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## Seasonal dynamics of extracellular polymeric substances (EPS) in surface sediments of a diatom-dominated intertidal mudflat (Marennes-Oléron, France).

Guillaume Pierre, Jean-Michel Zhao, Francis Orvain, Christine Dupuy, Géraldine Klein, Marianne Graber, Thierry Maugard

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1 **Seasonal dynamics of extracellular polymeric substances (EPS) in surface**  
2 **sediments of a diatom-dominated intertidal mudflat (Marennes-Oléron,**  
3 **France).**

4 **Guillaume Pierre** <sup>1,2\*</sup>, **Jean-Michel Zhao** <sup>2</sup>, **Francis Orvain** <sup>3</sup>, **Christine Dupuy** <sup>2</sup>,  
5 **Marianne Graber** <sup>2</sup>, **Thierry Maugard** <sup>2</sup>

6 (1) Clermont Université, Université Blaise Pascal, Institut Pascal, UMR 6602 CNRS - Polytech Clermont-  
7 Ferrand, 24 avenue des Landais, BP206, F-63174 Aubière cedex, France

8 (2) UMR 7266 CNRS - ULR LIENSs. Université de La Rochelle, UFR Sciences, Bâtiment Marie Curie,  
9 avenue Michel Crépeau, 17042 La Rochelle, France.

10 (3) Université de Caen Basse-Normandie, CNRS FRE 3484 BioMEA, esplanade de la Paix, 14032 Caen,  
11 France.

12 \* Tel: +33 (0) 4 73 40 74 22 Fax: +33 (0) 4 73 40 78 29 E-mail: guillaume.pierre@univ-bpclermont.fr

13 **Abstract**

14 Numerous field-based investigations have highlighted that the production of Extracellular  
15 Polymeric Substances (EPS) is physico-chemically and ecologically essential for intertidal  
16 mudflats. EPS are largely secreted by marine benthic diatoms and their quantity and quality  
17 are environmental-dependant. This paper focused on the dynamic pathways, concentration  
18 rates and monosaccharides composition of colloidal, bound and residual carbohydrates  
19 extracted by using a cationic exchange resin from a diatom-dominated intertidal mudflat  
20 (Marennes-Oléron, France) during two different sampling periods: winter (February 2008)  
21 and summer (July 2008). A wide range of biotic and abiotic parameters were also studied to  
22 better understand the effect of environmental parameters, *e.g.* chlorophyll *a*, salinity, pore  
23 water, emersion time, luminosity, C:N ratio or tidal coefficient. Multiple colorimetric assays  
24 coupled to gas chromatographic analyses were carried out to perform the biochemical

25 characterizations. Firstly, the quantity of carbohydrates produced during winter was more  
26 important than during summer. Yet, more proteins were found during summer especially for  
27 the colloidal and bound fractions. Further investigations showed that the dynamic pathways  
28 were equivalent between winter and summer: bound carbohydrates (BC) quantities increased  
29 during the sediment emersion periods on the contrary to colloidal carbohydrates (CC) which  
30 tended to drop throughout the emersion time. The quality in monosaccharides was fraction-  
31 dependant, whatever the season. CC were always glucose-rich confirming their role of carbon  
32 source. BC were mainly composed of rhamnose whose the ratio increased during the  
33 emersion period, thus conferring adhesive properties to the extracellular matrix bounding  
34 diatoms cells. Residual carbohydrates (RC) were composed of various monosaccharides and a  
35 major increase of glucose content was found at the end of emersion, corresponding to  
36 intracellular C-storage in prevention to immersion times. Summer-RC were composed of  
37 fucose, a monosaccharide specific to these fractions and which was non-present during the  
38 winter campaign. Environmental parameters, as salinity, pore water, tidal coefficient could  
39 have a significant impact on the concentration rates and pathways of carbohydrates.

40 *Key words:* Extracellular polymeric substances (EPS), carbohydrates, diatoms, seasonal  
41 impacts, mudflat

42

### 43 **1. Introduction**

44 Intertidal mudflats are extremely productive areas and may provide up to 50 % of the primary  
45 and secondary production of estuaries (Underwood and Kromkamp, 1999), especially due to  
46 the formation of diatom-dominated biofilms at their surface (Falcatore and Bowler, 2002).  
47 Subject to periodic tidal exposure, the physical and chemical properties of these biofilms  
48 change (Christie et al., 2000; de Brouwer and Stal, 2001). Thus, some authors noted that

49 variations in sediment dynamics and compositions could result from seasonal fluctuations  
50 (Frostick and McCave, 1979), and particularly Extracellular Polymeric Substances (EPS)  
51 composing benthic biofilms. Benthic biofilms are composed of water, microalgae,  
52 prokaryotes (bacteria, archaea), other eukaryotic microbes, virus and inorganic particles  
53 entangled in an EPS matrix (Yallop et al., 1994). This EPS matrix is rich in a wide variety of  
54 polysaccharides, proteins, glycoproteins, lipids and nucleic acids (Wingender et al. 1999).  
55 Besides, marine diatoms are known to produce various kinds of EPS depending on the  
56 environmental conditions and the tidal periods (Underwood et al., 2004). On one hand,  
57 colloidal EPS are excreted in the microenvironment of the benthic biofilms (Underwood et al.,  
58 1995) and are used by different microorganisms, *e. g.* the glucose-rich exudates consumed by  
59 heterotrophic bacteria (van Duyl et al., 1999; Hofmann et al. 2009). On the other hand, bound  
60 EPS are secreted as mucilaginous slime coating the cells (Underwood and Paterson, 2003).  
61 These EPS play key roles in microbial and physico-chemical defense (Decho, 1990), in the  
62 motility of epipelagic diatoms (Stal and Défarge, 2005), in cells/cells or cells/substratum  
63 adhesion (Wimpenny et al. 2000) and sediment biostabilisation (Spears et al., 2008). Several  
64 studies have reported large variability of EPS composition, abundance and properties, thus  
65 highlighting the scientific challenge to extract and analyze consistently EPS by biochemical  
66 methods (Azerado et al., 2003; de Brouwer and Stal, 2004). In this way, Underwood and  
67 Paterson (2003) have pointed the importance of the biochemical methodology to accurately  
68 reflect the biological utility or relevance of EPS in benthic biofilms. Recently, an alternative  
69 method for *in situ* EPS extraction and determination (Takahashi et al., 2009), applied during  
70 two field studies (Pierre et al., 2010 ; Pierre et al., 2012), has shown the possibility to separate  
71 three major classes of EPS through the use of a cationic exchange resin (Fig. 1). Moreover,  
72 the use of diluted polar solvents in these works allowed the size separation of EPS: short-  
73 chain to long-chain oligosaccharides, *i.e.* low molecular weight (LMW) compounds and high

74 molecular weight (HMW) compounds (Decho, 2000; Bellinger et al., 2005). Such  
75 characterization of EPS is a necessary step to determine and understand their roles in benthic  
76 biofilms, especially if we focus on the influence of biotic and abiotic parameters.

77 The goal of this study was to investigate the temporal distribution and compositional changes  
78 of EPS, in relation to seasonal dynamics. A challenging aim was also to compile numerous  
79 data obtained on the same field to clearly show the impact of seasons on the EPS composition  
80 of a microphytobenthic biofilm and, *a fortiori*, on the trophic involvement of intertidal  
81 mudflats. The EPS samples were collected from a diatom-dominated biofilm (Marennes-  
82 Oléron Bay, France) during different ecological periods in the chemistry and biology of tidal  
83 flats, *i.e.* in February 2008 (winter) and July 2008 (summer), when the microphytobenthic  
84 biofilm development and its EPS composition are supposed to be drastically dissimilar.

## 85 **2. Material and methods**

### 86 *2.1. Field sampling*

87 The sediment samples were collected from Marennes-Oléron Bay (Atlantic Coast of France),  
88 during only one week in February 2008 (winter) due to strong field constraints and two weeks  
89 in July 2008 (summer) at low tide (Fig. 2). The field sampling was organized as a chessboard  
90 where square sampling units (2m-side) separated by alleys (2 m in width) were defined. Every  
91 day, three squares were randomly considered for spatial heterogeneity. Sediment samples  
92 from each square were collected by using core diameter of 20 cm. Three cores of each  
93 selected square were sampled every hour during the tidal periods. For each core, the top 1cm  
94 was collected three times and pooled. After each sampling, sediment pools were brought back  
95 from the field for an immediate EPS extraction on fresh sediments. Biochemical analyses  
96 were then performed in triplicate on the colloidal, bound and residual fractions (864  
97 fractions).

98 *2.2. Biotic and abiotic measurements*

99 The chlorophyll *a* concentration in the sediment was measured using fluorometry method  
100 (Lorenzen, 1966). Light was measured using a Li-Cor sensor which was recorded every  
101 minute during sampling days. Salinity, pore water, C:N ratio were also followed. Enumeration  
102 of bacteria was performed by microscopy, after 4,6-diamidino-2-phenylindole  
103 dihydrochloride (DAPI) labeling (x 1000, Axioskop, Zeiss) using the method of Porter and  
104 Feig (1980). All missing data were due to experiment constraints.

105 *2.3. Material*

106 Solvents, cationic resin (Dowex Marathon C), assay kits, protein (Bovine Serum Albumin,  
107 BSA) and carbohydrate standards (dextran, dextran sulfate, glucose, galactose, rhamnose,  
108 fucose, fructose, xylose, arabinose, ribose, mannose, *myo*-inositol, glucuronic and  
109 galacturonic acids) were obtained from Sigma-Aldrich. The DB-1701 J and W Scientific  
110 column (30 m, 0.32 mm, 1  $\mu$ m) for Gas Chromatography-Mass Spectrometry analysis  
111 (GC/MS) was obtained from Agilent.

112 *2.4. EPS extraction and fractionation*

113 The extraction method (Takahashi et al., 2009) was done immediately after sampling and  
114 sediment mixing on the field. 20 mL of fresh mudflat was continuously mixed with 20 mL of  
115 Artificial Sea Water (ASW 30 Practical Salinity Units) during 1 h in darkness at 4 °C and  
116 then centrifuged at 3500 g and 4 °C for 10 min. The supernatant containing colloidal EPS was  
117 collected and stored at 4 °C. 20 mL of ASW and 1 g of activated Dowex Marathon C,  
118 previously prepared in Phosphate Buffer Saline for 1 h in the dark, was added to the sediment  
119 pellet. The samples were mixed gently at 4 °C for 1 h in the dark and then centrifuged at 3500  
120 g and 4 °C for 10 min. A supernatant containing the bound EPS and a cap containing  
121 intracellular and residual polymers were obtained. The cap was then frozen. The residual

122 polymers were extracted from the frozen sediment samples by sonication at 100 W for 3 min  
123 on ice after resuspension in 20 mL in ASW. For each fraction (colloidal, bound and residual  
124 polymers), absolute ethanol at -20 °C was added to the sample to obtain a final ethanol  
125 concentration of 75 % (v/v). The solution was gently mixed and stored overnight at -20 °C.  
126 The solution was then centrifuged at 3500 g and 4 °C for 15 min to obtain a supernatant  
127 (LMW fraction) and a precipitate pellet (HMW fraction). Finally, the fractions were dried  
128 under air flow and stored at -20 °C.

### 129 *2.5. Carbohydrate, uronic acid and protein analysis*

130 Total sugar content was determined by the phenol-sulfuric acid assay, using glucose as a  
131 standard (Dubois et al., 1956). Total sugar amounts for the fractions were measured and  
132 normalized to chlorophyll *a* (chl *a*), thus allowing the overestimation of diatom EPS,  
133 comparing to other EPS sources (Underwood and Paterson, 2003). Uronic acid content was  
134 determined using the meta-hydroxydiphenyl method (MHDP), with galacturonic and  
135 glucuronic as standards (Blumenkrantz and Asboe-Hansen, 1973; Filisetti-Cozzi and Carpita,  
136 1991). Protein content was determined by the bicinchoninic acid (BCA) method, using bovine  
137 serum albumin (BSA) as a standard (Smith et al., 1985).

### 138 *2.6. Gas Chromatography coupled to Mass Spectrometry (GC/MS) to characterize the* 139 *carbohydrate of EPS fractions*

140 EPS fractions were solubilized in 5 mL of ultrapure water, dialyzed (6-8 KDa) and freeze-  
141 dried. EPS were then dissolved in 2 M HCl at 50 mg/mL and heated at 90 °C for 4 h. The  
142 preparation was then freeze-dried and stored at -20 °C. Analyses were carried out by GC/MS  
143 using a Varian CP-3800 GC/Varian Saturn 2000. 400 µL of pyridine and 400 µL of BSTFA:  
144 TMCS (99: 1) was added to 2 mg of purified monosaccharides. The solution was mixed for 2  
145 h at room temperature, then injected into a DB-1701 J&W Scientific column (30 m, 0.32 mm,

146 1  $\mu\text{m}$ ) at a flow of 1 mL/min. The helium pressure was 8.8 psi. The temperature of the  
147 injector was set at 250 °C. The rise in temperature in the oven was programmed for a first step  
148 at 150 °C for 0 min, then an increment of 10 °C/min up to 200 °C with a final step at 200 °C  
149 for 35 min. The ionization was performed by Electronic Impact (EI, 70 eV), the trap  
150 temperature was set at 150 °C and the target ion was fixed at 40-650 m/z.

## 151 *2.7. Statistical analysis*

152 All statistical analyses were run using the statistical software XLStat (Addinsoft). One-way  
153 ANOVA was used to analyze changes in carbohydrate and uronic acid amounts among abiotic  
154 parameters for each day (sampling location and emersion time). Data transformations (root)  
155 were performed to check application conditions (normality) each time it was required. Post  
156 hoc procedures (Tukey test) were performed to analyze pairwise differences. *t* and Z-tests  
157 were conducted to determine significant differences between values of variables at the  
158 beginning and at the end of the emerged period. Pearson correlation tests were done with the  
159 complete data set to investigate the relationships between the different EPS fractions with  
160 biotic (bacterial abundance, Chl *a*) and abiotic (luminosity, salinity) parameters. Principal  
161 component analyses were used to highlight and group fractions with closed monosaccharides  
162 distributions.

## 163 *2.8. Abbreviations and nomenclature*

164 In accordance with the literature (Underwood et al., 2010), the term “carbohydrates” refers to  
165 the total sugars (neutral and acidic sugars) determined by Dubois assays and GC/MS. The  
166 term “uronic acids” corresponds only to the uronic assays clearly measured by the BCA  
167 method or GC/MS analyses. Concerning the abbreviations, W and S correspond to winter and  
168 summer, the two sampling periods. LMW and HMW refer to the size of the carbohydrates:  
169 low molecular weight and high molecular weight. CC, BC and RC refer to the carbohydrates

170 extracted from the three main fractions: colloidal carbohydrates (CC) are water-extractable,  
171 bound carbohydrates (BC) are obtained by using Dowex Marathon C, and residual  
172 carbohydrates (RC) correspond to the fractions rich in intracellular carbohydrates and  
173 refractory EPS.

### 174 **3. Results and discussion**

#### 175 3.1. Relationships between EPS and environmental parameters: a seasonal heterogeneity?

176 The total carbohydrate quantities and the contribution of neutral carbohydrates, uronic acid  
177 and proteins were determined for the three main EPS fractions extracted in winter and  
178 summer (Fig. 3). At this step, it is noteworthy that the sugar amounts were normalized to Chl  
179 *a* in order to overestimate diatom EPS production, compared to other EPS sources (Haubois et  
180 al., 2005). In average and regardless the emersion time, more colloidal carbohydrates were  
181 extracted from the sediment in winter ( $5.28 \mu\text{g} \cdot \mu\text{g chl } a^{-1}$ ) than summer ( $2.04 \mu\text{g} \cdot \mu\text{g chl } a^{-1}$ ).  
182 The same observations were done for the bound and residual carbohydrates since 2.5 times  
183 more carbohydrates were found in the winter samples (Fig. 3A). Concerning the relative  
184 contribution of neutral and acidic sugars, the results showed that neutral sugars were the main  
185 component of the fractions both in summer and winter although the ratios varied during these  
186 two periods. For the colloidal and bound fractions, the neutral sugar ratios dropped from 78 %  
187 to 61 % and 87 % to 63 % respectively (relative % w/w) due to the presence of proteins in the  
188 fractions extracted in summer.

189 However, if we focus only on the carbohydrate part, no significant change ( $p < 0.01$ ) was  
190 observed for the neutral sugar ratios. Previous papers noted that variations in sediment  
191 dynamics resulted from seasonal changes in biological influences (Frostick and McCave,  
192 1979), EPS quality and quantity being excellent indicators to measure the impact of these  
193 modifications. In this way, correlations were found between the level of EPS and the diatom

194 biomass in the sediment, *i.e.* EPS quantities reduced when the diatom biomass decreased  
195 (Staats et al., 2001). Ambient light climate play an essential role on the photosynthetic power  
196 of epipelagic diatoms, which move up to the sediment (Serôdio et al., 1997). Microphytobenthic  
197 biofilms can have high rates of photosynthesis and a large part of the photo-assimilated  
198 carbon is excreted into the environment as exopolymers (Underwood and Paterson, 2003).  
199 During our sampling periods, the luminosity was around  $1600 \pm 400 \mu\text{mol photons.m}^{-2}.\text{s}^{-1}$  in  
200 summer and  $440 \pm 250 \mu\text{mol photons.m}^{-2}.\text{s}^{-1}$  in winter. The level of chl *a* measured on the  
201 sediment was significantly lower ( $p < 0.01$ ) in summer ( $8.1 \pm 1.2 \mu\text{g.g dry sediment}^{-1}$ ) than  
202 in winter ( $20.2 \pm 0.9 \mu\text{g.g dry sediment}^{-1}$ ). This difference was attributed to the intense level  
203 of grazing by *Peringia ulvae* whose the density was higher in summer ( $17.2 \text{ ind.m}^{-2} \pm 7.08$ )  
204 than in winter ( $5.77 \text{ ind.m}^{-2} \pm 2.99$ ). Moreover, the classical normalization with chl *a*  
205 enhanced the gap between the values in summer and winter. Besides, intertidal mudflats are  
206 living areas whose the primary trophic production is non-negligible. Indeed, diatoms and their  
207 EPS production are responsible for 40 to 50 % of the total global primary production in  
208 marine systems (Medlin, 2002). So, biota living in the intertidal zone are adapted to use EPS  
209 as food or as protection against environmental dynamics which dominate tidal flats (Widdows  
210 and Brinsley, 2002). During summer periods, consumers of EPS (carbon source) are much  
211 more active and directly responsible in a drop of sediment stability (Andersen, 2001). This  
212 greater summer consumption could also explain the differences between the amounts of  
213 carbohydrates measured in summer and winter.

214 Thirdly, the amounts of proteins detected during summer were interesting because specific of  
215 this period (Fig.3, B). Yet, proteins were also found in colloidal and bound fractions collected  
216 in summer. During summer, pore water in the sediment was significantly lower than in winter  
217 ( $p < 0.05$ ) and positive correlations were found with S-LMW CC and S-LMW BC (Table 1,  $p$   
218  $< 0.05$ ). Some authors have suggested that EPS (carbohydrates and proteins) could protect

219 microphytobenthos from high desiccation levels and salinity (Spears et al., 2008). Thus, we  
220 concluded that the presence of proteins combined to specific carbohydrates during summer  
221 could be a response to desiccation stress, explaining the positive correlations found (Table 1).  
222 These fractions could be correlated to osmoregulatory and hydration properties to fight  
223 against extensive salinity. Besides, proteins can also be used as additional C-storage which  
224 can explain their presence in residual fractions, composed of intracellular (chrysolaminaran)  
225 and refractory EPS.

226 A number of authors have pointed at the rapid changes, in order to hour, which occur at the  
227 surface sediments during periodic tidal exposures but the patterns varied even if colloidal  
228 carbohydrates are often involved (Taylor and Paterson, 1998; Christie et al., 2000, Hofmann  
229 et al. 2009, Pierre et al. 2012). The fate of colloidal and bound carbohydrate amounts between  
230 the beginning and the end of the emersion periods during winter and summer was then  
231 investigated in this work (Fig. 4). LMW colloidal carbohydrate amounts tended to decrease  
232 during the emersion period. This drop observed in winter was not significant ( $p < 0.05$ ) but  
233 significantly visible in summer ( $p < 0.01$ ). Besides, HMW colloidal carbohydrate amounts  
234 remained stable all over the emersion time, in winter or summer. These results were  
235 contradictory to other works which reported an increase of colloidal EPS in the early part of  
236 the tidal exposure and near to the end of the emersion (Taylor, 1998). Nevertheless,  
237 Underwood and Paterson (2003) nuanced these results and argued that the evolution of  
238 colloidal EPS during the emersion period was often misinterpreted due to the extraction  
239 techniques used. In this way, previous studies did not specifically work on colloidal EPS but  
240 on fractions containing a part of bound and residual polymers. The Dowex extraction that we  
241 performed allowed a fine separation between true colloidal and bound EPS surrounding  
242 diatom cells and/or entangled in the EPS matrix (Fig. 1). We already demonstrated that the  
243 use of this procedure *in situ* averted mixing a part of colloidal EPS with LMW bound EPS

244 (Pierre et al., 2012). It can be estimated that the levels and production patterns of colloidal  
245 EPS that we found in the present study were then closer to their function(s) and fate. Thus,  
246 many authors highlighted that colloidal EPS were C-sources in the trophic network of tidal  
247 mudflats, especially for heterotrophic bacteria and other micro-consumers (van Duyl et al.,  
248 1999; Hanlon et al. 2006; Pierre et al. 2012).

249 On the other hand, the amounts of bound carbohydrates increased all over the tidal exposure.  
250 Even if this observation was not significant for W-LMW BC due to a lack of experimental  
251 values ( $p < 0.05$ ), significant increases were observed in summer ( $p < 0.01$ ). Owing to the use  
252 of the Dowex procedure, it was possible to distinguish the fate of true colloidal carbohydrates  
253 whose the amounts dropped and bound carbohydrates whose the concentrations increased  
254 during the emerged period. A part of this result was already observed (Pierre et al., 2012),  
255 leaving us to suggest that bound fractions could be involved in the formation and adhesion of  
256 the microphytobenthic biofilm. The role of microphytobenthos as sediment stabilizers was  
257 well documented (Underwood and Paterson, 1993; Paterson and Black, 1999, Thornton et al.,  
258 2002; Widdows and Brinsley, 2002; Spears et al., 2008) and a reduction in erosion state was  
259 already correlated with increasing of carbohydrates concentrations (Sutherland et al., 1998).  
260 Numerous authors suspected the role of colloidal EPS in general on the sediment dynamics  
261 but not of a very specific and separate part of these exopolymers. Nevertheless, recent works  
262 brought colloidal and especially bound EPS to the fore, whose the production could be light-  
263 dependant (Giroldo et al., 2003; Underwood and Paterson, 2003; Hofmann et al. 2009; Pierre  
264 et al., 2011). Anyway, we noted that no light-dependant production was found for the bound  
265 fractions on the contrary to colloidal fractions whose the production was tidal coefficient-  
266 dependant (Table 1,  $p < 0.05$ ).

267 Numerous authors try to understand how the composition and dynamic of EPS produced by  
268 benthic diatoms change depending on environmental conditions (Underwood and Paterson,

269 2003). Thus, biochemical nature, production pathways, role and fate of EPS in intertidal  
270 ecosystems are widely studied. In general, all authors point that chemical and physical  
271 properties of EPS produced *in situ* vary significantly in response to biotic and abiotic  
272 parameters. That is why important abiotic and biotic parameters were followed in this study,  
273 as chl *a*, light, salinity, pore water, bacterial abundance, tidal coefficient, C:N ratio, in view of  
274 understanding their involvement in EPS dynamic productions. We previously showed that  
275 salinity, pore water, light and chl *a* have to be considered when the seasonal dynamics of EPS  
276 are studied. But it was also interesting to note that tidal coefficients could have a significant  
277 impact (Table 1) which was logical since tidal coefficients influence the degree of periodic  
278 tidal emersions and the light degree. Besides, no significant patterns were found for bacterial  
279 abundance or C:N ratios. It was not possible to correlate C:N ratios with the presence of  
280 marine bacteria/microalgae, terrestrial or degraded organic matter.

### 281 3.2. Monosaccharide composition of EPS: roles and functions of the different fractions

282 Monosaccharide composition of EPS was investigated for colloidal, bound and residual  
283 fractions extracted in winter and summer (Fig. 5). The monosaccharide composition was  
284 heterogeneous depending on the concerned fractions and the low standard deviations (< 5 %)   
285 indicated that the types and quantities of EPS were finely controlled during the short-term  
286 emerged periods. Colloidal carbohydrates were rich in glucose (> 50%), xylose, galacturonic  
287 acid and inositol, regardless the sampling period. Indeed, colloidal carbohydrates are known  
288 as carbon trophic sources due to their high quantity of glucose (Abdullahi et al., 2006;  
289 Hofmann et al. 2009). Nevertheless, we noted that the colloidal carbohydrates collected in  
290 summer were richer in glucose than in winter (+ 4%, *p* value [5%] = 0.003). The same  
291 observation was also done for bound and residual fractions (+ 40 and 45 % respectively). On  
292 the one hand, the high glucose ratio in residual fractions (22 to 42 %) was not surprising since  
293 these samples correspond essentially to intracellular carbohydrates. The majority of the

294 glucosyl units found in these fractions are components of storage polymers as  
295 chrysolaminaran (Underwood and Paterson, 2003). On the other hand, it seemed important to  
296 focus on the increase of glucose content in colloidal and bound fractions during summer. We  
297 suggested that the phenomenon was a seasonal response to environmental needs due to C-  
298 consumers, as nematods, hydrobia or herbivorous zooplankton, which are more abundant and  
299 more active in summer. Thus, colloidal carbohydrates dropped during the emersion time (Fig.  
300 4) but glucose content were higher (Fig. 5). It is therefore possible that carbohydrate EPS are  
301 selectively used by heterotrophic bacteria depending on season. In this way, bacteria could  
302 use the different monosaccharide as substrate but with different degrees of induction. Besides,  
303 the ratios of structural hexoses (xylose and mannose) and uronic acids (galacturonic acids)  
304 were lower in summer for colloidal and bound fractions.

305 Rhamnose was the monosaccharide widely found in LMW (> 25 %) and HMW (18 to 34 %)   
306 bound carbohydrates in winter and summer. The high levels of deoxy sugars in this fraction  
307 were already observed in winter on the same sampling site (Pierre et al., 2011) and other  
308 studies (Hanlon et al., 2006). Owing to their surface-active properties, deoxy sugars can  
309 promote the biostabilisation of sediments (Zhou et al, 1998; Giroldo et al, 2003) by  
310 facilitating the coagulation of particles and macromolecules (Khodse et al., 2007). Rhamnose  
311 could also play a role of biochemical sensor in microphytobenthic biofilms as a target of  
312 proteins involved in cell-cell transmission signals. However, the level of rhamnose was lower  
313 in summer for the HMW bound fractions, *i.e.* for carbohydrates closely attached to diatom  
314 cells. This observation suggested a potential lower biostabilisation of the sediment in summer  
315 (LMW BC: 25.6 %, HMW BC: 18 %).

316 Fucose, a monosaccharide missing during winter samplings, was found in residual fractions  
317 (10 %) collected in summer. We previously attributed the lack of fucose to the physiological  
318 state of the growing biofilm studied in winter (Pierre et al., 2012). During summer, colloidal

319 and bound fractions did not contain fucose, in contrary to many works (Abdullahi et al., 2006;  
320 Hanlon et al., 2006; Hofmann et al., 2009; Takahashi et al., 2009). It is relevant to highlight  
321 that fucose is a monosaccharide involved in the metabolic pathways occurring during the  
322 degradation of  $\beta$ -1,3-glucan storage in diatom cells (Granum, 2002), which could explain its  
323 unique presence in residual fractions.

324 In the same way, another surprising sugar, inositol, was also found in the different samples  
325 and particularly in colloidal fractions (7 to 17 %). As we already reported (Pierre et al., 2012),  
326 this monosaccharide could be a growth factor for heterotrophic microorganisms involved in  
327 the microphytobenthic biofilm formation and synergistic relationships between benthic  
328 diatoms and bacteria (Lubarsky et al., 2010). Moreover, its production seemed to be non-  
329 seasonal dependant (- 5 %).

330 A principal component analysis (ACP) along dominant sugar vectors was performed to  
331 clearly identify the differences in monosaccharide distributions of the EPS fractions (Fig. 6).  
332 Three distinct clusters were highlighted by ACP (C1 to C3). C1 was a cluster constituted of  
333 carbohydrates rich in glucose and inositol. Colloidal carbohydrates were sorted in C1.  
334 However, a significant seasonal difference was found between the monosaccharide  
335 distribution of W-LMW CC and S-LMW CC. Light and pore water could play a significant  
336 role in this dissimilarity (Table 1,  $p < 0.05$ ). So, the monosaccharide distribution of LMW CC  
337 could be seasonal dependant (no cluster). These fractions are extremely labile and can be  
338 selectively consumed by heterotrophic bacteria. Cluster 2 represented fractions rich in  
339 rhamnose, xylose and galacturonic acid. Bound fractions, excepting S-HMW BC, belonged to  
340 C2. Previously, we noted that the rhamnose content was lower in S-HMW BC and we  
341 suggested that this ratio could play a major impact on sediment biostabilisation.

342 It was noteworthy that residual carbohydrates were rich in glucose. However, galacturonic  
343 acid, galactose, fucose and mannose were ACP sugar vectors to take into account to sort these  
344 fractions (C3). Anyway, the results highlighted a strong heterogeneity of carbohydrate  
345 distributions in residual fractions which could be further investigated.

### 346 3.3. *In situ* modification of monosaccharides distribution: specific pathways?

347 The mechanisms allowing diatoms to rapidly change the EPS types being produced are not  
348 well understood (Underwood and Paterson, 2003; Hofmann et al., 2009). Moreover, this is the  
349 selective consumption of carbohydrate EPS by heterotrophic bacteria which seems to play the  
350 major role in these EPS changes (Giroldo et al., 2003). Previously, we showed that four major  
351 monosaccharides (glucose, xylose, galacturonic acid, rhamnose) characterized the different  
352 fractions with some ratios above 20 %. Fucose was only found during summer specifically in  
353 residual fractions. Moreover, these monosaccharides were representative sugars vectors in  
354 ACP analyses to identify clusters. That is why the standing stocks of these five  
355 monosaccharides were followed during the emerged periods in order to highlight possible  
356 specific pathways of selective consumption (Fig. 7). The first important observation to note  
357 was that the same monosaccharide distribution patterns were observed during winter and  
358 summer. Combined to our previous conclusions, this major result indicated that the  
359 distribution of monosaccharides in the fractions seemed to follow the same process regardless  
360 the season and also that the carbohydrate production, in term of amounts, could vary in  
361 response to environmental parameters (cf. part 3.2.). Bacterial interactions, adhesion and  
362 biofilm formation, protection against salinity or migration into the sediment could be  
363 ecological and physico-chemical phenomena involving EPS changes.

364 In details, the results showed that all over the emersion periods, glucose amounts stayed stable  
365 whatever a possible consumption of colloidal carbohydrates by heterotrophic bacteria.

366 Galacturonic acid amounts dropped and rhamnose concentrations slightly increased for  
367 colloidal fractions extracted in winter and summer. The evolution in the galacturonic acid  
368 distribution was interesting and suggested that colloidal fractions were not only used as C-  
369 sources. Uronic acids are involved in several marine environmental processes including the  
370 production of macroaggregates, microbial adhesion and biofilm formation, binding of  
371 extracellular enzymes or ion sequestration (Decho, 1990; Sutherland, 2001; Bhaskar et al.,  
372 2005). This drop of uronic acids amounts could have an impact on the biofilm community,  
373 maybe by protecting diatoms from extreme salinity conditions.

374 If we focus on bound fractions, rhamnose amounts increased during the emersion time. We  
375 extensively discussed about the potential role of deoxy sugars in part 3.2 (cell-cell  
376 interactions) and this observation seemed to confirm the involvement of rhamnose in adhesion  
377 phenomena or in the motility of diatom cells. Finally, residual fractions, rich in intracellular  
378 carbohydrates and refractory EPS were very complex in accordance with the literature. The  
379 important point to highlight was the slight drop of glucose amounts during the first hours of  
380 emersion, followed by a large increase of glucose ratios. This result could be due to the use of  
381 C-storage during the emerged period (metabolism) then to a drastic storing of carbon to  
382 survive during periods of darkness (Hanlon, 2006).

#### 383 **4. Conclusion**

384 Based on extensive data set (> 800 fractions), this paper focused on the seasonal dynamics of  
385 EPS and extensively carbohydrates produced on an intertidal mudflat during two different  
386 sampling periods (winter and summer). Firstly, colloidal carbohydrates, well known as C-  
387 sources for heterotrophic bacteria and other C-consumers, were steadily produced suggesting  
388 that an ecological equilibrium maybe existed between carbon production and consumption.  
389 The increase of bound carbohydrate levels during emersion times could show their

390 involvement in adhesion/cohesive properties and/or in the locomotive properties of diatom  
391 cells which have to migrate back into the sediment at the end of emersion time. These  
392 dynamic pathways were identical between winter and summer, even if the data numbers  
393 allowed us to significantly confirm this hypothesis only for summer. The presence of proteins  
394 during summer was correlated to an additional C-storing for diatoms cells (residual fractions)  
395 and to a potential protection against high desiccation and salinity degrees. A number of  
396 abiotic/biotic parameters seemed to have impact on EPS dynamics and production as salinity,  
397 pore water, light or tidal coefficient. At last, GC/MS analyses showed that colloidal  
398 carbohydrates were glucose-rich, bound carbohydrates were significantly composed of  
399 rhamnose and residual fractions presented a wide variety of monosaccharides. All the  
400 fractions were richer in glucose during summer than winter, maybe to response to greater  
401 trophic needs. ACP analyses allowed refining the results and highlighted that colloidal  
402 carbohydrates formed a first cluster (glc, ino). Cluster 2 (xyl, rham) was constituted of bound  
403 carbohydrates and cluster 3 (fuc, gal. acid, gal, man) of residual carbohydrates. The evolution  
404 of five representative monosaccharides (glc, gal. acid, xyl, rham, fuc) was also followed in  
405 function of the tidal emersion periods and the results showed again that the distribution  
406 pathways did not change in winter or summer in contrary to monosaccharide amounts. We  
407 noted that the levels of galacturonic acid varied for colloidal and bound fractions, which could  
408 have an effect on binding forces, protection (via ion sequestration for instance) and cell-cell  
409 interactions in microphytobenthic biofilms. Besides, C-storage consumption and C-storing  
410 (glc, fuc, man) were observed in residual fractions during the emerged periods. To conclude,  
411 many results were consistent with the literature and additional information were obtained  
412 concerning the influence of seasonal parameters on EPS productions and dynamics. It should  
413 be interesting to check whether these conclusions could be observed on a much larger data  
414 set, specifically targeting light, salinity and pore water, tidal coefficient as major parameters.

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420 06-BLAN-0393-01).

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552

553 **Table 1.** Matrix of Pearson's correlation coefficients between carbohydrate contents of the  
554 different fractions (LMW/HMW: low and high molecular weight, CC: colloidal  
555 carbohydrates, BC: bound carbohydrates, RC: residual carbohydrates) and biotic/abiotic  
556 parameters measured on the sediment and during the two sampling periods (W: winter, S:  
557 summer). Values of r significant at \*  $p < 0.05$ , \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$ .

558

559 **Figure 1.** Location hypothesis of the different EPS fractions collected by the Dowex method,  
560 adapted from the model of Underwood & Paterson (2003).

561 **Figure 2.** Location of the study area (Brouage mudflat, France) where samples of surficial  
562 intertidal sediment were collected.

563 **Figure 3.** Total carbohydrate quantities ( $\mu\text{g} \cdot \mu\text{g chlo } a^{-1}$ ) and relative contribution (% w/w)  
564 of neutral carbohydrates (light grey), uronic acid (dark grey) and proteins (grey) for each EPS  
565 fraction extracted during (A) winter and (B) summer campaigns. Bars represent the  
566 heterogeneity of the three sampling sites.

567 **Figure 4.** Comparison of carbohydrates quantities ( $\mu\text{g} \cdot \text{g dry sediment}^{-1}$ ) during winter and  
568 summer of the extracted EPS fractions, at the beginning of the emerged periods with their  
569 values at the end of the emerged period. Abbreviations W, S, LMW, HMW, CC, BC and RC  
570 correspond respectively to winter, summer, low molecular weight, high molecular weight,  
571 colloidal carbohydrates, bound carbohydrates and residual carbohydrates. Regression lines are  
572 used to read the graphs. Data points in the upper left part correspond to variable which  
573 increased over the emerged period; data points in the lower right part correspond to variable  
574 which decreased during the same period. Data points close to the line: no change over the  
575 emerged period.

576 **Figure 5.** Monosaccharide composition (% w/w) of the different fractions extracted in winter  
577 and summer. See fig 4. for abbreviations meaning.

578 **Figure 6.** Principal component analysis scatter plot grouping monosaccharide profiles of the  
579 different fractions along dominant sugar vectors. See fig 4. for abbreviations meaning.

580 **Figure 7.** Effect of the emersion time (average on the three sampling sites) on the major  
581 monosaccharide contents. Glucose, xylose, galacturonic acid, rhamnose and fucose are  
582 representative monosaccharides reported in fig. 6 as characteristics of the physicochemical  
583 properties of the colloidal, bound and residual fractions. The variability within true sample  
584 replicates was less than 5 %. See fig 4. for abbreviations meaning.

585

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587 **TABLE 1**

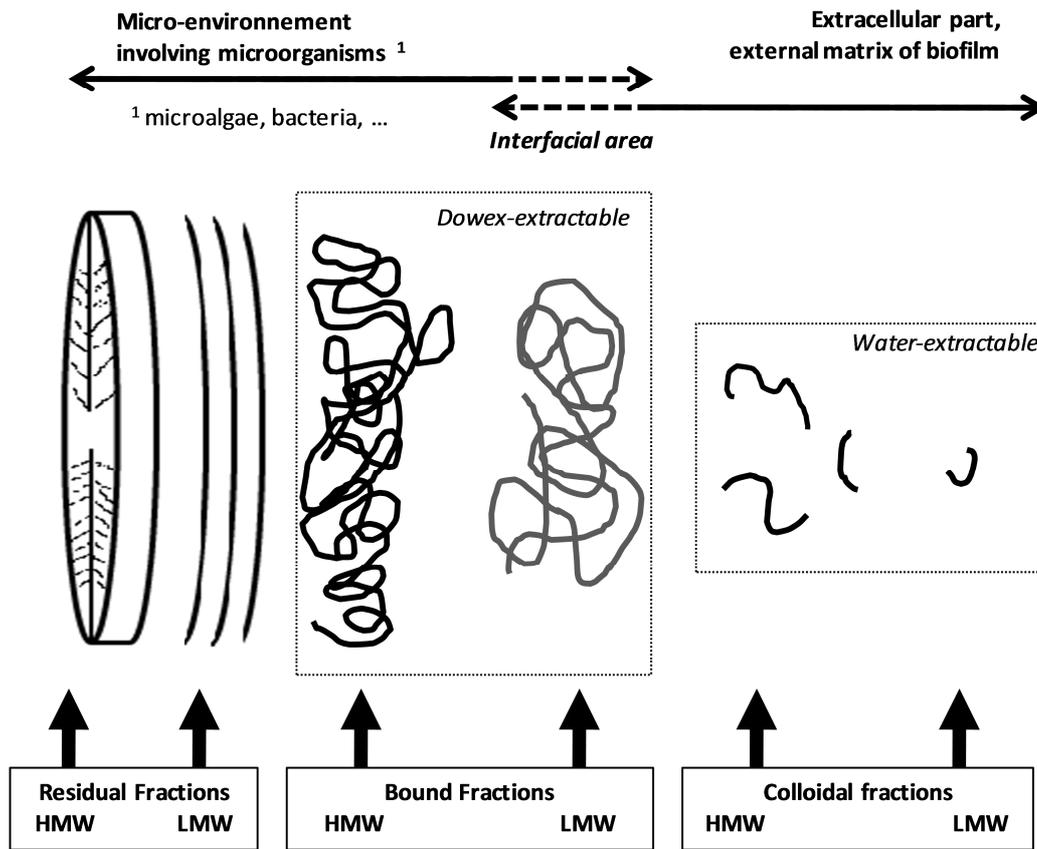
<b>a) Winter</b>	W-LMW CC	W-HMW CC	W-LMW BC	W-HMW BC	Light	Salinity	Pore water	Bacterial abundance	Tidal coefficient	C:N
Chl <i>a</i>	-0.114	0.291	-0.087	0.007	0.257	0.417	0.556	-0.524	-0.391	nd
W-LMW CC	x	<b>0.656*</b>	<b>0.852**</b>	-0.185	<b>0.698*</b>	-0.387	0.083	-0.323	0.200	nd
W-HMW CC	x	x	<b>0.795**</b>	0.276	0.347	-0.393	-0.176	-0.126	0.427	nd
W-LMW BC	x	x	x	0.189	0.422	<b>-0.733*</b>	-0.241	-0.222	0.585	nd
W-HMW BC	x	x	x	x	-0.621	-0.272	-0.433	0.514	<b>0.776**</b>	nd
Light	x	x	x	x	x	0.097	0.522	<b>-0.682*</b>	-0.423	nd
Salinity	x	x	x	x	x	x	0.618	-0.008	<b>-0.763*</b>	nd
Pore water	x	x	x	x	x	x	x	-0.558	<b>-0.654*</b>	nd
<b>b) Summer</b>	S-LMW CC	S-HMW CC	S-LMW BC	S-HMW BC	Light	Salinity	Pore water	Bacterial abundance	Tidal coefficient	C:N
Chl <i>a</i>	<b>0.409*</b>	0.108	-0.122	<b>-0.375*</b>	0.037	0.306	<b>0.600***</b>	<b>0.405*</b>	0.163	0.086
S-LMW CC	x	-0.033	-0.062	0.097	0.027	0.061	<b>0.362*</b>	0.307	0.091	0.090
S-HMW CC	x	x	<b>-0.576***</b>	-0.304	0.288	0.120	0.293	-0.072	<b>0.536***</b>	-0.175
S-LMW BC	x	x	x	0.292	0.014	0.128	<b>-0.338*</b>	-0.136	-0.024	-0.046
S-HMW BC	x	x	x	x	<b>-0.344*</b>	-0.249	-0.090	-0.114	-0.267	0.163
Light	x	x	x	x	x	<b>0.330*</b>	-0.196	-0.033	<b>0.507**</b>	-0.231
Salinity	x	x	x	x	x	x	<b>-0.421*</b>	0.103	<b>0.417*</b>	0.066
Pore water	x	x	x	x	x	x	x	0.317	-0.009	-0.030

588 *nd: non determined*

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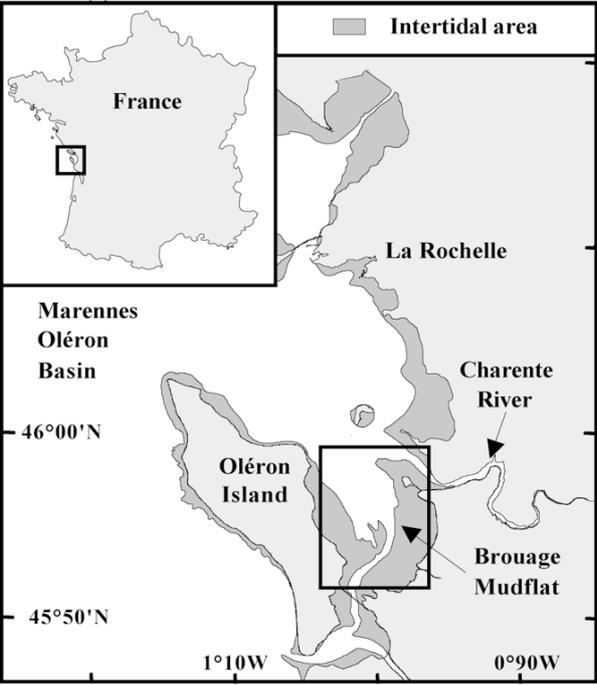
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**FIGURE 1**



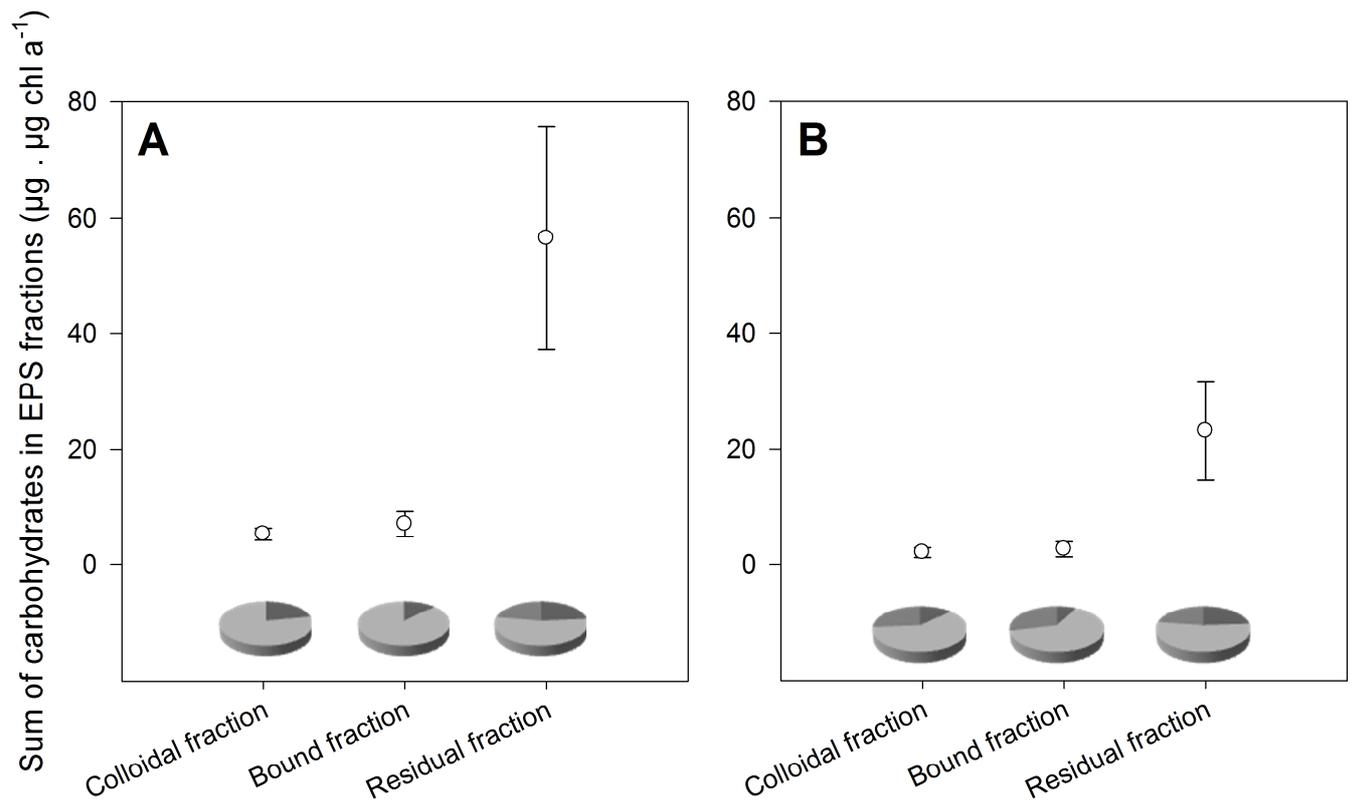
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**FIGURE 2**

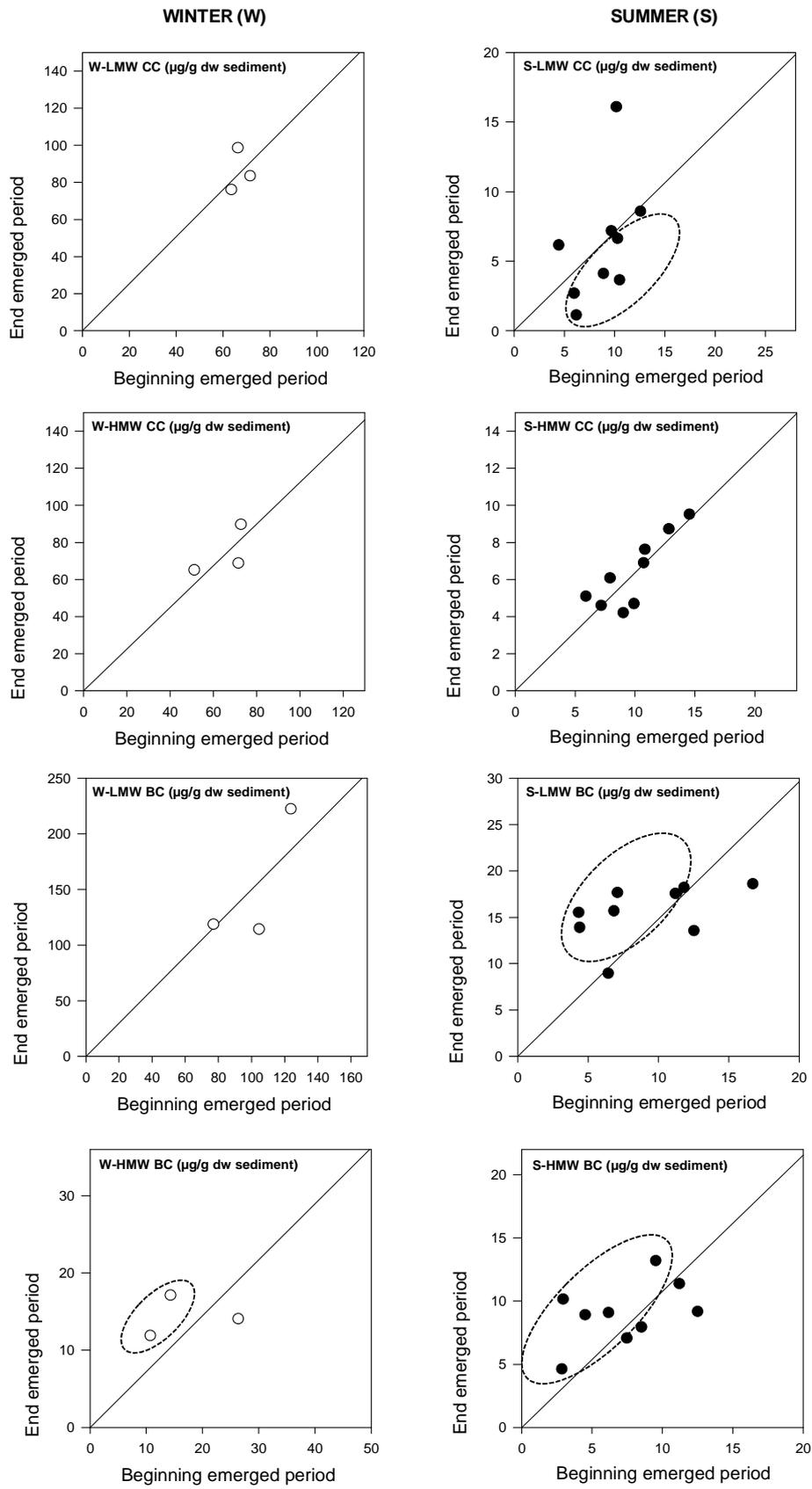


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**FIGURE 3**

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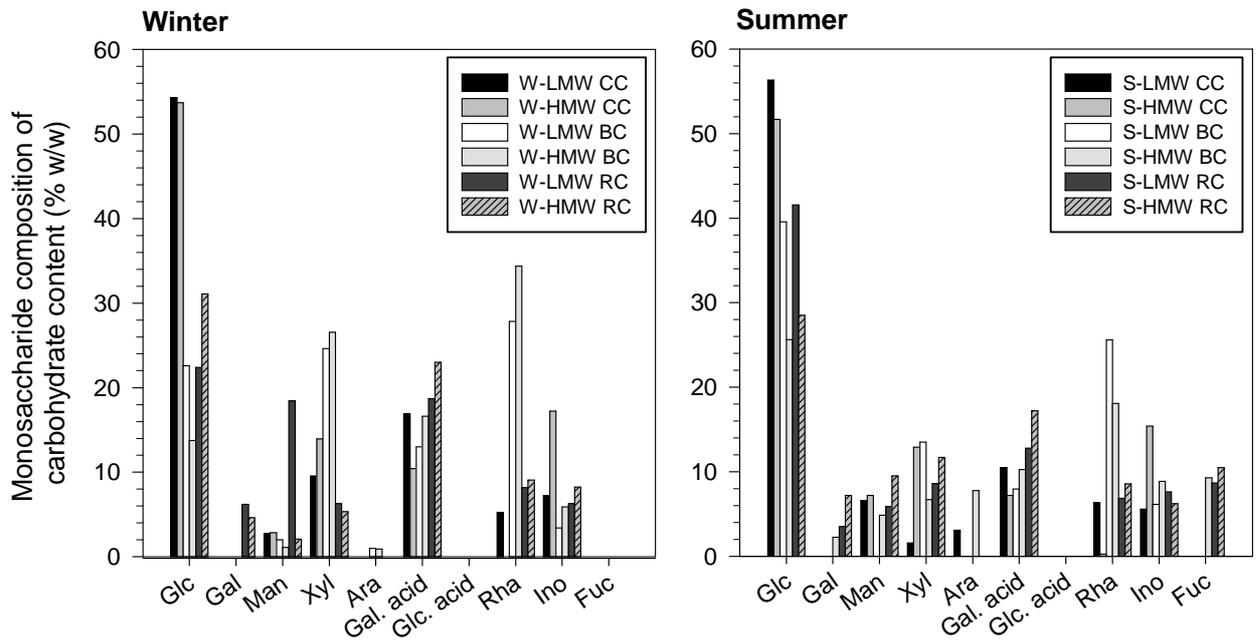


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**FIGURE 4**

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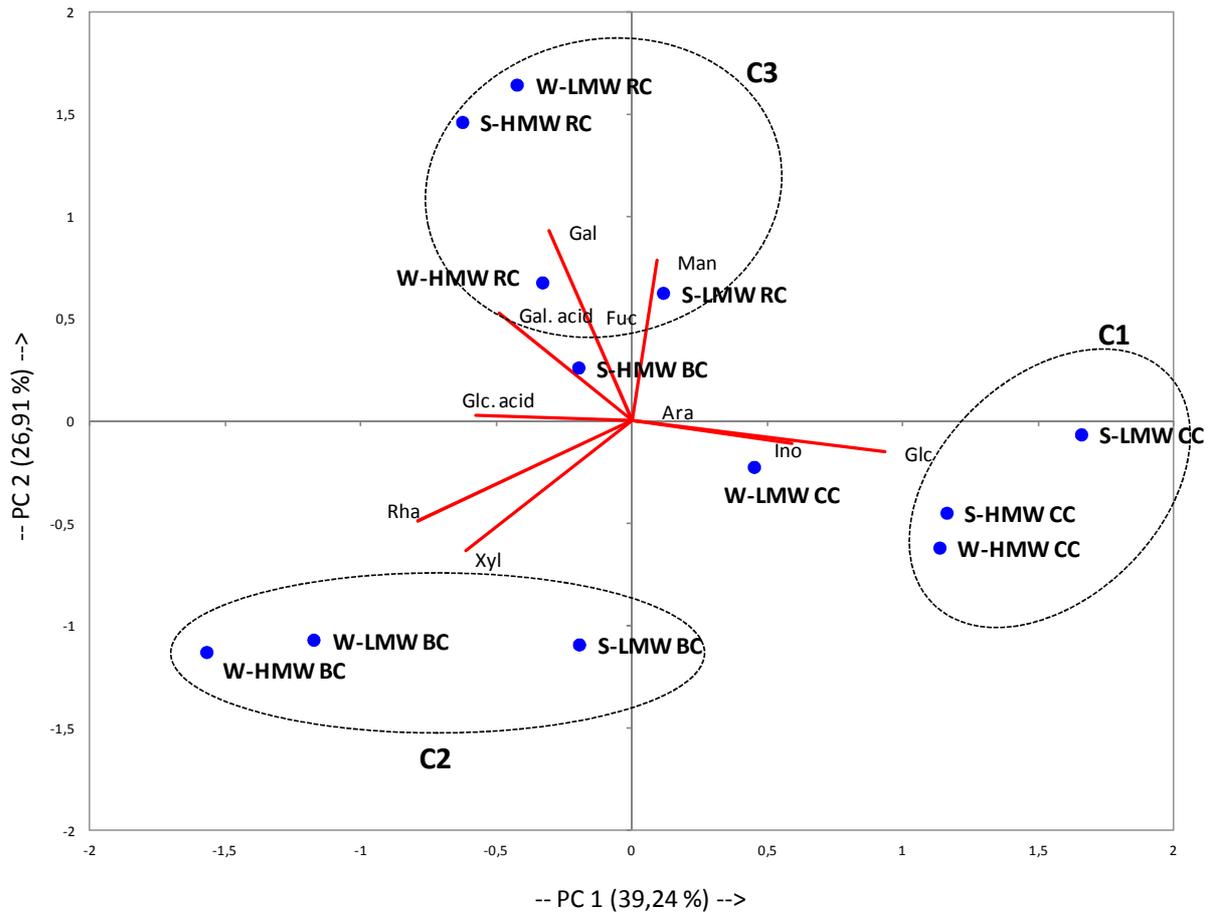


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**FIGURE 5**

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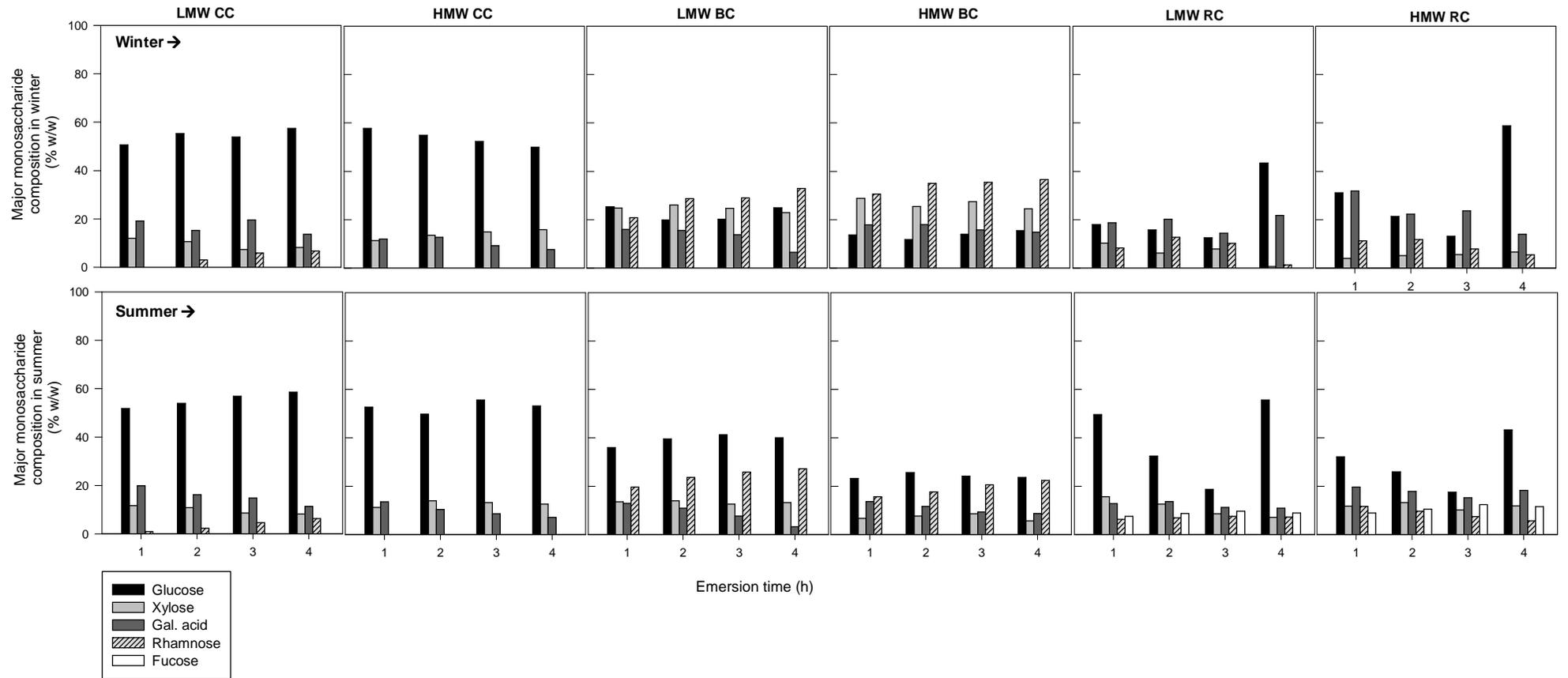
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**FIGURE 6**

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FIGURE 7