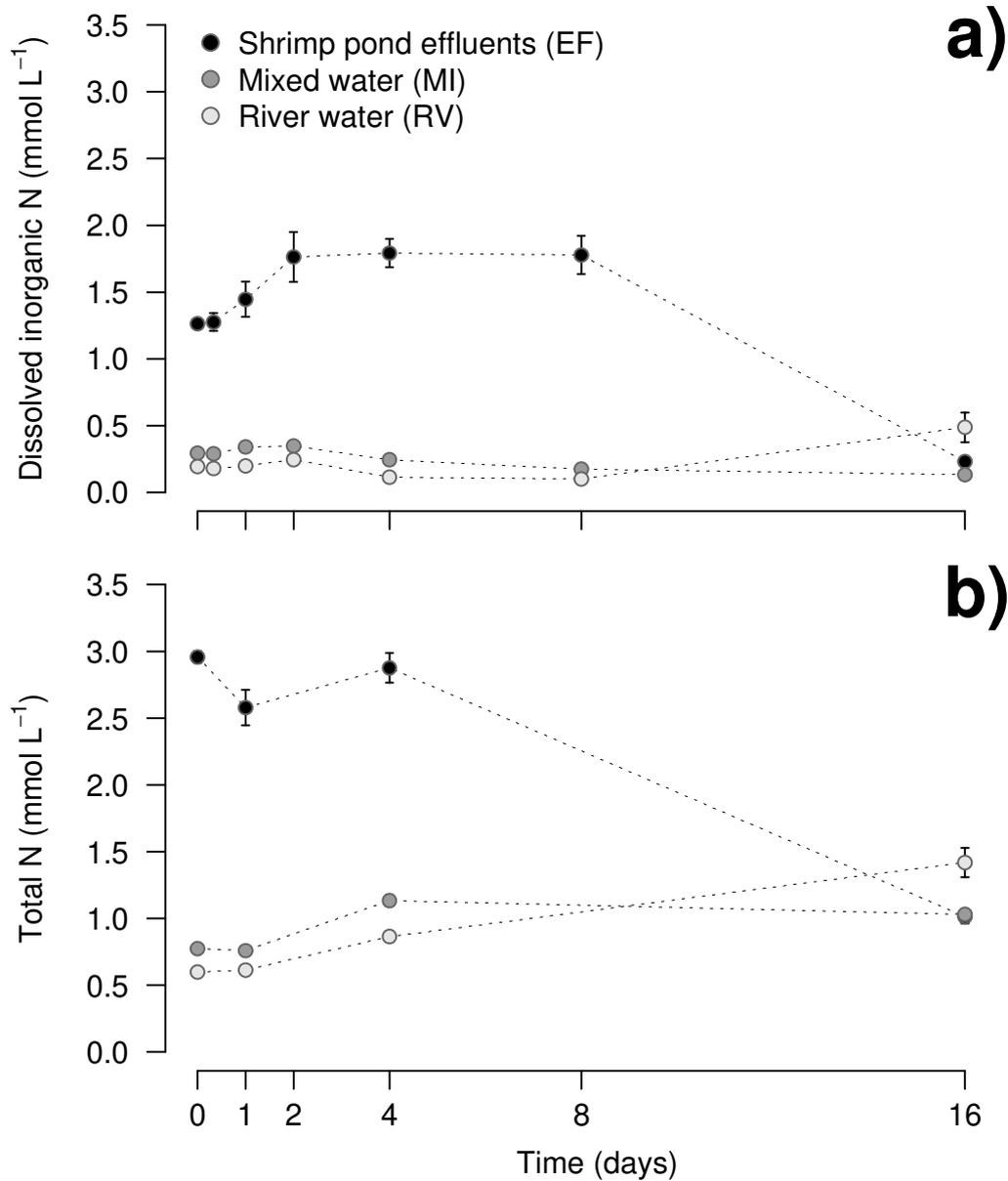
230 Concentrations of POC were about 3 times higher in EF ($10.1 \pm 0.8 \text{ mg L}^{-1}$)
231 compared to RV ($3.3 \pm 0.3 \text{ mg L}^{-1}$; Fig. 2a). It rapidly dropped to 6.1 ± 0.2 to during the
232 first 24h of the experiment in EF, while it slightly increased in MI and RV, reaching $5.7 \pm$

233 0.5 mg L⁻¹ and 6.4 ± 0.8 mg L⁻¹, respectively in MI and RV at the end of the experiment
234 (Fig. 2a).

235

236 Table 1: Water parameters at the beginning of the experiment (T0) in each experimental
237 condition

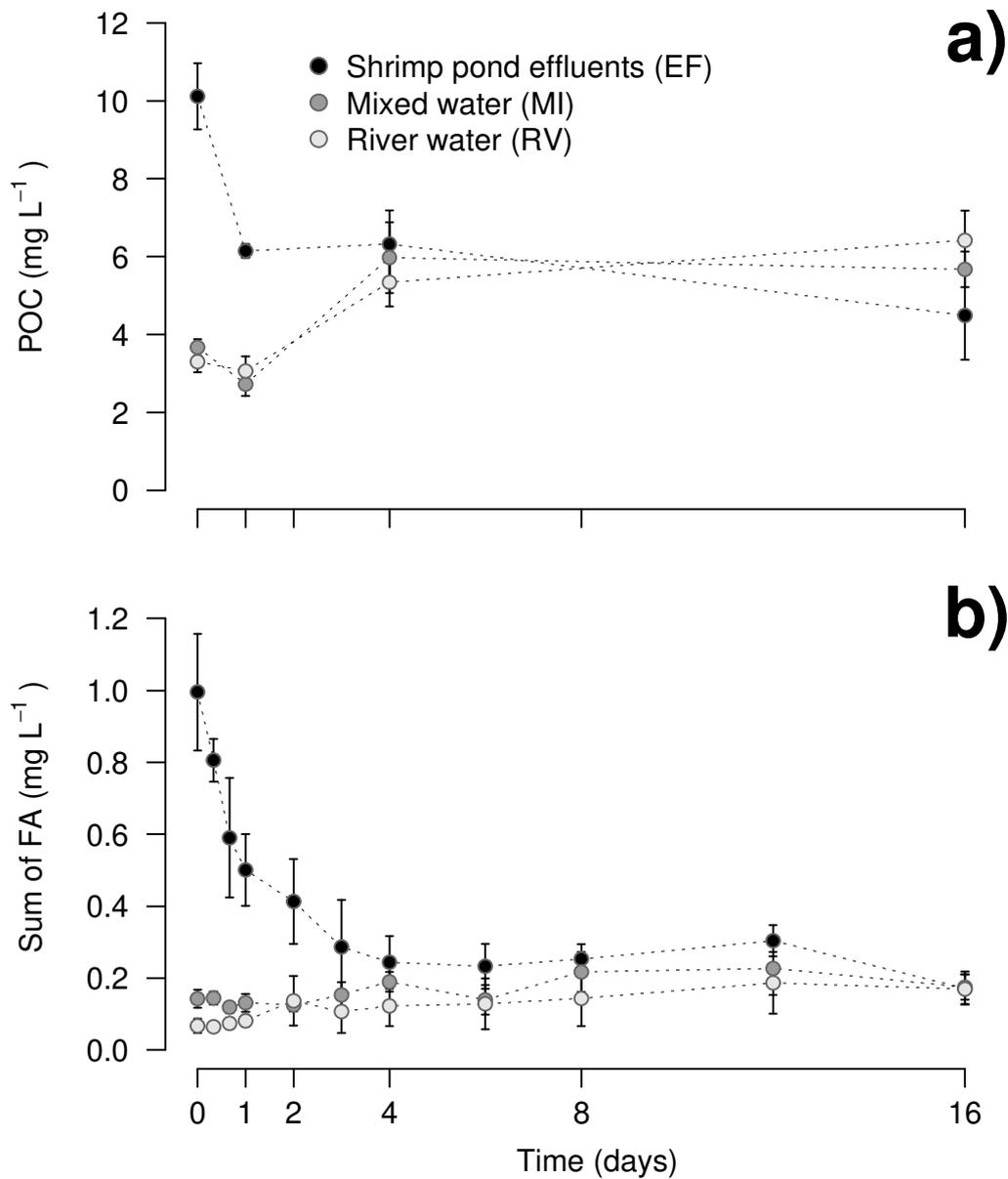
Parameter	Shrimp pond effluents (EF) (n = 4)	Mixed water (MI) (n = 4)	River water (RV) (n = 4)
Salinity	10.1 ± 0.0	12.8 ± 0.0	13.2 ± 0.0
pH	8.35 ± 0.01	7.53 ± 0.00	7.45 ± 0.01
SPM (mg/L)	91.2 ± 10.3	175.3 ± 3.7	204.6 ± 4.3
C/N (mol/mol)	7.0 ± 0.0	9.0 ± 0.4	9.7 ± 1.4



238

239 Figure 1: Dynamics of a) dissolved inorganic nitrogen ($\text{NH}_4^+ + \text{NO}_2^- + \text{NO}_3^-$; mmol L^{-1})
 240 and b) total nitrogen (dissolved inorganic nitrogen + particulate organic nitrogen; mmol L^{-1})
 241 in the three different treatments.

242



243

244 Figure 2: Dynamics of a) particulate organic carbon (mg L⁻¹) and b) sum of FA (mg L⁻¹)

245 in the three different treatments.

246

247 3.2. Fatty acids

248 Identified FA in SPM samples included saturated fatty acids (e.g. 16:0 and 18:0),
249 polyunsaturated fatty acids (PUFA; e.g. 18:2 ω 6 and 20:5 ω 3), monounsaturated fatty
250 acids (e.g. 16:1 ω 7 and 18:1 ω 9), and branched fatty acids (BrFA; e.g. iso-15:0 and
251 anteiso-15:0) (Table 2). Total FA (TFA) concentrations at T0 were about 15 times higher
252 in EF ($1.00 \pm 0.16 \text{ mg L}^{-1}$) compared to RV ($0.07 \pm 0.02 \text{ mg L}^{-1}$; Fig. 2b). High
253 proportions of PUFA were identified in EF ($32.9 \pm 1.1\%$), with a dominant contribution of
254 C₁₆ PUFA, 18:3 ω 3 and 20:5 ω 3 (Table 2). In contrast, RV was dominated by saturated
255 FA ($56.8 \pm 4.1\%$) and exhibited low PUFA proportions ($8.8 \pm 0.7\%$; Table 2).

256 The concentrations of TFA and specific or groups of PUFA rapidly decreased
257 during the first day of incubation, gradually decreased from T24 to D4 and then only
258 slowly degraded over these periods (Fig. 2b and Fig. 3). The degradation of fatty acids
259 thus apparently occurred in three steps, similarly to the observations of Matsumoto
260 (1983) during the incubation of river water. Consequently, we fitted three degradation
261 rate constants for each treatment. Degradation rate constants were highest in the EF
262 condition, while they were lower or not significantly different from 0 in the MI condition
263 and always not significant in the RV condition (Table 3). A 50% loss of TFA was
264 measured in EF during the first 24h of the experiment, reaching 75% at D4, while TFA
265 remained almost stable or even increased in MI and RV during the entire experiment
266 (Fig. 2b and Table 3). Concentrations of total PUFA, 18:3 ω 3 and 20:5 ω 3 followed the
267 same trend as TFA (Fig. 3), while relative contributions remained almost stable between
268 T0 and T24 in EF, and dropped rapidly after T24 (Fig. 3). In contrast, total PUFA and
269 18:3 ω 3 relative proportions decreased since the first hours of the experiment in MI (Fig.

270 3) and almost did not changed in RV during the entire experiment. Concentrations of
271 BrFA and 18:1 ω 7 remained almost stable during the entire experiment, while relative
272 proportions increased in all conditions (Fig. 3). Relative proportions of 18:1 ω 7 showed a
273 sharp increase in MI from $5.3 \pm 0.3\%$ at T0 to a maximum of $23.6 \pm 0.8\%$ at D4,
274 reaching values higher than in EF ($12.8 \pm 1.1\%$) and RV ($15.8 \pm 2.4\%$) at the same
275 moment (Fig. 3).

276 Table 2: Relative proportions of main FA in each experimental condition at T0 (mean \pm
 277 SD). Only FA with proportions > 0.5% in at least one condition are displayed.

Fatty acids (%)	Shrimp pond effluents (EF) (n = 3)	Mixed water (MI) (n = 4)	River water (RV) (n = 4)
<i>Saturated</i>			
12:0	0.2 \pm 0.1	0.3 \pm 0.1	0.6 \pm 0.8
14:0	9.7 \pm 0.3	6.8 \pm 1.4	4.7 \pm 1.4
15:0	0.9 \pm 0.1	1.3 \pm 0.2	2.4 \pm 0.5
16:0	22.2 \pm 0.6	28.3 \pm 1.4	33.9 \pm 1.4
17:0	0.4 \pm 0.1	0.9 \pm 0.2	1.3 \pm 0.4
18:0	1.9 \pm 0.3	4.6 \pm 0.8	9.6 \pm 1.2
19:0	0.0 \pm 0.0	0.3 \pm 0.1	0.8 \pm 0.6
20:0	0.2 \pm 0.1	0.4 \pm 0.1	0.8 \pm 0.1
22:0	0.2 \pm 0.0	0.6 \pm 0.1	1.3 \pm 0.2
24:0	0.1 \pm 0.0	0.9 \pm 0.4	1.0 \pm 0.5
ΣSFA	35.9 \pm 1.3	44.6 \pm 1.6	56.8 \pm 4.1
<i>Monounsaturated</i>			
16:1 ω 9	2.9 \pm 0.1	2.9 \pm 0.4	3.6 \pm 1.3
16:1 ω 7	15.4 \pm 0.2	13.1 \pm 0.4	9.3 \pm 1.3
16:1 ω 5	0.4 \pm 0.0	0.5 \pm 0.0	0.8 \pm 0.2
17:1 ω 9	0.1 \pm 0.0	0.2 \pm 0.1	0.6 \pm 0.3
18:1 ω 11	0.3 \pm 0.0	0.2 \pm 0.1	1.2 \pm 1.3
18:1 ω 9	3.6 \pm 0.1	5.0 \pm 0.7	5.7 \pm 0.5
18:1 ω 7	3.7 \pm 0.2	5.3 \pm 0.3	7.3 \pm 2.4
ΣMUFA	27.2 \pm 0.1	28.2 \pm 1.1	29.5 \pm 3.7
<i>Polyunsaturated</i>			
16:2 ω 6	1.1 \pm 0.2	0.5 \pm 0.1	0.4 \pm 0.2
16:2 ω 4	2.3 \pm 0.1	1.6 \pm 0.1	0.6 \pm 0.1
16:3 ω 4	2.5 \pm 0.1	1.6 \pm 0.1	0.5 \pm 0.1
16:3 ω 3	2.4 \pm 0.2	1.4 \pm 0.1	0.2 \pm 0.0
16:4 ω 3	1.3 \pm 0.1	0.9 \pm 0.2	0.3 \pm 0.2
18:2 ω 9	tr.	tr.	0.7 \pm 0.1
18:2 ω 6	3.4 \pm 0.1	3.2 \pm 0.3	1.1 \pm 0.2
18:3 ω 3	7.3 \pm 0.4	5.2 \pm 0.6	0.6 \pm 0.1
18:4 ω 3	2.6 \pm 0.1	1.8 \pm 0.1	0.5 \pm 0.2
20:2 ω 9	tr.	tr.	0.8 \pm 0.4
20:4 ω 6	1.4 \pm 0.1	1.2 \pm 0.1	0.5 \pm 0.1
20:5 ω 3	5.0 \pm 0.3	3.4 \pm 0.2	1.6 \pm 0.3
22:6 ω 3	1.5 \pm 0.2	0.3 \pm 0.4	0.4 \pm 0.4
ΣPUFA	32.9 \pm 1.1	22.3 \pm 0.6	8.8 \pm 0.7
<i>Branched</i>			
15:0iso	1.6 \pm 0.0	1.6 \pm 0.2	1.5 \pm 0.2
15:0anteiso	0.5 \pm 0.0	0.8 \pm 0.1	1.2 \pm 0.1
16:0iso	0.4 \pm 0.0	0.5 \pm 0.0	0.7 \pm 0.1
17:0iso	1.0 \pm 0.2	1.5 \pm 0.2	0.8 \pm 0.1
ΣBrFA	4.0 \pm 0.1	4.9 \pm 0.3	4.9 \pm 0.4
Others	3.7 \pm 0.4	3.0 \pm 0.8	3.0 \pm 1.6

tr. = traces
 SFA = saturated fatty acids
 MUFA = monounsaturated FA

PUFA = polyunsaturated FA

BrFA = branched FA

278 Table 3: Degradation rate constants (k in d⁻¹) of specific fatty acids in the three different
279 treatments.

		Total FA	PUFA	C ₁₆ PUFA	18:3 ω 3	20:5 ω 3
Shrimp pond effluents	k1 (T0 -> T24)	0.73 ± 0.13	0.79 ± 0.14	1.02 ± 0.16	0.77 ± 0.15	0.73 ± 0.15
	k2 (T24 -> D4)	0.26 ± 0.07	0.41 ± 0.06	0.39 ± 0.08	0.46 ± 0.06	0.53 ± 0.06
	k3 (D4 -> D16)	n.s.	0.10 ± 0.01	0.12 ± 0.02	0.20 ± 0.02	0.14 ± 0.02
Mixed water	k1 (T0 -> T24)	n.s.	0.39 ± 0.12	0.47 ± 0.12	0.76 ± 0.13	0.33 ± 0.14
	k2 (T24 -> D4)	-0.13 ± 0.05	n.s.	n.s.	n.s.	0.15 ± 0.07
	k3 (D4 -> D16)	n.s.	0.03 ± 0.01	0.06 ± 0.02	0.13 ± 0.02	0.09 ± 0.02
River water	k1 (T0 -> T24)	n.s.	n.s.	n.s.	n.s.	n.s.
	k2 (T24 -> D4)	n.s.	n.s.	n.s.	n.s.	n.s.
	k3 (D4 -> D16)	n.s.	n.s.	n.s.	n.s.	n.s.

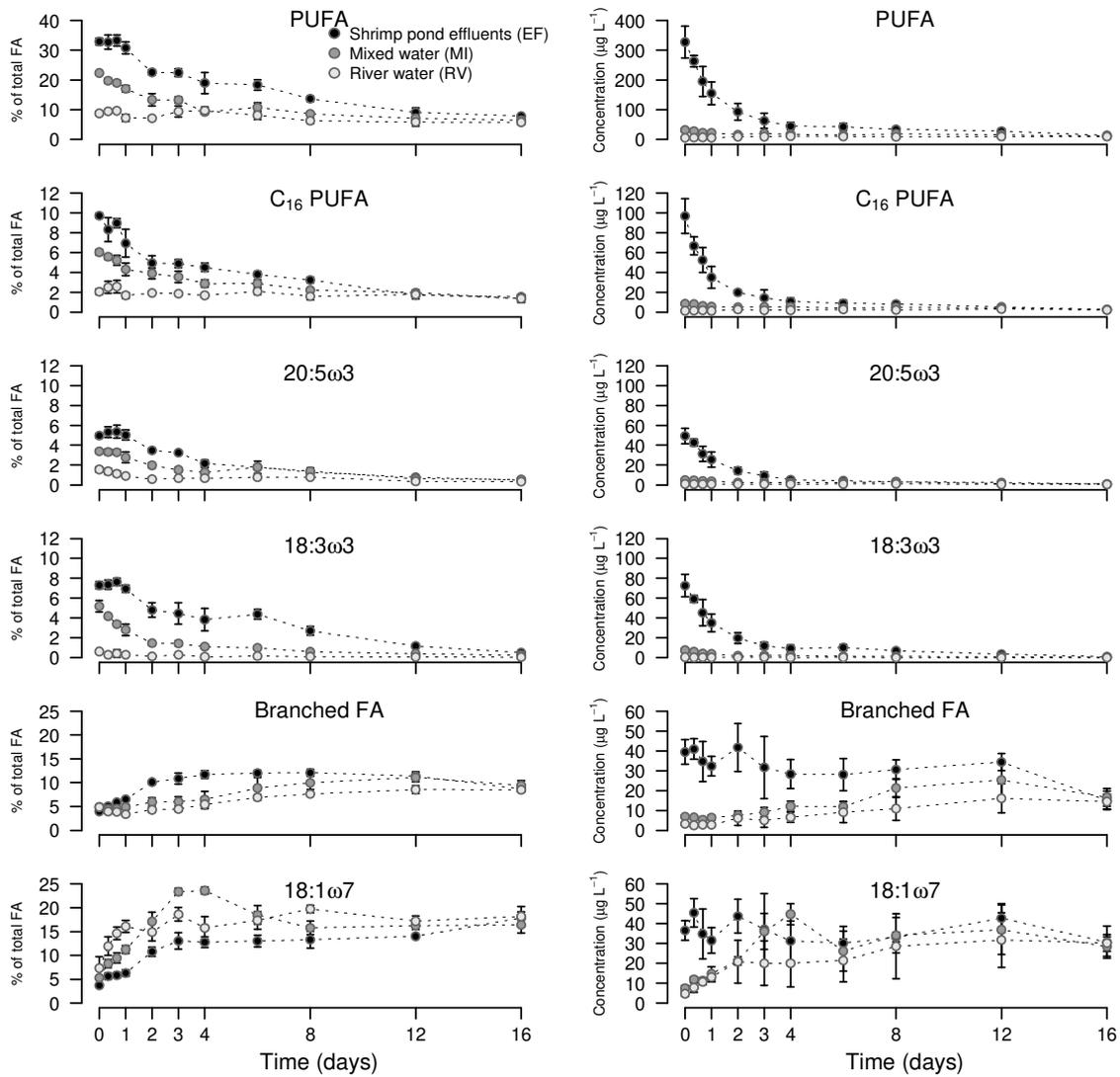
n.s. = not significantly different from 0

PUFA = polyunsaturated fatty acids

280

281 3.3 Stable isotopes $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$

282 $\delta^{13}\text{C}$ ratios of SPM at T0 were lower in EF ($-27.89 \pm 0.15\text{‰}$) compared to RV ($-$
283 $25.66 \pm 0.04\text{‰}$; Fig. 4a). It decreased in EF from T0 to T24 ($-38.35 \pm 0.09\text{‰}$) and then
284 increased until the end of the experiment, while it slightly increased during the entire
285 experiment in MI and RV (Fig. 4a). $\delta^{15}\text{N}$ ratios of SPM were higher in EF ($9.14 \pm 0.07\text{‰}$)
286 compared to RV ($5.16 \pm 0.73\text{‰}$; Fig. 4b). It increased until D4 up to $14.2 \pm 0.3\text{‰}$ in EF,
287 and then decreased to $10.0 \pm 0.6\text{‰}$ at D16, while it oscillated between 4.7‰ and 8.4‰
288 in MI and RV during the entire experiment.

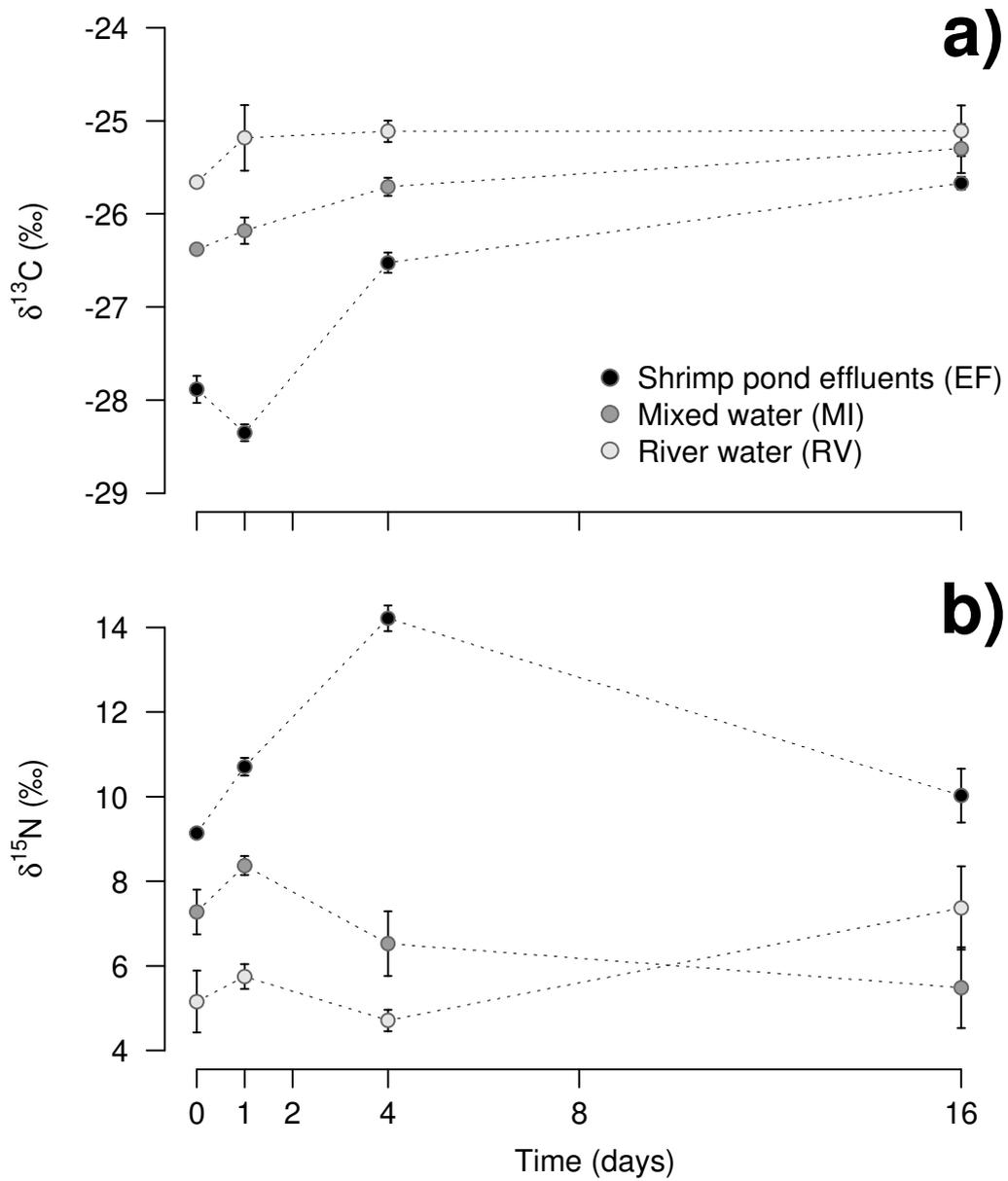


289

290 Figure 3: Dynamics of relative proportions (%) and concentrations ($\mu\text{g L}^{-1}$) of specific

291 fatty acids (20:5 ω 3, 18:3 ω 3 and 18:1 ω 7) and FA groups (polyunsaturated FA, C₁₆ PUFA

292 and Branched FA) in the three different treatments.



293

294 Figure 4: Dynamics of a) $\delta^{13}\text{C}$ (‰) and b) $\delta^{15}\text{N}$ (‰) of SPM in the three different

295 treatments.

296 4. Discussion

297 4.1. Composition of SPM sources at T0

298 High PUFA proportions in EF ($32.9 \pm 1.1\%$; Table 2) and C/N ratios close to the
299 Redfield ratio (6.6; Table 1), show that a substantial fraction of SPM is constituted by
300 living phytoplankton (Canuel, 2001; Moynihan et al., 2016; Napolitano et al., 1997).
301 Shrimp ponds generally exhibit high primary production (Burford et al., 2003) and the
302 elevated level of DIN we measured in EF (Fig. 1) provides ideal conditions for
303 phytoplankton growth. In our experiment, dominant algal cells were diatoms and green
304 algae, as indicated by the high proportions of PUFA 20:5 ω 3, C₁₆ PUFA, and PUFA
305 18:3 ω 3, indicative, respectively for the two first of Bacillariophyceae, and for the latter of
306 Chlorophyceae (Dalsgaard et al., 2003). In contrast, low POC concentrations (Fig. 2a)
307 and low PUFA relative proportions in RV ($8.79 \pm 0.7\%$, Table 2) show that SPM was in
308 an advanced state of decomposition (Canuel, 2001; Jaffé et al., 1995), as we previously
309 highlighted in this ecosystem (David et al., 2019). Lower $\delta^{13}\text{C}$ and higher $\delta^{15}\text{N}$ in EF
310 compared to RV may be attributed to shrimp pellets with high proportion of carbon
311 originating from terrestrial plants with low $\delta^{13}\text{C}$ and high proportion of nitrogen
312 originating from fish trashes with high $\delta^{15}\text{N}$. However, other measurements in the area
313 revealed high variability of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of SPM in shrimp ponds (-22.9 to -31.8 for
314 $\delta^{13}\text{C}$ and -0.6 to 20.7 for $\delta^{15}\text{N}$; unpublished data) suggesting that initial values may be
315 poorly informative in this study and that focus is mainly to be given to changes in
316 isotopic values through time. Total concentration of FA in RV at T0 ($0.07 \pm 0.02 \text{ mg L}^{-1}$)
317 was much below the findings of Matsumoto (1983) who studied the decomposition of
318 organic compounds in a highly polluted river of Japan (about 1 mg of TFA per L).

319 Actually, although the estuary is subjected to a strong anthropogenic pressure (Nguyen
320 et al., 2019), the sewage effluents released within Ho Chi Minh City are strongly diluted
321 before entering the mangrove area (David et al. 2019). In contrast, TFA in EF at T0
322 ($1.00 \pm 0.16 \text{ mg L}^{-1}$) were about similar to the concentrations measured by Matsumoto
323 (1983) in river water at the beginning of their experiment, thus possibly allowing relevant
324 comparisons.

325

326 4.2. Degradation of shrimp pond effluents

327 The high degradation rate of FA in EF indicates that algal lipids were rapidly
328 degraded once photoautotrophic production was made impossible (in the absence of
329 light). However, the sustaining of PUFA relative proportions during the first 24h of the
330 experiment (from T0 to T24) followed by their gradual decay from T24 to D16 (Fig. 3)
331 suggests that two different processes are successively involved in FA degradation. In
332 complete darkness, living algal cells consume lipids from intracellular storage
333 compartments (Brown et al., 1996; Eltgroth et al., 2005), possibly allowing them to
334 survive during a few days using triacylglycerols and galactolipids as an energy source
335 (Manoharan et al., 1999). In the present study, phytoplankton from shrimp pond
336 effluents most probably survived during the first 24h of the experiment (from T0 to T24),
337 leading to the maintaining of their PUFA relative proportions. In contrast, the decrease of
338 PUFA proportions after T24 suggests the death of algal cells, as usually observed
339 during microbial degradation of phytoplankton (Ding and Sun, 2005; Pan et al., 2017,
340 2014). The dynamics of $\delta^{13}\text{C}$ confirms the succession of these two processes, with
341 decreasing values during the first 24h of the experiment and increasing values thereafter

342 (Fig. 4a). Pan et al. (2014) actually measured decreasing values of phytoplankton $\delta^{13}\text{C}$
343 during cell respiration, and increasing values during its microbial degradation.

344 Degradation rate constants k_1 of FA during the first 24h of the experiment (Table
345 3) were much higher than those measured during the respiration phase by Pan et al.
346 (2014), suggesting that experimental conditions may have conducted algal cells to
347 rapidly depleting their energy stocks. As a consequence, natural populations of
348 microalgae in our experiment died after 24h, while algal cells cultured in laboratory
349 conditions may last weeks in darkness (Manoharan et al., 1999, Pan et al., 2014). The
350 setting up of the experiment may have stressed natural microalgal communities and
351 induced a rapid collapse of their populations. Nevertheless, such situation probably
352 better reflects what happens once effluents are released in the river than the
353 suppression of light alone in sterile laboratory cultures. During the first degradation
354 phase, k_1 remained lower than values measured in a highly urbanized river of Japan by
355 Matsumoto (1983; up to 1.5 d^{-1}) and the 50% loss of FA was higher than measured
356 during the first 24h in a sewage-seawater mixture by Marty et al. (1996), where initial FA
357 concentration was about half that of our experiment. Such results suggest that
358 decomposition rates may be both sensitive to initial concentration and the conditions in
359 which effluents are placed.

360 The increase of BrFA and FA 18:1 ω 7 relative proportions, indicative of bacteria
361 (Dalsgaard et al., 2003; Kaneda, 1991; Meziane and Tsuchiya, 2000), along with the
362 maintaining of their concentrations (Fig. 2), indicate that the bacterial compartment
363 remained active during the entire experiment. Bacterial OM mineralization led to the
364 release of DIN in EF during the first 2 days of the experiment (Fig. 1a). The preferential

365 consumption of the lighter isotope by heterotrophic bacteria increases $\delta^{15}\text{N}$ in SPM
366 during decomposition (De Brabandere et al., 2002; Caraco et al., 1998), and most
367 probably induced the $\delta^{15}\text{N}$ increase in shrimp pond effluents SPM during our experiment
368 (Fig. 4b). However, the decrease of DIN concentrations at the end of the experiment
369 (Fig. 1a) was unexpected. This nitrogen was not transferred to the SPM (Fig. 1b) and
370 the nitrogen loss can only be explained by the implementation of a denitrification
371 process, leading to the release of gaseous forms of nitrogen to the atmosphere.
372 Denitrification generally occurs only in anaerobic condition (Chen et al., 2012), which
373 should not have happened in our experiment in which water was continuously mixed
374 with pumps. Nevertheless, mature biofilms may have developed on the bucket walls and
375 in SPM microniches during the experiment and supplied a microbial habitat that contains
376 both aerobic and anaerobic conditions where denitrification could take place (Li et al.
377 2017).

378

379 4.3. Mixing of shrimp farm effluents with river water

380 Conversely to EF, significantly null decomposition rates in RV (Table 3) indicated
381 that organic matter was poorly processed during the experiment and that SPM was in an
382 advanced state of decomposition, as we already evidenced in a previous study (David et
383 al. 2019). The SPM is thus poorly reactive and the transformation processes measured
384 in MI can be mainly attributed to the input of shrimp pond effluents. In the absence of
385 interaction effect between EF and RV (e. g. RV inhibiting the degradation of EF or EF
386 inducing a priming effect promoting the degradation of RV), we expected measured FA
387 to remain between the values obtained for EF and RV during the entire experiment.

388 In MI, no maintaining of PUFA relative proportions was measured during the first
389 24h of the experiment, conversely to EF (Fig. 3), suggesting that phytoplankton cells
390 immediately died and started to decompose just after water mixing. This is confirmed by
391 the $\delta^{13}\text{C}$ of SPM, which immediately increased in MI due to microbial degradation, unlike
392 in EF where cell respiration and microbial degradation occurred successively and
393 showed two different trends in SPM $\delta^{13}\text{C}$ variation (Fig. 4a). This immediate cell death in
394 MI was not due to light depletion, as there was no light available in both MI and EF, but
395 rather to the mixing of the two different water masses. It may have occurred because of
396 a virus present in RV or the sudden change in environmental conditions (pH, salinity,
397 etc.) and/or increased oxidative stress (yet of poorly understood origin; Escher et al.
398 2013) that may have activated programmed cell death (Bidle and Falkowski, 2004).
399 Although the volume of shrimp pond effluents released in the environment is unknown, it
400 is necessarily lower than 10% (the ratio used in our experiment) of this large river
401 discharge. The changes in environmental conditions undergone by the shrimp pond
402 effluents released in mangrove channels are thus at least as intense as in our
403 experiment and immediate cell death would most probably also occur in natural
404 conditions. Decomposition rates k_1 were lower than those measured for EF (Table 3)
405 but we may expect the fraction originating from the 90% of river water not to being
406 decomposed. During the second step (T24 to D4), decomposition rates k_2 were mostly
407 not significantly different from 0 and total FA even increased (Table 3), along with a two-
408 fold increase in POC concentrations (Fig. 2a), suggesting that the input of shrimp pond
409 effluents in river water may have stimulated primary producers and lipid synthesis.

410 The FA 18:1 ω 7 showed an interesting dynamic in MI since its relative proportion
411 increased between T0 and D4 up to values that exceed proportions measured in both
412 EF and RV (Fig. 3). Its temporal trend was different from that of the BrFA's one, which
413 never exceeded either that of EF or RV and suggesting that specific bacterial strains
414 may have grown in MI, probably as a result of substrate availability brought by EF and/or
415 the bacterial inoculum brought by RV. The FA 18:1 ω 7 is commonly used as a bacterial
416 biomarker in natural environments (Dalsgaard et al., 2003; Meziane and Tsuchiya,
417 2000). Marty et al. (1996) measured the degradation of FA from urban wastes in
418 seawater and also found a significant production of FA 18:1 ω 7 during the first days of
419 their experiment followed by its decrease after 5 days of incubation. Studying the
420 decomposition of pure phytoplankton strains, Pan et al. (2014) found that FA 18:1 ω 7
421 became a major component (20-30% of total FAs) during cells microbial degradation.
422 Such elevated proportions of the FA 18:1 ω 7 were previously found in phospholipids of
423 alphaproteobacteria, such as *Paracoccus denitrificans*, which are involved in aerobic
424 denitrification (Zelles, 1997). Further analysis (e.g. DNA) would be required to
425 investigate the presence of aerobic denitrifying bacteria in decomposing shrimp pond
426 effluents, which may have consequences on the release of greenhouse gases in the
427 atmosphere such as NO and N₂O (Ji et al., 2015; Queiroz et al., 2019). In natural
428 conditions, Aschenbroich et al. (2015), found a higher concentration of FA 18:1 ω 7 in
429 mangrove sediments receiving shrimp pond effluents during the active farm period
430 compared to the non-active period and also attributed this increase to the grow of
431 specific bacterial strains. We thus suggest that the FA 18:1 ω 7 represents a potential
432 biomarker of anthropogenic substance (i. e. shrimp farm effluents or urban sewage)
433 releases in natural waters. Finally, shrimp farm effluents are rich in PUFA, that are of

434 high economic value. Developing methods to recover lipids from these effluents may
435 bring additional income to farmers, as well as avoiding excessive inputs of potential
436 polluting substances to the nearby environments.

437

438 5. Conclusion

439 Our study confirms that shrimp pond effluents are enriched in phytoplankton and
440 nitrogen compared to mangrove waters. Placed in dark conditions, phytoplankton cells
441 first depleted their intracellular storage lipids, leading to a 50% loss of FA during the first
442 24h of the experiment with little effect on relative proportions. Then, cells death led to
443 changes in FA relative proportions with preferential degradation of PUFA, leading to a
444 total of 75% FA loss after 4 days of incubation. In contrast to shrimp pond effluents
445 alone (EF), the mixture of shrimp pond effluents + river water (MI) induced immediate
446 phytoplankton cells death, most likely due to the sudden change in environmental
447 conditions and representative of the processes that could happen in the natural
448 environment. The degradation of SPM was then accompanied by the growth of specific
449 bacterial communities (yet undetermined and characterized by especially high levels of
450 the FA 18:1 ω 7), as indicated by higher relative proportions of the FA 18:1 ω 7 compared
451 to that of EF or RV taken in isolation. This result confirms our hypothesis that the mixing
452 of waters favors specific bacterial strains and/or specific degradation pathways. As a
453 consequence, shrimp ponds effluents release in mangrove waters may impact the
454 nutritional quality of SPM by modifying the balance of fatty acids that filtering organisms
455 are expected to feed on. High proportions of the FA 18:1 ω 7 in mangrove sediments
456 were also associated to the nearby shrimp farming activity in New Caledonia

457 (Aschenbroich et al. 2015). We suggest that the FA 18:1 ω 7 represents a potential
458 biomarker of anthropogenic substance (e.g. shrimp farm effluents, urban sewage)
459 releases in natural waters, thus confirming our hypothesis that specific fatty acids may
460 be used as tracers of shrimp farm effluent releases (although not exclusively targeting
461 shrimp farm effluents) in marine environments.

462

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