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1 **Factors influencing prokaryotes in an intertidal mudflat**
2 **and the resulting depth gradients**

3
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19
20 Running title: Prokaryote drivers in mudflats

21 1 Highlights

- 22 ▪ Strong stratification in two horizons of microbial densities and activities in the first
23 10 cm of the sediment.
 - 24 ▪ A gradual transition could correspond to an environmental ecocline rather than an
25 ecotone.
 - 26 ▪ Bottom-up-control of the prokaryotic community revealed by the variation partitioning
27 analysis.
- 28

29 2 Abstract

30 Intertidal mudflats are rich and fluctuating systems. The upper 20 cm support a high
31 diversity and density of microorganisms that ensure diversified roles. The depth profiles of
32 microbial abundances and activities were measured in an intertidal mudflat (Marennes-Oléron
33 Bay, SW France) at centimeter-scale resolution (0-10 cm below the sediment surface). The aim
34 of the study was to detect microbial stratification patterns within the sediments and how this
35 stratification is shaped by environmental drivers. Two sampling dates, *i.e.* one in summer and
36 another in winter, were compared. The highest activities of the microbial communities were
37 observed in July in the surface layers (0-1 cm), with a strong decrease of activities with depth.
38 In contrast, in February, low microbial bulk activities were recorded throughout the sediment.
39 In general, prokaryotic abundances and activities were significantly correlated. Variation
40 partitioning analysis suggested a low impact of predation and a mainly bottom-up-controlled
41 prokaryotic community. Hence, in the top layer from the surface to 1–3.5 cm depth, microbial
42 communities were mainly affected by physicochemical variables (*i.e.*, salinity, phosphate and
43 silicate concentrations). Below this zone and at least to 10 cm depth, environmental variables
44 were more stable and prokaryotic activities were low. The transition zone between both layers
45 probably represents a rather smooth gradient (environmental ecocline). The results of our study
46 provide a better understanding of the complex interactions between micro-organisms and their
47 environment in a fluctuating ecosystem such as an intertidal mudflat.

48 **Keywords:** intertidal mudflat, sediment depth, microbial communities, benthic ecology

49 **3 Introduction**

50 In temperate zones, intertidal mudflats are among the most productive coastal
51 ecosystems due to the development of an active microphytobenthic biofilm at the surface of the
52 sediment (Admiraal, 1984; Underwood and Kromkamp, 1999). Several factors drive the high
53 productivity levels such as incident light and large nitrogen-rich inputs from the continent
54 (Underwood and Kromkamp, 1999), in these complex ecosystems. The knowledge about the
55 relationship between the microphytobenthos and the activity of prokaryotic communities,
56 although recognized as of paramount importance for determining the productivity of these
57 ecosystems (Agogu e et al., 2014; Decho, 2000; McKew et al., 2013; Orvain et al., 2014a), is
58 still largely insufficient (Van Colen et al., 2014). Marine coastal sediments harbor among the
59 most diverse and abundant prokaryotic communities (Whitman et al., 1998; Zinger et al., 2011).
60 The abundances and activities of these microbial communities seem to vary along a vertical
61 gradient at a restricted vertical scale (*e.g.*, < 20 cm) under the influence of the 1) organic matter
62 composition and quality and electron acceptor availability (Kristensen, 2000), 2) physical
63 properties of the sediments, 3) bioturbation and bioirrigation activities 4) bottom-up and top-
64 down trophic controls, and 5) climatic conditions.

65 The dominant source of carbon for heterotrophic microorganisms in temperate intertidal
66 mudflats is derived from microphytobenthic activities (*i.e.*, photosynthesis and exopolymeric
67 substance production) (Underwood and Kromkamp, 1999). This organic matter production is
68 primarily ensured by epipellic (*i.e.*, motile free-living) diatoms and quickly transferred to other
69 biological compartments (Middelburg et al., 2000). The microphytobenthic biofilm has such a
70 relevant effect on prokaryotic communities at low tide that it drastically modifies the
71 remineralization and fluxes of inorganic nutrients across the sediment surface (Middelburg et
72 al., 2000).

73 In muddy fine-grained low-permeable sediments, where advection fluxes are almost
74 absent, physical transport of solutes is mainly driven by molecular diffusion within the
75 interstitial water. The top sediment layers show a strong consumption of oxygen by
76 organotrophic microorganisms and by reoxidation of reduced compounds such as Fe^{2+} , Mn^{2+} ,
77 H_2S (Soetaert et al., 1996). Hence, oxygen does not diffuse below the first few millimeters in
78 mudflats where deeper sediment are most often anoxic (Bertics and Ziebis, 2010). Other
79 inorganic electron acceptors, including the nutrient nitrate, can thus be used deeper in the
80 sediment by dissimilatory processes (*e.g.*, denitrification) for anaerobic mineralization. Hence,
81 microbial communities may exhibit vertical patterns in the nature and rate of their activity in
82 response to changing biogeochemical conditions, implicating different prokaryotic assemblage.

83 Moreover, infauna activity plays a crucial role in the modulation of microbial activity
84 in sediments by disturbing the vertical gradients of oxygen, organic matter and inorganic
85 nutrients (Bertics and Ziebis, 2009; Gilbertson et al., 2012; Jones et al., 1996). As an example,
86 prokaryotic activity has been shown to be increased by both bioirrigation and bioturbation
87 activities in a coastal lagoon of the Santa Catalina Island (CA, USA) (Bertics and Ziebis, 2009).

88 Furthermore, prokaryotes may strongly vary under trophic controls. The impact of the
89 availability of resources (*e.g.*, organic matter and/or inorganic nutrients) is defined as the
90 bottom-up control of the microbial communities (Fuhrman and Hagström, 2008), and may
91 strongly change at both spatial and temporal scales. On the other hand, top-down control is
92 described as grazing pressure primarily carried out by meiofauna or viruses (*i.e.*, prokaryotic
93 cell lysis) in intertidal mudflats. Among the few studies focusing on the balance of bottom-
94 up/top-down control in mudflats, the role of top-down control by meiofauna seems to be
95 significant and could be more important than bottom-up control (Fabiano and Danovaro, 1998),
96 although a local study indicated that grazing pressure did not represent a crucial control of
97 bacterial community (Pascal et al., 2009). In a microcosm study, De Mesel et al. (2004)

98 highlighted that both trophic controls have to be considered as bacterial community structure is
99 a function of substrate but the relative abundance of each taxa is influenced by the grazing
100 activities of bacterivorous nematods.

101 Finally, in intertidal zones and especially in macrotidal systems, the alternation of
102 emersion and immersion produces drastically fluctuating conditions, particularly during low
103 tide at the sediment surface. For example, temperature, a key factor impacting prokaryotic
104 metabolism in coastal sediments (Hubas et al., 2007), can fluctuate significantly within 6 hours
105 of a low tide (until 16°C of amplitude measured at the sediment surface in the Marennes Oléron
106 mudflat, France, Orvain et al. (2014a)). Moreover, in these shallow ecosystems, other climatic
107 conditions such as wind or waves can strongly disturb the global (*i.e.*, biotic and abiotic) vertical
108 zonation of the sediment (Dupuy et al., 2014).

109 The aims of this study were 1) to describe stratification patterns of the activities and
110 densities of prokaryotic communities in coastal mudflats at cm-scale spatial resolution and 2) to
111 statistically disentangle the relative contributions of environmental variables and meiofauna
112 abundances in the different depth layers as possible drivers for these prokaryotic activities and
113 densities. This work was focused on an intertidal mudflat in Marennes-Oléron Bay (SW France)
114 sampled twice at low tide, during representative summer and winter conditions, respectively in
115 assessed how the patterns of prokaryotic densities and activities varied with depth and to
116 identify the impact of physicochemical variables and potential grazing pressure on the
117 stratification observed.

118 **4 Materials and Methods**

119 *4.1 Study site and sampling*

120 Sediment cores were sampled in Marennes-Oléron Bay on the Atlantic French coast (1 km
121 from the shore) (N 45° 54' 53"; W 01° 05' 23"). The intertidal mudflat is characterized by the
122 presence of parallel ridges and runnels and sampling was performed on ridges at low tide. Two
123 sampling dates were compared at a similar tidal range (5.5 ± 0.2 m): 1) on July 5, 2012, high
124 temperature and incident irradiance and 2) on February 11, 2013, low temperature and incident
125 irradiance.

126 On each sampling date, triplicate 15-cm diameter cores were sliced *in situ* into five layers
127 using a piston inserted below the core from 0 to 10 cm below the sediment surface (bsf) (D1 =
128 0-0.5 cm; D2 = 0.5-1 cm; D3 = 1-2 cm; D4 = 2-5 cm and D5 = 5-10 cm). Samples were
129 homogenized and subdivided using 50-ml sterile syringes with cut-off tips for further analysis
130 (storage conditions differed according to the variable, see Supp info Table S1). Triplicate cores
131 12-cm in diameter were simultaneously recovered for the determination of pore-water nutrient
132 concentrations. These cores were pre-drilled vertically at 0.5 cm resolution, and pore water was
133 collected at 0.5, 1, 1.5, 3.5 and 7.5 cm bsf, using the Rhizons® (Rhizosphere Research Products
134 Netherlands) method (Seeberg-Elverfeldt et al., 2005). The Rhizons were inserted horizontally
135 into the sediment core during 20 minutes to collect enough pore-water volume for subsequent
136 analysis.

137 *4.2 Physical and chemical analysis*

138 Incident irradiance and temperature at the surface of the sediment were assessed *in situ*
139 every 30 seconds with a universal light-meter and data logger (ULM-500, Walz Effeltrich,
140 Germany) equipped with a plane light/temperature sensor (accessory of the ULM-500) and a
141 plane cosine quantum sensor (Li-COR, USA). Depth temperature profiles were measured every

142 30 seconds during all the sampling period with five 3.1-cm length Hobo sensors (Hobo Pro V2,
143 USA) fixed on a homemade stick that was vertically pushed into the sediment to position the
144 sensors at 5 different depths (0.5 cm, 1 cm, 2 cm, 5 cm and 10 cm bsf).

145 At the laboratory, pore-water pH and salinity (using the Practical Salinity Scale) were
146 measured in the supernatant after centrifugation (15 min, 3,000 $\times g$ at 8 °C) with a pH probe
147 (Eutech Instruments PC150, USA) and a conductivity meter (Cond 3110, TetraCon 325, WTW,
148 Germany), respectively. Sediment density and porosity were evaluated by weighing 50 ml of
149 fresh sediment before and after drying (48 h at 60 °C). Porosity was calculated as the ratio of
150 the volume of water divided by the total volume of sediment. After removal of salts and organic
151 matter, the mean grain size of the sediment was measured by a laser granulometer (Mastersizer
152 2000, Malvern Instruments, U.K.) and evaluated using the GRADISTAT program (Blott and
153 Pye, 2001) according to the Folk and Ward theory (Folk and Ward, 1957).

154 Total organic carbon (TOC) and total nitrogen (TN) contents were measured on
155 lyophilized samples by oxidic combustion at 950°C (Strickland and Parsons, 1972) using a CHN
156 elemental analyzer (Thermo Fisher Flash EA 1112, Waltham, MA, USA). Samples for TOC
157 were decarbonated (in hydrochloric acid, HCl 1N) prior to combustion to remove the inorganic
158 carbon. Because the decarbonation could biased the TN content analysis, subsamples were ran
159 before and after decarbonation to validate the TN measurement.

160 Two exopolymeric (EPS) fractions (colloidal and bound) were extracted in two steps:
161 colloidal EPS were extracted using fresh sediment mixed with an equal volume of artificial
162 seawater, then bound EPS were extracted using the residual sediment mixed with Dowex resin
163 (Takahashi et al., 2009). Before quantification of EPS-proteins and -carbohydrates, each extract
164 was vacuum-evaporated over 6h (Maxi Dry plus, Heto, Denmark). Colloidal and bound EPS-
165 protein concentrations were determined using the bicinchoninic acid assay (Smith et al., 1985).
166 Colloidal and bound EPS-carbohydrate concentrations were determined according to the

167 phenol-sulfuric acid method (Dubois et al., 1956). The four resulting fractions colloidal EPS-
168 proteins, bound EPS-proteins, colloidal EPS-carbohydrates and bound EPS-carbohydrates were
169 expressed in $\mu\text{g g}^{-1}$ sed DW. Colloidal EPS correspond to the sum of colloidal EPS-proteins
170 and colloidal EPS-carbohydrates. Bound EPS correspond to the sum of bound EPS-proteins
171 and bound EPS-carbohydrates. Colloidal EPS and bound EPS were used for the calculation of
172 the ratio colloidal EPS / bound EPS. EPS-carbohydrates correspond to the sum of colloidal
173 EPS-carbohydrates and bound EPS-carbohydrates. EPS-proteins correspond to the sum of
174 colloidal EPS-proteins, bound EPS-proteins. EPS-carbohydrates and EPS-proteins were used
175 for the calculation of the ratio EPS-carbohydrates/EPS-protein.

176 Total protein content was determined in sediment (stored at $-20\text{ }^{\circ}\text{C}$) after extraction (30
177 min, in the dark, $+4\text{ }^{\circ}\text{C}$ in $0.2\text{-}\mu\text{m}$ -filtered seawater) using Lowry Peterson's modification assay
178 (Sigma-Aldrich). Ammonium (NH_4^+), nitrites (NO_2^-), nitrates (NO_3^-), phosphate (PO_4^{3-}), and
179 silicate ($\text{Si}(\text{OH})_4$) concentrations were determined using an autoanalyzer (Seal Analytical,
180 GmbH Nordertedt, Germany) equipped with an XY-2 sampler according to Aminot and
181 K rouel (2007).

182 4.3 Biotic parameters

183 Chlorophyll *a*, used as a proxy of algal biomass, was assessed by fluorimetry (640 nm,
184 Turner TD 700, Turner Designs, USA) after extraction with 90% acetone. Chlorophyll *a*
185 concentrations were expressed as $\mu\text{g g}^{-1}$ sediment dry weight (DW) according to Lorenzen
186 (1966). Prokaryotic abundance was evaluated by flow cytometry after a cell extraction
187 procedure described by Lavergne et al. (2014).

188 Analyses of the two potential extracellular enzymatic activities, β -glucosidase and
189 aminopeptidase, were determined by spectrofluorimetry (Boetius, 1995) (SAFAS Scientific
190 Instruments, Monaco) [excitation/emission = β -glucosidase activity: 365 nm/460 nm; and

191 aminopeptidase: 340 nm/410 nm]. For β -glucosidase activity, slurry sediment samples were
192 incubated in triplicate using 4-Methylumbelliferyl β -D-glucopyranoside (500 $\mu\text{mol L}^{-1}$ final
193 conc.) as a substrate at three different incubation times: 15, 45, and 75 min. For aminopeptidase
194 activity, slurry sediment samples were incubated in triplicate with L-leucine β -naphthylamide
195 hydrochloride (300 $\mu\text{mol L}^{-1}$, final conc.) as a substrate at three different incubation times: 10,
196 30, and 60 min. Final concentrations of 4-Methylumbelliferyl β -D-glucopyranoside and L-
197 leucine β -naphthylamide hydrochloride were determined previously to represent saturation
198 levels and maximum yield velocities (V_{max}) (Boetius and Lochte, 1996).

199 Incorporation of [methyl- ^3H] thymidine into DNA was measured as a proxy of benthic
200 bacterial production (Garet and Moriarty, 1996; Pascal et al., 2009). Briefly, 30 μl of fresh
201 sediment slurry (vol/vol; 0.2- μm -filtered seawater) was incubated with ^3H -thymidine $0.74 \times$
202 10^6 Bq for 1 h at *in situ* temperature (22°C in July and 12°C in February). Blank controls were
203 stopped just after the addition of labelled ^3H -thymidine with 8 ml of cold 80% ethanol. After
204 incubation, samples were stopped with 8 ml of cold ethanol (80%). After two washes with 80%
205 cold ethanol by mixing and centrifugation (15 min, 4 500 g, +4°C), slurries were transferred
206 with 2 mL of ice-cold TCA (5%, trichloroacetic acid) onto a polycarbonate filter (Nuclepore
207 0.2 μm , 25 mm, Millipore, NJ, USA). Subsequently, the filters were washed four times with
208 5% ice-cold TCA. Afterwards, the filters were transferred into scintillation vials containing 2
209 ml 0.5N chlorhydric acid and incubated 16 h at +95°C (Garet and Moriarty, 1996). Supernatant
210 (0.5 mL) was transferred in a new scintillation vial with 5 mL of scintillation fluid (Ultima
211 Gold, Perkin-Elmer, MA, USA). The amount of radioactivity in each vial was measured using
212 a scintillation counter (Perkin-Elmer, USA). Benthic bacterial production was finally expressed
213 as $\text{pmol } ^3\text{H Thy g}^{-1} \text{ sed DW h}^{-1}$ using a conversion factor 4.51×10^{-13} (Ci dpm $^{-1}$) evaluated
214 experimentally to account for counter efficiency.

215 For meiofaunal assemblage determination, samples from each depth (60 mL) were
216 stored directly after sampling at room temperature in absolute ethanol, sieved through 50 μm
217 before staining with rose Bengal and observation under stereo microscope (Zeiss). Foraminifera
218 were counted in all the sediment samples, and for other meiofauna organisms (*i.e.*, juvenile
219 gastropods, copepods, ostracods, nematods, foraminifera, and juvenile bivalves), samples were
220 diluted prior to counting. Abundances were expressed as individuals (ind.) per cm^3 .
221 Additionally, six 20-cm diameter PVC cores were harvested at each date and sieved through 1
222 mm. The macrofauna was collected and stored in ethanol 60% for further identification. In the
223 current study, only the data of the abundance of the macrozoobenthic grazer, *Peringia ulvae*
224 (Pennant, 1777) are presented. The mean abundance of the six cores is expressed in ind m^{-2} .

225 4.4 Statistical analyses

226 All statistical analyses were performed with R software (R Core Team, 2013). In this
227 study, the results are presented as the means \pm standard error (SE) because the SE evaluates the
228 mean estimation imprecision. To evaluate the effect of temperature on thymidine incorporation
229 rate, Q_{10} values were calculated at each sampling depth (Lomas et al., 2002) using the Equation
230 1.

$$231 \text{ Equation 1: } Q_{10} = (R2/R1)^{(10/t_2-t_1)}$$

232 Where $R2$ and $R1$ are the thymidine incorporation rates and t_2 and t_1 are the incubation
233 temperatures in July and February, respectively. This factor indicating the increase of a process
234 rate with 10°C increase of temperature is more powerful when calculated with large dataset
235 and/or used with regression data (Hubas et al., 2007; Lomas et al., 2002). In the current study,
236 as the replicates are independent between the two sampling dates, Q_{10} factor was calculated for

237 all the possible combinations ($n=3^3$). Then, a student test for one sample was used to evaluate
238 whether the Q_{10} values were significantly different from 1.

239 Pearson tests were used to test whether the distribution of two variables was similar.
240 The significant variation of environmental and prokaryotic variables among sediment depths
241 and sampling dates was evaluated by two-way ANOVA (using sampling date - 2 levels - and
242 depths - 5 levels - as factors) followed by multiple comparison tests (Tukey HSD test) and
243 variance homogeneity and residuals normality were tested. For the two variables “chlorophyll
244 a” and “ Q_{10} of thymidine incorporation”, the ANOVA assumptions were violated, the variables
245 were ln-transformed and two-way ANOVA followed by Tukey HSD test was performed. For
246 the two variables “EPS-carbohydrates/EPS-protein” and “colloidal EPS/ bound EPS”, the
247 ANOVA assumptions were violated and transformation was not possible, non-parametric
248 Friedman test was thus run followed by the Nemenyi post-hoc test for multiple joint samples
249 (Nemenyi, 1963; Sachs, 1997) using the “PMCMR” package (Pohlert, 2014).

250 Multivariate principal component analysis (PCA) was performed for July sampling and
251 February sampling separately with 8 environmental variables using the “FactoMineR” package
252 (Husson et al., 2013). Then, in order to define sediment horizons using the basis of each PCA
253 obtained, a hierarchical clustering analysis was applied using the HCPC function of the
254 “FactoMineR” package (Husson et al., 2013).

255 Finally, in order to disentangle the impacts of the environmental variables and
256 meiofaunal group abundance, both taken individually as well as shared, on the distribution of
257 prokaryotic density and activities, variation partitioning was performed (Borcard et al., 1992;
258 Ramette, 2007; Volis et al., 2011) using the varpart function of the “vegan” packages (Oksanen
259 et al., 2013)). First, one response table and three explanatory tables were built and composed
260 as follows. The response table corresponds to the “prokaryotic” table (P table) containing
261 prokaryotic abundance (PA), thymidine incorporation (Thy.inc), aminopeptidase activity

262 (AMA), and β -glucosidase activity (BGA) and was standardized to unit variance. The
263 explanatory “meiofauna” table (M table) containing abundances of juvenile gastropods,
264 copepods, ostracods, nematods, foraminifera, and juvenile bivalves was $\log_{10}(x + 1)$
265 transformed to normalize the distribution. And the explanatory table of the “environmental
266 variables” (E table) (standardized) contains temperature, salinity, pH, the ratio DIN:PO_4^{3-} , the
267 ratio TOC:TN , total protein content, porosity, EPS-carbohydrates/EPS-protein and colloidal
268 EPS/bound EPS.

269 Using forward selection procedure (Legendre and Legendre, 1998) with the function
270 `forward.sel` in the package ‘`packfor`’ (Dray et al., 2013), we selected the variables that
271 influenced the most the response table (Ramette and Tiedje, 2007). The final explanatory tables
272 was thus composed as follows: the table E containing phosphate and silicate concentrations as
273 well as salinity and the table M containing abundance of juvenile gasteropods. The variation
274 partitioning evaluates diverse components of variation of a set of response variables: 1) the pure
275 effect of each individual explanatory table without the effect of the other explanatory table; 2)
276 the redundancy of the two explanatory tables which is the part of the variance explained by both
277 explanatory tables; and 3) the residual effects unexplained by the chosen variables (Borcard et
278 al., 1992; Volis et al., 2011). In this set of data, it is expected that the distribution of abundances
279 and activities of prokaryotes (P table) responds linearly to the explanatory variables, thus we
280 used the linear-based PCA and redundancy analysis (RDA) for the analysis. The total variance
281 to be explained was evaluated by a PCA with the abundances and activities of prokaryotes (P
282 table). RDA was used to assess the amount of variation of the P table explained by the two
283 explanatory variables (as constraining variables). Using partial RDA (pRDA), the effect of a
284 set of variable (an explanatory table) could be removed from the analysis if selected as a
285 covariable. This is an important issue of this multivariate analysis that, in the present case,
286 evaluates for example the effect of meiofauna on prokaryotic variables without the effect of the

287 environmental variables. These environmental variables such as salinity could indeed be
288 important factors for both prokaryotic and meiofaunal communities and the partition of these
289 effects allows to quantify the pure effect of the meiofauna without the shared variation with
290 environmental variables. Finally, the significance of each ordination was tested by an ANOVA
291 like permutation test using 9999 permutations (Volis et al., 2011).

292 **5 Results**

293 *5.1 Environmental conditions and variation of physicochemical variables*

294 The air temperature and incident irradiance at the surface of the mudflat were $28 \pm$
295 0.9 °C and 1800 ± 156 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and 10.5 ± 1.1 °C and 611 ± 292 $\mu\text{mol photons m}^{-2}$
296 s^{-1} during the samplings in July and in February, respectively (Supp info Fig. S1 and Table
297 S2). The sediment was predominantly silt-clay (mean of 91.2%), with an average grain size of
298 11.17 ± 0.34 μm and a porosity of 0.73 ± 0.01 .

299 Two-way ANOVA reveals that all the physicochemical variables (presented in the Table
300 1) were significantly different between the two sampling dates ($p < 0.05$) except the Colloidal
301 EPS / bound EPS ratio. Significant variations with sediment depth are highlighted by Tukey's
302 post hoc test (Table 1, see Supp info Fig. S2 to Fig. S4 for detailed profiles).

303 Then, two principal component analysis (PCA) were performed using 8 variables (*i.e.*,
304 presented in the Table 1 except grain size, porosity and algal biomass) aiming at describing the
305 interactions within the physicochemical variables for each sampling date and a hierarchical
306 clustering analysis based on the ordinations obtained was used to group the samples. In July,
307 the two first dimensions of the PCA together explained 66.68 % of the observed variability in
308 the dataset (Figure 1a). The first dimension was mostly characterized by TOC:TN, pH, salinity
309 and temperature and differentiated the samples in two groups from 0 to 1 cm bsf on one hand

310 and the samples from 1 to 10 cm bsf on the other hand (Figure 1c). In February, the two first
311 dimensions of the PCA together explained 68.94 % of the observed variability in the dataset
312 (Figure 1b). The first dimension was mostly characterized by TOC:TN, pH and temperature
313 and differentiated the samples in two groups from 0 to 2 cm bsf on one hand and the samples
314 from 2 to 10 cm bsf on the other hand (Figure 1d).
315 In both cases, DIN:PO_4^{3-} and EPS-carbohydrates/EPS-proteins ratios have information
316 represented in both dimensions of the ordinations.

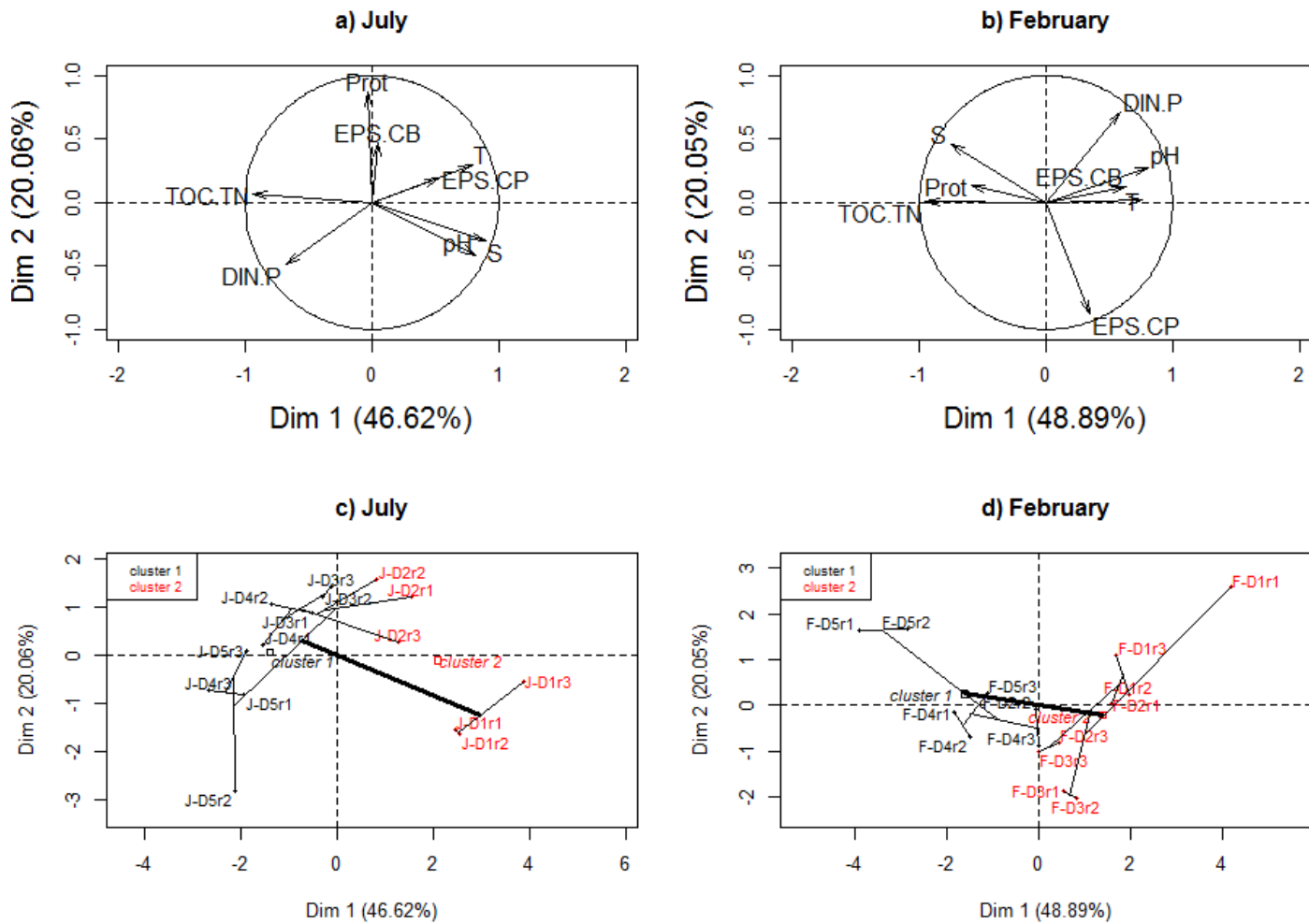
317 **Table 1.** Average of each environmental variables and algal biomass (\pm SE) along sediment depths. Two-way ANOVA reveals always significant d
 318 letters in bold font indicate Tukey's post hoc test for each sampling date.

	Grain size¹		Porosity		Salinity		pH		Temperature		TOC:TN²		DIN:PO₄³⁻		car						
	μm								$^{\circ}\text{C}$												
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE		Mean					
<i>July 5th, 2012</i>																					
D1 (0-0.5 cm)	9.1	0.3	0.78	0.02	a	45.4	2.3	a	7.5	0.1	a	22.2	2.9	a	6.06	0.03	a	9.10	3.86	a	2.0
D2 (0.5-1 cm)	8.7	0.5	0.72	0.01	ab	37.5	0.2	b	7.0	0.0	b	22.2	0.9	a	6.27	0.03	a	54.65	21.12	ab	4.0
D3 (1-2 cm)	9.3	0.1	0.69	0.03	ab	34.3	0.1	b	6.7	0.1	b	21.9	0.6	a	6.45	0.03	a	21.88	1.09	b	1.0
D4 (2-5 cm)	10.7	0.2	0.69	0.00	b	33.5	0.8	b	6.6	0.0	c	21.5	0.3	ab	6.62	0.09	a	175.10	100.58	ab	1.0
D5 (5-10 cm)	11.0	0.4	0.68	0.02	b	33.4	0.5	b	6.9	0.1	b	20.8	0.2	b	6.59	0.04	a	241.58	79.25	ab	0.0
<i>February 11th, 2013</i>																					
D1 (0-0.5 cm)	14.4	NA	0.77	0.03	a	24.3	0.2	ab	7.9	0.0	a	9.5	2.1	a	6.75	0.17	a	57.09	14.26	a	0.0
D2 (0.5-1 cm)	13.2	NA	0.75	0.00	ab	23.5	0.8	a	7.2	0.1	b	8.8	1.2	ab	7.20	0.08	a	18.66	7.84	abc	0.0
D3 (1-2 cm)	10.5	NA	0.79	0.03	ab	23.6	1.0	ab	7.2	0.2	b	9.2	0.5	ab	7.35	0.13	b	6.87	2.06	ab	0.0
D4 (2-5 cm)	11.9	NA	0.71	0.02	b	26.3	1.8	ab	7.0	0.1	b	8.8	0.7	ab	7.47	0.13	b	11.52	1.74	bc	0.0
D5 (5-10 cm)	13.0	NA	0.69	0.01	b	29.5	1.7	b	7.0	0.1	b	8.4	0.2	b	8.00	0.16	b	19.54	6.14	c	0.0

¹Note that triplicates were not available for grain size for February sampling (NA: not available)

²TOC:TN : ratio of total organic carbon (TOC) to total nitrogen (TN). TOC and TN are in $\mu\text{g g}^{-1}$ sed DW

³ EPS-carbohydrates/EPS-protein and colloidal EPS/bound EPS are ratios without unit. Colloidal EPS-proteins, bound EPS-proteins, colloidal EPS-carbohydrates



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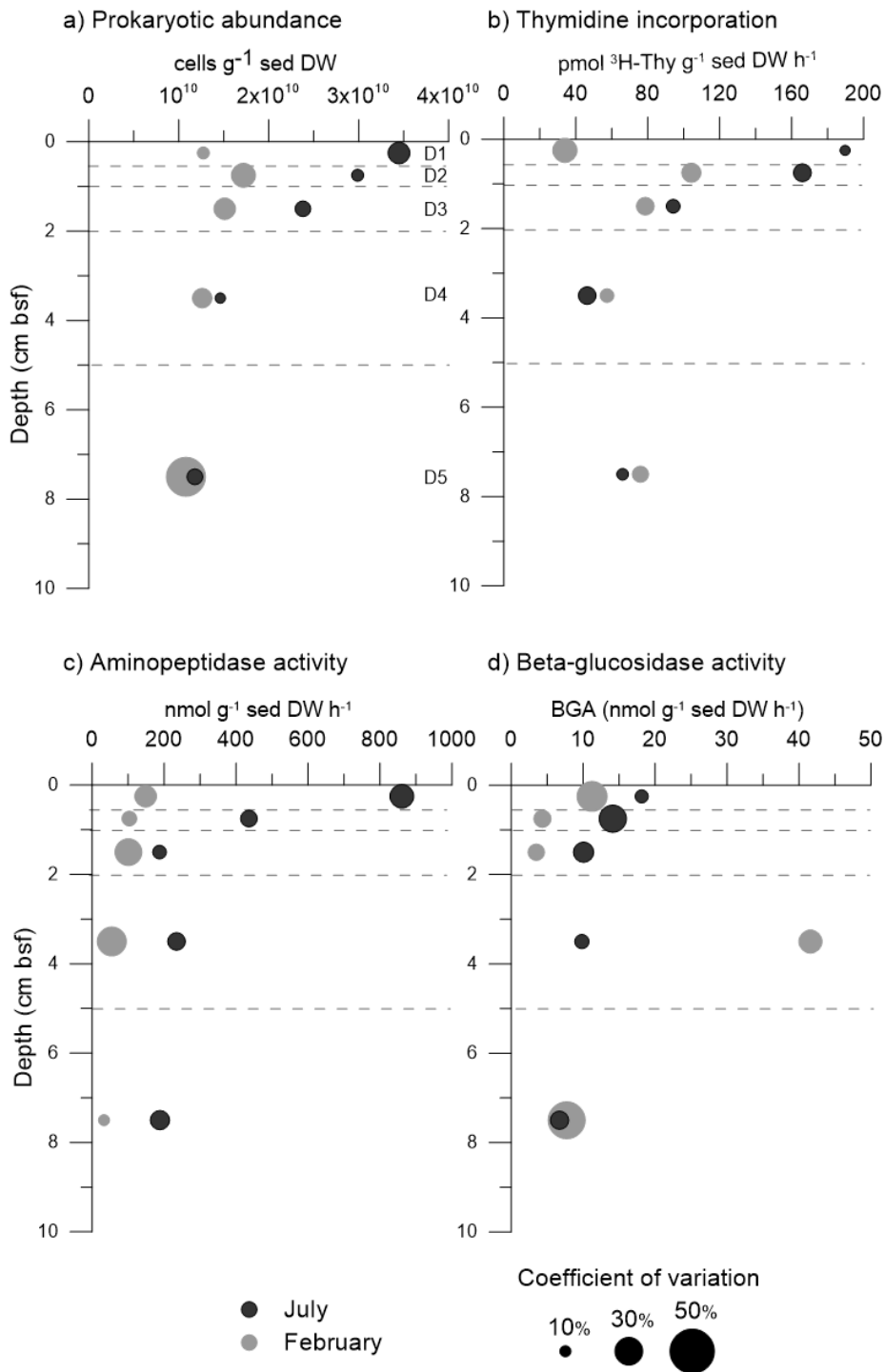
321 **Figure 1.** Principal component analysis (PCA) ordination calculated using 8 physico-chemical
 322 variables for a and c) 15 samples in July and b and d) 15 samples in February. a and b)
 323 Ordination of the variables and correlation circle. b and d) Position of the observations in the
 324 ordination; tree calculated hierarchical classification on principle components and the different
 325 clusters evaluated using 10000 iterations . T: temperature; S: salinity; Prot: total protein
 326 concentration; EPS.CB: colloidal EPS/bound EPS ratio; EPS.CP: EPS-carbohydrates/EPS-
 327 proteins ratio; DIN.P: DIN:PO₄³⁻ ratio; TOC:TN: ratio of total organic carbon (TOC) and total
 328 nitrogen (TN). PCA and hierarchical classification were performed using “FactoMineR”
 329 package (Husson et al., 2013).

330 5.2 *Prokaryotic abundances and activities*

331 Prokaryotic abundances ranged from $1.18 \pm 0.20 \times 10^{10}$ to $3.45 \pm 1.05 \times 10^{10}$ cells g⁻¹ sed DW
332 in July with maximum values in the surficial sediment layer (0-0.5 cm below the sediment
333 surface) (Figure 2). Abundances were significantly lower in February than in July (two-way
334 ANOVA, $F=24.16$, $p < 0.001$, Supp info Table S3) with values between $1.08 \pm 0.75 \times 10^{10}$ and
335 $1.72 \pm 0.50 \times 10^{10}$ cells g⁻¹ sed DW and a peak recorded between 0.5 and 1 cm bsf. In July,
336 thymidine incorporation (a proxy for benthic bacterial production) decreased with depth from
337 189.69 ± 8.15 to 46.31 ± 9.93 pmol ³H-Thy g⁻¹ sed DW h⁻¹. In February, thymidine
338 incorporation was lower but showed a similar decrease with depth. For both sampling dates,
339 thymidine incorporation and prokaryotic abundance distribution profiles were very similar
340 (Pearson test, $n=30r^2 = 0.806$, $p < 0.001$) (Figure 2). The impact of a 10°C-increase on
341 thymidine production was expressed by using Q₁₀. The temperature had a strong impact on
342 thymidine production between 0 and 0.5 cm bsf (average value of Q₁₀=6.265). Then, between
343 0.5 and 1 cm bsf, temperature effect was less important (average value of Q₁₀=1.589) but
344 significantly different from 1 (t-test one sample, $t= 3.4589$, $p = 0,009$). Between 1 and 10 cm
345 bsf, the temperature had no effect as Q₁₀ values were not significantly different from 1 (t-test
346 one sample, $p > 0.01$).

347 Variance analysis (two-way ANOVA) showed that potential aminopeptidase activity was
348 significantly higher in July ($F=75.29$, $p < 0.001$, Supp info Table S3) (mean for all depth: 381.31
349 ± 78.64 nmol g⁻¹ sed DW h⁻¹) than in February (mean for all depth: 88.02 ± 13.60 nmol g⁻¹ sed
350 DW h⁻¹) and that in July, these activities were significantly different in the surface sediment
351 compared to the deeper layers (Tukey HSD test, $p < 0.001$) (Figure 2). Potential β-glucosidase
352 activity was generally low throughout all the sediment depths. Values ranged from 6.71 ± 1.09

353 to 18.14 ± 1.42 nmol g⁻¹ sed DW h⁻¹ in July and from 3.49 ± 1.21 to 41.59 ± 8.32 nmol g⁻¹ sed
354 DW h⁻¹ in February (Figure 2).



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Figure 2. Prokaryotic abundances, production and activities along a vertical depth

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gradient below the sediment surface (bsf). All points represent the middle of each layer. The

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coefficient of variation is displayed as bubble size. Black bubbles represent values for July 5,

359

2012, and gray bubbles represent values for February 11, 2013.

360 5.3 *Algal biomass*

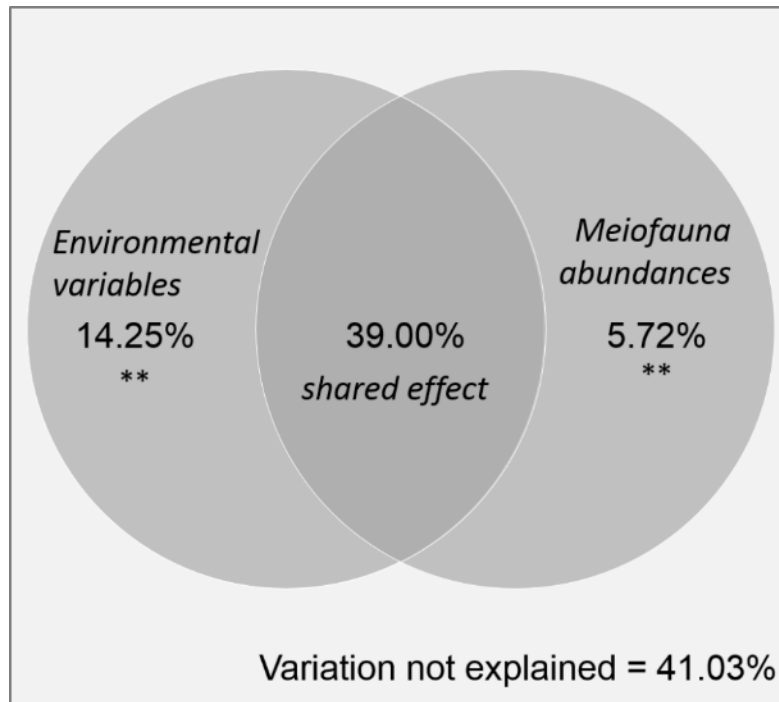
361 The algal biomass on the surface (D1) was $69.5 \pm 2.4 \mu\text{g Chl } a \text{ g}^{-1} \text{ sed DW}$ $59.4 \pm 1.7 \mu\text{g}$
362 $\text{Chl } a \text{ g}^{-1} \text{ sed DW}$ during the samplings in July and in February, respectively (Table 1). The
363 highest standard errors were recorded in D1, resulting probably from the patchiness distribution
364 of the microphytobenthos observed in the field. The algal biomass showed an exponential
365 decrease with values never exceeding $17.40 \mu\text{g Chl } a \text{ g}^{-1} \text{ sed DW}$ under 0.5 cm bsf (Table 1).

366 5.4 *The distribution of fauna abundances*

367 The abundance of six meiofaunal groups was recorded: nematods, copepods, ostracods, small
368 gastropods, small bivalves and foraminifera (Supp info Fig S5). The most abundant were the
369 nematods (maximum abundance= 1060 ind cm^{-3}) and foraminifera (maximum abundance= 57
370 ind cm^{-3}). The abundances of groups investigated decreased with depth increase (Supp info Fig.
371 S5). Higher abundances were recorded in July except for copepods and ostracods. Additionally,
372 the macrozoobenthic grazer, *Peringia ulvae* (Pennant, 1777) present at the surface of the
373 sediment appeared to be more abundant in February (1908 ind m^{-2}) than in July (528 ind m^{-2}).

374 5.5 *Factors influencing prokaryotic activities and densities*

375 All variables used in the variation partitioning analysis (E table: salinity, phosphate and
376 silicate concentrations and M table: juvenile gastropods) had a significant effect on prokaryotic
377 activity and abundance (Supp info Table S4). The environmental variables (E table) explained
378 14.25 % of the variance of distribution of the prokaryote-related variables without the
379 component variations shared with the meiofauna abundance (M table). While meiofaunal
380 abundances explained 5.72% of the variation of prokaryotic variables. Collectively, phosphate
381 and silicate concentrations, salinity, and abundances of little gastropods explained 59% of the
382 prokaryotic abundances and activity variations (Figure 3).



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Figure 3. Venn diagram based on a variation partitioning from prokaryotic variables (*i.e.*, prokaryotic abundance; thymidine incorporation; aminopeptidase activity; and beta-glucosidase activity). The external square represents the whole variation of the prokaryotic table. Each circle represents the explanatory tables and values are the part of the variation explained by each explanatory table. The variables used in the analysis was previously selected by forward selection and final tables included: Environmental variable table: Salinity, PO_4^{3-} concentrations, and silicate concentrations; and meiofauna table: abundances of juvenile gastropods. Statistically significant pure fraction of variation of prokaryotes communities are presented as: <0.01 ** (ANOVA like permutation test, 9999 permutations) and details are given in Supp info Table S4.

394 **6 Discussion**

395 The muddy sediments in Marennes-Oléron Bay support high microbial activities and
396 production rates as is typical for fine-grained sediments (Böer, 2008; Llobet-Brossa et al.,
397 1998). This study shows depth gradients of prokaryotic abundances and activities in the top 10-
398 cm of these coastal mudflats based on the analyses of depth layers chosen to characterize
399 centimetre-scale processes. The stratification was particularly pronounced for the sampling in
400 July and this appeared to be related to depth variation of abiotic and biotic environmental
401 variables. Using the set of these environmental variables in the different depth layers, we have
402 studied how they could statistically explain the differences of prokaryotic abundances and
403 activities in the sediment. However, we had to exclude grain size and oxygen. The former
404 showed hardly any variation with depth, while the latter is known to show mm-scale variation
405 close to the surface that was not adequately measured in this study. Nevertheless, a sufficiently
406 large panel of biotic and abiotic variables were available for disentangling the contributions of
407 these environmental variables for driving prokaryotic abundances and activities.

408 *6.1 Relative impact of environmental variables and meiofauna: the main driving factors*

409 A forward selection identified that prokaryotic abundances and activities were
410 significantly influenced by salinity, phosphate and silicate concentrations as well as juvenile
411 gasteropod abundances. Above all, the resulting variation partition, underlined that the
412 interaction among physicochemical variables and meiofaunal abundance is high and has a
413 significant impact on prokaryotic abundances and activities (Figure 3). While the gasteropod
414 juveniles are not abundant in this study, their distribution significantly affects the prokaryotic-
415 related variables and is strongly related to physicochemical variables (*i.e.*, large part of variance
416 explanation shared with environmental table).

417 Nitrites or nitrates are more often identified as forcing factors for prokaryotic
418 communities in sediments (Böer et al., 2009), however, in the current study, the use of variation
419 partition shows that others inorganic nutrients such as silicates and phosphates significantly
420 influenced the prokaryotic activities and abundances. Interestingly, the phosphate
421 concentrations appeared to limit the prokaryotic activities (*e.g.*, thymidine incorporation,
422 aminopeptidase and beta-glucosidase activities) more than nitrogen-related nutrients in bottom
423 layers in July and in surface in February (*i.e.* DIN:PO₄³⁻ ratio>16; Supp info Figure S2).

424 In a previous study, Pascal et al. (2009) showed that only 6 % of the total bacterial
425 biomass was controlled by consumers in the first 1 cm of the sediment surface, suggesting a
426 major effect of resources in the Marennes-Oléron mudflat. Our statistical results suggest that
427 the activities and abundances of benthic prokaryotes in the first 10 cm of sediment were more
428 influenced by physicochemical properties of the sediment (*i.e.*, inorganic nutrients and salinity)
429 rather than by predation pressure by meiofauna (Supp info Table S4). The variation partitioning
430 that we propose statistically identifies that bottom-up control (represented by physicochemical
431 variables) had stronger influence on prokaryotic activities than top-down control by meiofauna
432 and that the shared interactions between the two trophic controls are of major importance. In
433 the current study, it appears that physicochemical properties of the sediment that varied with
434 depth strongly stratified the biotic communities. The high part of variation explained by the two
435 trophic controls could reflect this influence of physicochemical variables on both prokaryotic
436 activities and abundances and meiofauna abundances. However, it could also be due to the fact
437 that meiofauna could slightly modify the vertical stratification of organic matter, inorganic
438 nutrient or EPS composition. Other proxies can be used to identify factors that drive microbial
439 communities. For example, Pace & Cole (1994) proposed that a strong positive correlation
440 between prokaryotic biomass and production rates indicates bottom-up control. This relation

441 can thus be successfully applied to understand the relationships in benthic microbial ecology,
442 although other factors such as organic matter should also be considered.

443 6.2 *Two horizons, two different stories*

444 The principal component analysis followed by hierarchical clustering based on
445 physicochemical variables confirmed a vertical zonation mainly described by organic matter
446 composition (*i.e.*, the TOC:TN ratio), pH and salinity (Figure 1). Collectively, our results
447 showed that the upper 10 cm of the sediment was divided into two clearly different horizons
448 with thickness varying between the samplings in July and February. The surface horizon,
449 separated from the bottom one by a transition layer is thicker in February (2 cm) than in July (1
450 cm). The position of the transition zone proposed here was therefore dependent on the thickness
451 of the sampling layers in our study and was expected to fluctuate from 1 cm to 3.5 cm bsf
452 (middle of our sampling layer).

453 The biotic and abiotic variables in the surface horizon differed between the two
454 sampling dates. In July, prokaryotic and environmental variables (*e.g.*, prokaryotic abundance,
455 thymidine incorporation, aminopeptidase activity, EPS-carbohydrates and salinity) were high
456 compared to February (Table 1 and Figure 2). For example, in July, aminopeptidase activity
457 was particularly high compared to other studies (as reviewed by Danovaro et al. (2002)) but
458 comparable with aminopeptidase activities recorded in the Balearic Sea (Tholosan et al., 1999).
459 Thymidine incorporation, used as a proxy of benthic bacterial production, drastically increased
460 with an increase of 10°C (*i.e.*, high Q₁₀ value). On the basis of our results (Figure 2), we
461 hypothesized that in February, in the surface horizon (0-2 cm bsf), the prokaryotic communities
462 showing low metabolic activities were not able to sustain growth as a large part of their
463 metabolic energy was used for maintenance. In contrast, in July, as a result of higher
464 temperature, the high densities and high metabolic rates of prokaryotes seemed to be related to
465 metabolically active and growing populations. At low tide, prokaryotic populations in the

466 surface horizon are strongly influenced by external parameters (*e.g.*, light exposure,
467 resuspension and tidal cycle) and microphytobenthic activity. Although algal biomass (*i.e.*, as
468 measured by chlorophyll *a* concentration) was in the same range for both sampling dates, the
469 high microphytobenthic primary production in July (gross primary production: 6.0 ± 1.7 mg C
470 $\text{h}^{-1} \text{m}^{-2}$, CO₂ fluxes in benthic chambers measurement method, pers. comm. from J. Lavaud)
471 had probably enhanced the bacterial production in the sediment top layer (0-0.5 cm bsf). This
472 source of labile carbon may be quickly transferred to the bacterial compartment as shown
473 previously in sandy sediments (Cook et al., 2007) and intertidal flats (Middelburg et al., 2000).
474 Moreover, large amounts of EPS-carbohydrates were recorded in July compared to February
475 and these EPS may be produced by epipellic diatoms in response to nutrient limitation or photo-
476 protection (Smith and Underwood, 2000, 1998). Together, the high EPS-carbohydrates
477 concentrations, the low nutrient concentrations, and the DIN:PO₄³⁻ ratio below the Redfield
478 value (Redfield, 1958), suggested a nitrogen limitation for benthic micro-organisms in surface
479 in July.

480 While EPS-carbohydrates were dominant in July, EPS-proteins clearly increased in
481 February (as shown by the shift of the ratio EPS-carbohydrates/EPS-proteins, Supp info Figure
482 S3). At this date, both prokaryotic density and thymidine incorporation were low in the top
483 horizon (0-2 cm bsf, Figure 2) and this was not only due to the low sediment temperature
484 because higher bacterial production occurred deeper in the sediment despite a similar
485 temperature. A study in Marennes-Oléron mudflat (Orvain et al., 2014b) shows that a higher
486 proportion of EPS-proteins coincided with mass erosion events and higher abundance of the
487 macrozoobenthic grazer, *Peringia ulvae* (Pennant, 1777). These macrozoobenthic grazers may
488 disturb the sediment stability by grazing on biofilm and EPS-proteins may potentially
489 originated from shell mucus (Orvain et al., 2014b). Based on these features and on our results,
490 it may be possible that the highest abundance of *Peringia ulvae* (Pennant, 1777) recorded in

491 February provoked a high predation pressure (*i.e.*, predation pressure: 1.72 mg C h⁻¹, calculated
492 according to Pascal et al. (2009)) and an increase of EPS-proteins, hence inducing mass erosion
493 of the sediment. This erosion is associated with the release of diatoms and prokaryotes into the
494 water column (Guizien et al., 2014; Montanié et al., 2014; Shimeta et al., 2002) and may
495 therefore impact the surface of sediment in February by a decrease of prokaryotic density and
496 bacterial production. Finally, in our study, even if mass erosion of the sediment surface might
497 have occurred at seeing the sea state (Suppl. Info, Table S2) and the wind speeds (data not
498 shown), prokaryotic abundance could be lower because of the grazing of *Peringia ulvae*
499 (Pennant, 1777) or by viral lysis that has been reported to be responsible for the loss of 40 % of
500 bacterial production in Marennes-Oléron mudflat (Saint-Béat et al., 2013). These results
501 suggesting a mass erosion event that occurred in February are consistent with a thicker surface
502 horizon (from 0 to 2 cm bsf) compared to the one in July.

503 In the bottom horizon, between 1 or 2cm bsf (in July and February, respectively) and 10
504 cm bsf, all biotic and physicochemical gradients showed little variation with depth. For both
505 sampling dates, the thymidine incorporation used as a proxy of bacterial production was similar
506 below 2 cm depth despite high environmental differences. Indeed, temperature, salinity, and
507 the EPS-carbohydrates strongly decreased from July to February, and nutrient concentrations
508 also changed—specifically, phosphate and ammonium concentrations increased (Supp info
509 Figure S2). While this bacterial production was clearly lower in this bottom horizon compared
510 to the surface one we probably underestimated thymidine uptake in the anoxic layers because
511 the experiments were not performed under anoxic conditions while microorganisms may be
512 partially or strictly anaerobes. Despite this potential underestimation, bacterial communities
513 were able to maintain the same production level between 2 and 10 cm bsf in both sampling
514 dates, suggesting that the system may potentially contain a low and stable microbial bulk
515 activity in this horizon throughout the year independently of environmental changes.

516 6.3 *The transition zone*

517 The boundary layer may represent a transition zone between the surface horizon largely
518 influenced by external parameters and the bottom horizon corresponding to reduced sediment.
519 The current study proposes that the transition zone should represent the limit of influence of
520 weather conditions on sediment physicochemical properties and thus on prokaryotic activities
521 in the intertidal mudflat. The depth of this layer was expected to fluctuate weakly over the
522 seasons and among the low tide period. Notably, storms can destroy the vertical structure deeper
523 than the external parameter-influenced zone. Nevertheless, except during these rare but strong
524 events, the depth of this surface layer can be considered specific to intertidal muddy sediments.
525 Indeed, sandy sediments are generally permeable and allow advective fluxes of water through
526 the interstitial spaces (Musat et al., 2006) and thus exhibit a different depth profile compared to
527 muddy sediments. Except for transient storms, the transition layer is thus located at 1-3.5 cm
528 depth in intertidal muddy sediments.

529 Whether this transition zone represents an environmental ecotone or ecocline can be
530 discussed. These two terms have been largely used in ecology to characterize boundary zones
531 where gradients occur, but their definitions and how to use them are still unclear (Erdős et al.,
532 2011). Nevertheless, many authors agree that the term environmental ecotone defines a gradient
533 between two adjacent habitats characterized by rather abrupt changes and that it comprises
534 habitats that should be very specific for certain species (Attrill and Rundle, 2002; Erdős et al.,
535 2011; van der Maarel, 1990; Whittaker, 1967). In contrast, an environmental ecocline stands
536 for more gradual changes that may result from mixing of the two communities from the
537 neighboring habitats (Attrill and Rundle, 2002; Erdős et al., 2011; van der Maarel, 1990;
538 Whittaker, 1967). In the present study, the transition zone corresponded to a gradient zone at a
539 cm scale which we characterized by a gradual change of environmental variables such as
540 porosity or EPS ratios and a gradual change of microbial communities (*e.g.*, algal biomass,

541 enzymatic activities and prokaryotic abundance). Hence, following these definitions and our
542 findings, we should rather consider the identified transition zone as an environmental ecocline
543 boundary (Erdős et al., 2011).

544 6.4 Conclusion

545 The current study provided detailed snapshots of the depth gradients of prokaryotic
546 abundances and process rates at two sampling dates at low tide. The detailed stratification
547 pattern using a large ensemble of variables and different multivariate analyses allowed us to
548 decipher some of the major factors driving the densities and activities of microbial populations
549 in intertidal sediments. Thus, we succeeded in statistically explaining a large part of the
550 prokaryotic activity distributions by the environmental variables (*i.e.*, salinity and nutrients),
551 and to a lesser extent by consumers (meiofauna), suggesting that bottom-up control was more
552 important than top-down control. In general we observed that the top 10 cm of these muddy
553 sediments comprise two clearly different depth horizons that are separated by a transition zone.
554 Thus we identified a surface horizon, which appears variable in thickness between sampling
555 dates and where prokaryotic activities and densities are highly impacted by microphytobenthic
556 activities and physicochemical variables and, a deeper and more stable bottom horizon. The
557 transition appears to be gradual corresponding to an environmental ecocline rather than an
558 ecotone.

559 Nevertheless, one part of this distribution remained statistically unexplained (41% of
560 the variation is estimated to be unresolved by the chosen variables in the variation partitioning)
561 and further studies are needed to explore 1) other abiotic variables such as sulfate, iron oxide
562 or manganese oxide concentration, 2) prokaryotic activity and production dynamics throughout
563 the low tide period, and 3) other prokaryotic indices such as diversity or functional genes.

564 **7 Figure captions**

565 **Figure 1.** Principal component analysis (PCA) ordination calculated using 8 physico-
566 chemical variables for a and c) 15 samples in July and b and d) 15 samples in February. a and
567 b) Ordination of the variables and correlation circle. b and d) Position of the observations in the
568 ordination; tree calculated hierarchical classification on principle components and the different
569 clusters evaluated using 10000 iterations . T: temperature; S: salinity; Prot: total protein content;
570 EPS.CB: colloidal EPS/bound EPS ratio; EPS.CP: EPS-carbohydrates/EPS-protein ratio;
571 DIN.P: DIN:PO₄³⁻ ratio; TOC:TN: ratio of total organic carbon (TOC) and total nitrogen (TN).
572 PCA and hierarchical classification were performed using “FactoMineR” package (Husson et
573 al., 2013).

574 **Figure 2.** Prokaryotic abundances, production and activities along a vertical depth
575 gradient below the sediment surface (bsf). All points represent the middle of each layer. The
576 coefficient of variation is displayed as bubble size. Black bubbles represent values for July 5,
577 2012, and gray bubbles represent values for February 11, 2013.

578 **Figure 3.** Venn diagram based on a variation partitioning from prokaryotic variables
579 (*i.e.*, prokaryotic abundance; thymidine incorporation; aminopeptidase activity; and beta-
580 glucosidase activity). The external square represents the whole variation of the prokaryotic
581 table. Each circle represents the explanatory tables and values are the part of the variation
582 explained by each explanatory table. The variables used in the analysis was previously selected
583 by forward selection and final tables included: Environmental variable table: Salinity, PO₄³⁻
584 content, and silicate content; and meiofauna table: abundances of juvenile gastropods.
585 Statistically significant pure fraction of variation of prokaryotes communities are presented as:
586 <0.01 ** (ANOVA like permutation test, 9999 permutations) and details are given in Supp info
587 Table S4.

588 **8 Table caption**

589 Table 1. Average of each environmental variables and algal biomass (\pm SE) along
590 sediment depths. Two-way ANOVA reveals always significant differences between the two
591 sampling dates for all variables ($p < 0.05$), letters in bold font indicate Tukey's post hoc test for
592 each sampling date.

593

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