Prokaryotic abundance, cell size and extracellular enzymatic activity in a human impacted and mangrove dominated tropical estuary (Can Gio, vietnam)

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1 Title: Prokaryotic abundance, cell size and extracellular enzymatic activity
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4
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6
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26 **1. Abstract**

27 Extracellular enzymatic activities constitute the first and limiting step of
28 the whole process of organic matter (OM) cycling in aquatic ecosystems. This
29 study aims to identify the factors controlling prokaryotes ability to hydrolyse
30 OM in an Indo-Pacific tropical mangrove ecosystem (Can Gio, Vietnam).
31 Prokaryotic abundance and leucine-aminopeptidase exo-proteolytic activity
32 (EPA) were measured at vertical (from the sea-surface microlayer to bottom
33 waters), spatial (along a transect within the estuary) and seasonal (wet and dry
34 season) scales. Prokaryotic abundance ranged from 1.2 to 5.7 x 10^9 cells L^{-1} and
35 EPA ranged from 24 to 505 nmol L^{-1} h^{-1} that was relatively similar to other
36 highly productive ecosystems. The estuary was poorly stratified, most probably
37 because of high water turbulence. Yet, exo-proteolytic activity was significantly
38 higher in bottom waters, where higher loads of suspended particulate matter
39 were measured. Seasonal and spatial differences in EPA suggest that the nature
40 of OM transported by the Can Gio mangrove estuary affect EPA. The latter
41 seems to be increased by two "uncommon" situations: the input of fresh and
42 labile OM (*e.g.* shrimp farm effluents) or the lack of labile OM and the need to
43 hydrolyse refractory compounds (*e.g.* during the dry season).

44 **2. Introduction**

45 Prokaryotes and their ability to mineralise organic matter (OM) are
46 essential for controlling the fluxes of carbon and nutrients in coastal waters.
47 Yet, when, where, and how OM is decomposed into nutrients and CO_2 is not
48 fully understood in tropical mangrove ecosystems (Cai 2011). Extracellular
49 enzymatic activities constitute the limiting step of the whole process of OM
50 cycling (Arnosti 2011) and thus, the turnover rates of various compounds have
51 been used to evaluate the efficiency of the microbial community to mineralise
52 OM in coastal waters (Patel et al. 2000, Cunha and Almeida 2006, Bhaskar and
53 Bhosle 2008, Lamy et al. 2009). The leucine-aminopeptidase activity (exo-
54 proteolytic activity, EPA) is one of the most commonly used indicators of OM
55 hydrolysis. It represents the general ability of prokaryotes to hydrolyse proteins
56 (Hoppe 1993) and thus it informs on the efficiency of the ecosystem to recycle
57 nitrogen, which is generally the first limiting nutrient in marine production
58 (Fernandes 2011).

59 In the present study we explored vertical, spatial and seasonal variations
60 in the prokaryotic compartment of the Can Gio mangrove water column.
61 Prokaryotic abundances, cell sizes and EPA were measured at three layers of
62 the water column: (1) surface micro-layer (SML), (2) sub-surface layer and (3)
63 bottom layer. Our measurements were distributed from the downstream end
64 of Ho Chi Minh City to the South China Sea. We hypothesised that (i) the SML
65 prokaryotic compartment would be enriched and more active than underlying
66 waters, but rather similar or even reduced compared to the bottom water layer
67 (enriched in suspended particles); and that (ii) the prokaryotic compartment
68 and more particularly the EPA would vary at short spatial (a few kilometers)
69 and temporal (within one month) scales in the estuary, due to short-term and
70 spatial variations in suspended particulate matter (SPM) composition and

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71 origin (David et al. 2019). Revealing short-term spatial and temporal variations
72 in the ability of prokaryotes to mineralise OM and therefore making nutrients
73 available for primary producers will contribute to understanding OM cycling in
74 tropical estuaries.

75

76 **3. Materials and methods**

77 3.1. Study area

78 The Can Gio mangrove is located at the downstream end of Ho Chi Minh
79 City (Southern Vietnam; ~13 million inhabitants) and flooded by the Saigon-
80 Dong Nai River. The river discharges annually 37.4 × 10^6 m^3 of freshwater into
81 the South China Sea and its basin covers a total catchment area of 40.6 × 10^3
82 km^2 (12% of the total terrestrial area of Vietnam; Ringler et al. 2002). The
83 climate in Can Gio is monsoonal with a wet season from June to October and a
84 dry season from November to May. Tidal amplitude is variable over time and
85 ranges between 2 to 4 m depending on seasons and the distance from the sea
86 (Nam et al. 2014). In 2000, the UNESCO designated the 719.6 km^2 of the Can
87 Gio district as the first mangrove biosphere reserve in Vietnam and a land-use
88 regulation was established. The north-west border of the mangrove is fringed
89 by shrimp farms and salt producing lands, covering roughly 20% of the total
90 reserve surface area, while the rest of the district is preserved from
91 deforestation and mostly covered with mature trees of the species *Rhizophora*
92 *apiculata*.

93 We selected the study sites on the main estuarine channel to be at the
94 interface between land uses (Fig. 1): A) at the downstream end of Ho Chi Minh
95 City (10°39'55"N 106°47'30"E); B) between shrimp farms and the forested
96 mangrove area (10°34'19"N 106°50'11"E); C) in the centre of the mangrove

97 core area (10°31'04"N 106°53'13"E); D) and between the forested mangrove
98 area and the South China Sea coast (10°29'32"N 106°56'55"E). At all these sites,
99 the estuary has steep eroded banks, a depth of about 10-15 m and a width of
100 about 600 m. Site E exhibited very different physical characteristics. It was
101 located in a 1-3 m deep and 30 m large tidal creek within the mangrove core
102 area, which does not receive freshwater upstream inputs (10°30'24"N
103 106°52'57"E; Fig. 1).

104

105 ### 3.2. Sampling strategy

106 The study sites were sampled once per season, each one at one week
107 intervals and in alphabetical order. Samplings were performed from January
108 14th to February 3rd 2015 during the dry season and from September 22nd to
109 October 20th during the wet season. Exact sampling dates, corresponding tidal
110 coefficient and tidal variations can be found in David et al. 2018. Salinity, pH
111 and water temperature were measured at 5-min intervals during 24 h tidal
112 cycles using a Yellow Spring Instrument® meter (YSI 6920) and dissolved oxygen
113 (DO) was monitored similarly with a Hobo® data logger (HOBO U26-001), both
114 immersed 30 cm below the water surface and calibrated before each survey.

115 We collected sub-surface water from the bow of a sampling boat using a
116 10 L plastic bucket immersed 10 cm below the water level. We sampled bottom
117 water using a 2 L weighted bottle with a stopper pulled roughly 1 m above the
118 bottom of the estuary. Samples from the surface micro-layer were taken using
119 a glass plate sampler. The glass plate was immersed vertically and withdrawn at
120 an approximate rate of 20 cm s^{-1}, following the recommendations of Harvey
121 and Burzell (1972). It was then wiped between two polytetrafluoroethylene
122 squeegees fixed face to face to remove the adhering SML, which was falling by
123 gravity into a polyethylene bottle. This method was expected to recover the

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124 first 60-100 µm of the water column, which constitutes a water-air interface
125 where biological processes may differ from the rest of the water column. We
126 repeated the procedure of dipping, withdrawing and wiping the plate to obtain
127 a sufficient volume of sample for the analyses.

128 Sub-samples of water were vacuum-filtered through pre-combusted (5 h
129 at 450°C) and pre-weighted glass fibre filters (Whatman® GF/F 0.7 µm).
130 Suspended particulate matter concentration was measured gravimetrically
131 after freeze-drying the filters. Three sub-samples for bacterial counting and cell
132 size measurements were immediately preserved in 4.5 mL sterile Abdos Cryo
133 Vials®, buffered with 0.2 µm pre-filtered formalin (4 % final concentration) and
134 further stored at -25°C. Four and five replicates were analysed for EPA during
135 the dry season and the wet season, respectively (Table 1). Replicates consisted
136 of different water samples that were collected at 5-min intervals.

137

138 ### 3.3. Sample processing

139 Preserved samples were defrosted at ambient temperature and filtered
140 on 0.2 µm polycarbonate filters. Bacteria were stained using the 4'-6-diamino-
141 2-phenylindole (DAPI) fluorescent stain, following the method described by
142 Porter and Feig (1980). Ten to twelve randomly selected fields (to count at
143 least 500 cells) were photographed using a Leica epifluorescence microscope
144 coupled with a digital camera measuring light emission at 450 nm after
145 excitation at 350 nm. Prokaryotes numeration and cell size measurements
146 were performed with the open-source image-processing program ImageJ v1.5.
147 We binarised monochromatic pictures to automatically count nearly circular
148 aggregates of fluorescent pixels, which we assumed to be prokaryote cells. We
149 measured cell size using a Feret box enclosing the cell and considering that the

150 cell diameter roughly equals the average distance between maximal and
151 minimal Feret diameters (Loferer-Kröbbacher et al. 1998, Merkus et al. 2009).
152 The EPA was measured in the unfiltered fraction using the fluorigenic
153 substrate analogue L-leucine-methylcoumarinylamide (Leu-MCA; Hoppe 1993).
154 The substrate was added to 1.8 mL of water sub-samples and incubated on
155 board during 1 h at *in situ* temperature and low natural light intensity. At the
156 end of the incubation period, the enzymatic cleavage activity was stopped
157 using Sodium Dodecyl Sulfate (1 % final concentration). Controls in duplicates
158 were run similarly, except that the stopper solution was added before the
159 substrate. The cleavage of Leu-MCA resulted from the exoproteolytic activity
160 and was linearly related to the MCA fluorescence. Saturation curves were
161 carried out to determine the saturating substrates concentration and 800 µM
162 final concentrations were used for all samples. Since the substrate was
163 saturating in our study, results corresponded to potential activity rates. The
164 fluorescence was measured using a Varian Cary Eclipse spectrofluorometer
165 (excitation/emission of 380/440 nm) and transformed to hydrolysis activity
166 using a standard curve established with different concentrations of the
167 fluorochrome MCA. All laboratory analysis were performed within 9 months
168 after samples recollection.
169

170 ### 3.4. Data analyses

171 We performed univariate multiple comparisons using non-parametric
172 Kruskal-Wallis test due to variance heterogeneity and we identified groups of
173 samples differing from one another using Wilcoxon pairwise comparisons
174 (α = 5%, modified by Holm correction for multiple analyses). Statistical analyses
175 and graphical representations were performed using R 3.3.2 (R Core Team
176 2017).

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177 ## 4. Results and discussion

178 Salinity along the 40 km of the Can Gio mangrove estuary ranged from 0
179 to 26; water temperature ranged from 26 to 31 °C; dissolved oxygen saturation
180 ranged from 17 to 83 % and pH ranged from 6.5 to 7.8 (David et al. 2018,
181 Taillardat et al. 2019). All parameters were linearly correlated to salinity, which
182 increased from site A to site D and was roughly similar at sites C, D and E.
183 Dissolved oxygen saturation and pH increased with salinity and water
184 temperature decreased during the dry season and remained roughly stable
185 during the wet season. Both pH and dissolved oxygen reflected the mixing of
186 fresh and sea waters without significant perturbations induced by adjacent
187 inputs (e.g. from mangrove creeks). The acidic pH values measured in the
188 upper part of the estuary are likely due to the leaching of the surrounding
189 acidic sulfate soils along with the decay of organic inputs (Thanh-Nho et al.
190 2018). The latter being also responsible for dissolved oxygen levels down to 1.2
191 mgO_2 L^{-1}. These organic inputs may originate from domestic and urban
192 discharges of Ho Chi Minh City urban center and also from the industrial areas
193 located along the Saigon and Dong Nai Rivers (Thanh-Nho et al. 2018)

194

195 ### 4.1. Overall comparisons

196 Prokaryotic abundance ranged from 1.2 to 5.7 x 10^9 cells L^{-1} (Fig. 2). It
197 was slightly above the values previously reported in other tropical (Bhaskar and
198 Bhosle 2008) and sub-tropical mangroves (Bano et al. 1997, Williams and
199 Jochem 2006). In tidal creeks of the Indus River delta, Bano et al. (1997)
200 measured a prokaryotic abundance ranging from 1 to 4 x 10^9 cells L^{-1}. Bhaskar
201 and Bhosle (2008) counted 0.6 to 3.5 x 10^9 cells L^{-1} in a mangrove dominated

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202 estuary from the west coast of India. Williams and Jochem (2006) reported
203 0.27 to 2.91 x 10^9 cells L^{-1} in Florida Bay waterways.

204 The only recent record of exo-proteolytic activity in mangrove waters
205 was provided by Williams and Jochem (2006) in Florida Bay, and to the best of
206 our knowledge, no studies have ever been measuring EPA in Indo-Pacific
207 mangrove waters. The activities we measured, ranging from 24 to 505 nmol L^{-1}
208 h^{-1} in sub-surface waters (Fig. 2), were below those reported by Williams and
209 Jochem (2006) in Florida Bay, which ranged from 70 to 1650 nmol L^{-1} h^{-1}. They
210 were relatively similar to those measured by Rath et al. (1993) in an eutrophic
211 mangrove-influenced station in a barrier reef off Belize (247 to 306 nmol L^{-1} h^{-1}
212). Converted to cell-specific activity, the EPA we measured in sub-surface
213 waters ranged from 4.9 to 179.6 amol $cell^{-1}$, which is one order of magnitude
214 below the values measured by Williams and Jochem (2006) in Florida Bay (52.2
215 to 1571.7 amol $cell^{-1}$) or by Rath et al. (1993) off Belize (316.0 to 765.7 amol
216 $cell^{-1}$). Yet, the values of the present study were roughly similar to those
217 reported by Lamy et al. (2009) after a *Phaeocystis globosa* bloom in the eastern
218 English Channel (50 to 239.1 amol $cell^{-1}$). These results suggest that
219 prokaryotes of the Can Gio mangrove estuary efficiently mineralise proteins
220 and ease the availability of nitrogen for primary producers at a rate similar to
221 that of other highly productive ecosystems.

222

223 ### 4.2. Prokaryotic compartment stratification

224 No significant differences in prokaryotic abundance or cell size were
225 measured between the three water layers during the dry season (Fig. 3),
226 suggesting that the prokaryotic compartment is poorly stratified in the water
227 column. Yet, lower EPA in the surface micro-layer compared to underlying
228 waters and maximum values in the bottom (Fig. 3) suggest that prokaryotes

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229 function differently between water layers. The Can Gio mangrove estuary is
230 highly hydrodynamic, with current velocity reaching 1.7 m s^{-1} during ebb and
231 1.3 m s^{-1} in the opposite direction during flood (David et al. 2018). A strong
232 vertical mixing is thus to be expected and probably inhibits the establishment
233 of a prokaryote-enriched SML, as previously observed in a highly hydrodynamic
234 estuary of Portugal (Santos et al. 2011). In a site of the Ria de Aveiro estuary
235 (Portugal), with similar hydrodynamic characteristics as ours, Santos et al.
236 (2011) measured a similar abundance of prokaryotes between water layers but
237 lower prokaryotic productivity in the SML compared to underlying waters,
238 while the opposite was recorded when a structured SML could be established
239 due to lower hydrodynamism. We suggest that although prokaryotes may be
240 homogenously distributed in the upper layers of the water column in highly
241 hydrodynamic estuaries, they are less active at the water-air interface. Our
242 hypothesis that the SML would be more active than underlying waters is thus
243 invalidated, most probably because of the high hydronynamism of the Can Gio
244 mangrove estuary.

245 Higher EPA and suspended particulate matter concentrations in the
246 bottom compared to other layers (Fig. 3) suggest a particle-associated
247 behaviour of prokaryotes in bottom waters. Actually, prokaryotic cells are
248 influenced by the presence of particles in the water column, creating patches,
249 or “hotspots” of biomass where OM is intensely recycled (Long and Azam 2001,
250 Simon et al. 2002). In a tidal estuary exhibiting high loads of suspended matter,
251 particle-attached prokaryotes can reach up to 80% of total abundance (De
252 Souza et al. 2003). It has previously been observed that particle-attached
253 prokaryotes were more active than free-living cells (Grossart et al. 2007a,
254 2007b, Schapira et al. 2012). Although in our study no difference could be
255 evidenced in prokaryote abundance between water layers, the proportion of

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256 particle-attached cells may have been higher in the bottom compared to other
257 layers, due to higher loads of SPM (Fig. 3). Actually, a previous study (David et
258 al. 2019) showed a high relative contribution of branched fatty acids, indicative
259 of bacteria, in short settling-time particles, which are more hardly resuspended
260 and tend to remain closer to the estuary bottom. We thus suggest that short
261 settling-time particles are the sites of an intense prokaryotic
262 activity. Prokaryotes may however alternate from a free-living and a particle-
263 attached lifestyle (Grossart et al., 2010, Riemann and Winding, 2001) and both
264 populations may belong to the same community as no difference in cell size
265 was revealed between water layers (Fig. 3). Our hypothesis that the bottom
266 layer would be more active than upper waters is therefore confirmed, most
267 probably because of higher loads of SPM compared to other water layers.
268

269 ### 4.3. Link with organic matter quality

270 During the dry season, EPA was roughly 10 times higher than during the
271 wet season, while prokaryotic abundance did not differ and cells were only
272 slightly smaller (Fig. 2), which clearly indicates that the prokaryotic biomass did
273 not notably vary between seasons. We thus expect the specific activity of
274 prokaryotes to vary spatially and/or temporally rather than their abundances.
275 Exo-proteolytic activity is usually closely related to the prokaryotic abundance
276 (Cunha et al. 2000, Patel et al. 2000) and thus, seasonal stability in bacterial
277 stocks is surprising. Yet, intense top-down pressure (grazing or virus mortality)
278 could explain a decoupling between prokaryotic standing stock and activity
279 (Fuhrman and Noble 1995, De Souza et al. 2003, Pradeep Ram et al. 2018).
280 Aspects of top-down processes on prokaryotes would require further
281 investigations in mangrove ecosystems.

282 In the Can Gio mangrove estuary, organic matter exhibited higher lability
283 during the wet season, associated with a higher proportion of polyunsaturated
284 fatty acids indicative of fresh phytoplankton-derived OM, while during the dry
285 season, OM sources were mostly terrestrial (David et al. 2019). The observed
286 seasonal EPA variability could thus be related to the seasonal variation in the
287 composition of organic pools. Moreover, previous studies demonstrated
288 significant protease stimulation in the presence of terrestrial dissolved OM
289 (Traving et al. 2017) and humic-rich dissolved OM (Stepanauskas et al. 1999).
290 Baltar et al. (2017) demonstrated experimentally that prokaryotes fuelled with
291 mangrove-derived dissolved OM increased their EPA up to 400 nmol L^{-1} h^{-1}
292 after 4 days of incubation. In our study, the renewal of water in the mangrove
293 was most probably higher during the wet season compared to the dry season,
294 due to rain inputs and a higher discharge of the estuary. Thus, OM released by
295 mangrove leaves could be more rapidly diluted than during the dry season.
296 Higher leaching of mangrove leaves was actually measured in the Can Gio
297 mangrove during the wet season (Vinh et al. 2020). As a consequence, the EPA
298 did not increase to the same levels as recorded during the dry season,
299 characterized by heavier loads of OM of terrestrial origin. Similarly, high EPA at
300 site E during the wet season in comparison to the other sites (Fig. 2) was most
301 likely due to the high concentrations of mangrove-derived OM nearby the
302 mangrove forest. The ability of prokaryotes to mineralise OM thus varies at the
303 seasonal scale most probably as a response to OM quality (*i.e.* origin, lability).

304 Finally, the high EPA at site B in sub-surface water during both seasons at
305 high tide (Fig. 2) compared to its relative stability in other sites (except site E)
306 whatever the tidal stage considered suggest that OM differed at this site. Site B
307 is located down the area dedicated to shrimp farming (Fig. 1) and the intensive
308 shrimp production requires punctual water renewal in ponds, releasing

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309 wastewaters loaded with OM and nutrients to adjacent ecosystems (Anh et al.
310 2010). Shrimp pond effluents are constituted by fresh and highly labile OM that
311 stimulates bacterial growth (Vivier et al. 2019), which may explain the higher
312 EPA activity measured at site B. Our results suggest that both mangrove-
313 derived OM and shrimp pond effluents increased the release of extracellular
314 enzymes by prokaryotes. The aim of such enzymes is to convert compounds of
315 high molecular weight into monomeric substances, allowing them to be
316 transported through the cytoplasmic membrane. It is thus to be expected that
317 a higher degree of macromolecules polymerisation or organically less labile OM
318 would lead to a higher release of extracellular enzymes by prokaryotes. Baltar
319 et al. (2017) rather suggested that it is the high palatability (that is probably
320 linked to easier assimilation) of mangrove-derived dissolved OM that enhances
321 extracellular enzymes production. According to Findlay et al. (1991),
322 autochthonously produced OM, including exudates, intracellular contents and
323 biomass of primary producers are easily degraded by prokaryotes, while
324 allochthonous sources are relatively more complex and refractory to
325 prokaryote degradation. Our results suggest that EPA is enhanced in both
326 situations: when OM is mostly composed of macromolecules resistant to
327 degradation, and when OM is constituted by unusually high amounts of fresh
328 and labile OM.

329

330 4.4. Prokaryotes behaviour in mangrove ecosystems

331 Conceptually, there might be a “common” situation for prokaryotes in
332 mangrove ecosystems, where inputs of fresh labile organic matter are
333 sufficient to sustain their nitrogen demand. In the Can Gio mangrove this
334 “common” situation occurs during the wet season. It is probably reinforced by
335 the fact that the strong anthropogenic pressure brings easily available nitrogen

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336 to the ecosystem (Nguyen et al. 2019). Consequently, prokaryotes release low
337 amounts of exo-proteolytic enzymes. The situation becomes "uncommon"
338 during the dry season, when fresh and labile OM is getting more scarce.
339 Nitrogen demand is then fullfiled through higher release of exo-proteolytic
340 enzymes. Another "uncommon" situation is the input of OM from shrimps
341 farms, that may modify nutrient balance, and stimulate prokaryotes release of
342 exo-proteolytic enzymes. Yet, the focus on one mangrove ecosystem is
343 insufficient to conclude with certainty on prokaryotes behavior in these
344 understudied type of ecosystems (regarding prokaryotes) that are mangroves.
345 Further studies in other regions would need to confirm these preliminary
346 results in other regions, especially regarding anthropogenic releases that may
347 disturb the system and switch it to an unusual fonctionning. Later on, if this
348 switch is broadly confirmed in other ecosystems, its consequences on trophic
349 webs and nutrient cycling should be assessed.
350

351 ## 5. Conclusions

352 To the best of our knowledge, this study is the first to measure exo-
353 proteolytic activity in Indo-Pacific mangrove waters. The surface micro-layer of
354 the estuary, that we expected to serve as a receptacle for anthropogenic
355 pollutants and organic matter, is not well structured because of high water
356 turbulence. Yet, higher loads of suspended particulate matter in bottom waters
357 were correlated to higher EPA in this layer. The nature of OM transported by
358 the Can Gio mangrove estuary may affect the ability of prokaryotes to degrade
359 OM and recycle nitrogen, despite abundance and size of prokaryotic cells did
360 not vary. Releases of extracellular enzymes seem to be increased as a response
361 to "uncommon" situations induced by the input of fresh and labile OM (*e.g.*

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362 shrimp farm effluents) or by the lack of labile OM and the need to hydrolyse
363 refractory compounds (*e.g.* during the dry season).
364

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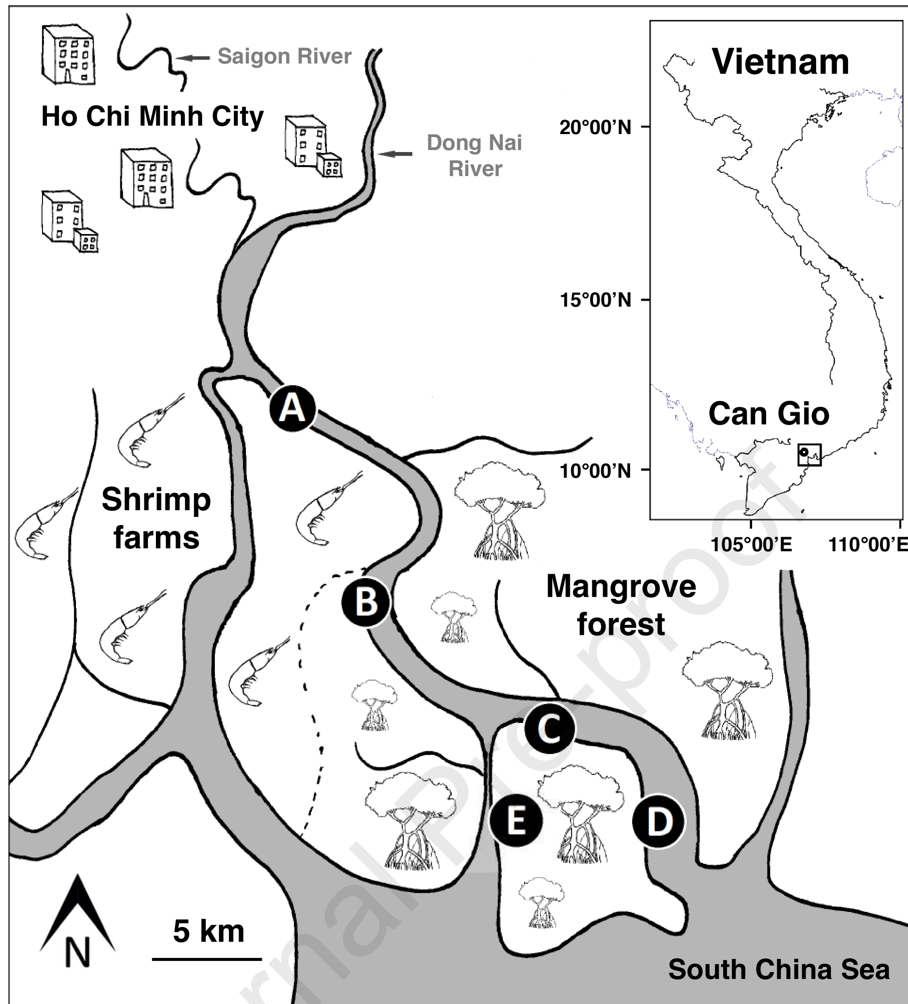

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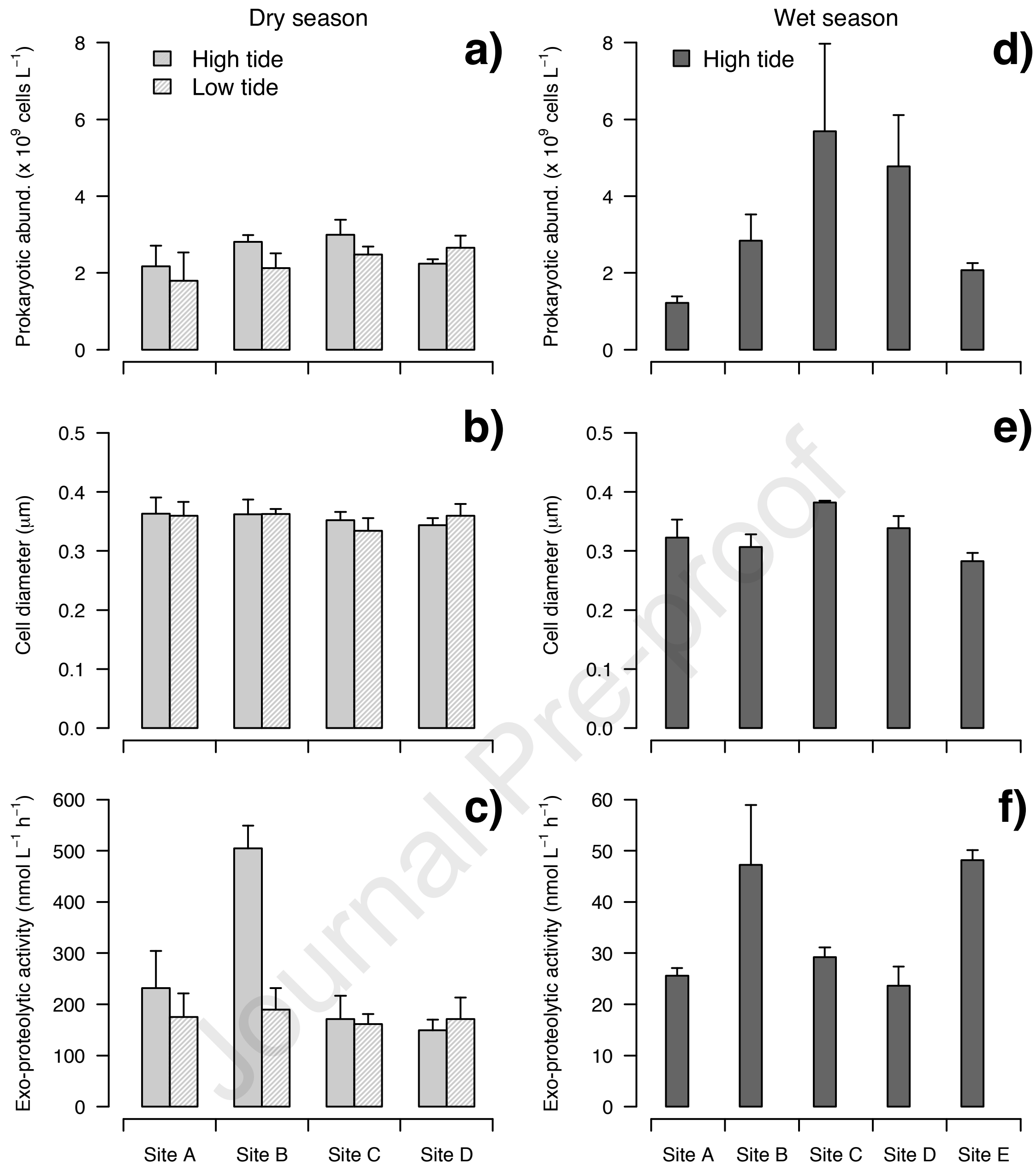
510 Fig. 1: Map of the sampling area in the Can Gio mangrove (Southern Vietnam).

511 A, B, C, D and E indicate the sampling stations along the estuary and within the

512 tidal creek



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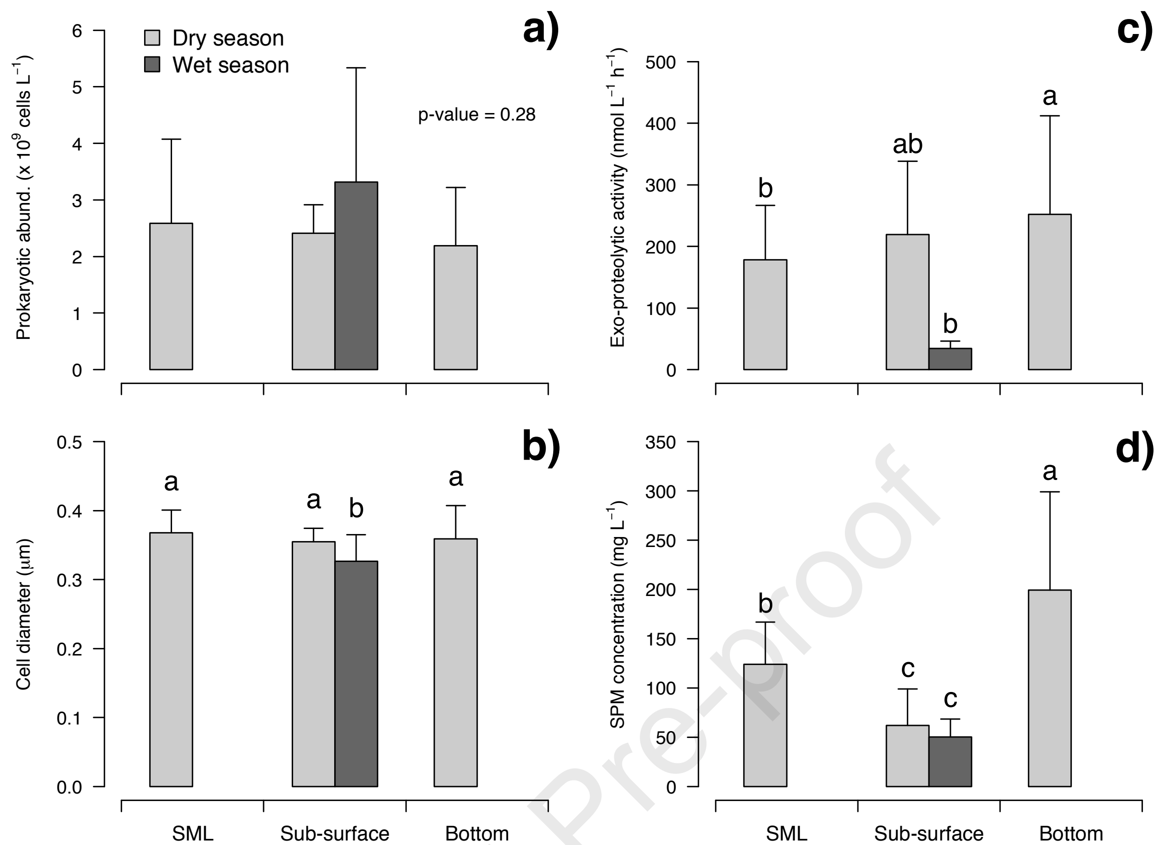
514 Fig. 2: Spatial variability in a) & d) prokaryotic abundance b) & e) cell diameter
515 and c) & f) leucine-aminopeptidase exo-proteolytic activity in the sub-surface
516 water layer of the Can Gio mangrove, during dry season (left panel) and wet
517 season (right panel). For a), b), d) and e) n = 3 for each bar; for c), n = 4 for each
518 bar; and for f), n = 5 for each bar. Statistical differences between groups were
519 not tested given the low amount of data.



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521 Fig. 3: Vertical variability in a) prokaryotic abundance b) cell diameter c)
522 leucine-aminopeptidase exo-proteolytic activity and d) suspended particulate
523 matter concentration in the three water layers of the Can Gio mangrove. For a)
524 and b), n = 24 for each bar during the dry season and n = 15 during the wet
525 season, and for c) and d), n = 32 for each bar during the dry season and n = 25
526 during the wet season. Letters indicate significant differences at α = 5%
527 (Kruskal-Wallis rank test plus Wilcoxon pairwise comparisons with Holm-
528 corrected α).

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529 Table 1: Sampling plan and sample distribution

Season	Study sites	Water layers	Tidal stage	EPA and SPM			Cell counts and size		
				Replicates[a]	Total per layer	TOTAL[b]	Replicates[a]	Total per layer	TOTAL[b]
Dry	A, B, C, D	SML, sub-surface, bottom	High tide + low tide	4	32	96	3	24	72
Wet	A, B, C, D, E	Sub-surface	High tide	5	25	25	3	15	15

[a]Replicates are different water samples collected at 5-min intervals

[b]TOTAL is the total number of samples

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Highlights

- Exo-proteolytic activity is similar to that of other highly productive ecosystems
- Bottom waters are more active that upper layers, especially the surface micro-layer
- Exo-proteolytic activity is enhanced by the input of labile organic matter
- The need to hydrolyse refractory compounds increases exo-proteolytic activity

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