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1 **Isotopic labelling of cultured macroalgae and isolation of ¹³C-labelled cell**
2 **wall polysaccharides for trophic investigations**

3

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14

15 **Abstract**

16 Macroalgae are considered as major primary producers in coastal environments, acting as a
17 global carbon sink. This abundant biomass contains up to 50% of storage or cell wall
18 polysaccharides, which therefore represent a reservoir of organic matter for potential algal
19 consumers. Tracking of natural isotopic abundance ($\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$) in macroalgae-colonized
20 habitats such as kelp forests and rocky shores previously evidenced the importance of the
21 algal resource to support local and adjacent trophic webs mostly *via* the microbial detrital
22 pathway. However, such bulk isotopic measures of natural abundances cannot inform
23 precisely on the microbial actors and processes at play for the degradation of selected algal
24 compounds. To overcome these limitations, we developed a stable isotope labelling procedure
25 for cultures of the brown alga *Laminaria digitata* and tested its ability to yield labelled
26 polysaccharides. Sporophytes of *L. digitata* were grown in controlled conditions for three
27 months in seawater regularly amended with ¹³C-labelled sodium bicarbonate. Elemental
28 analysis – isotope ratio mass spectrometry of algal specimens showed a significant

29 enrichment after 10 days of treatment, reaching a maximum of $\text{At}\%^{13}\text{C} = 3.5321\%$ after 80
30 days. Sequential polysaccharide extraction from this labelled algal biomass allowed retrieving
31 both alginate and fucose-containing sulphated polysaccharide fractions with high isotopic
32 enrichment ($\text{At}\%^{13}\text{C} = 3.6279\%$ and 3.5868% , respectively). This labelling protocol opens the
33 way for future studies combining coastal ecosystems trophic interactions and microbial
34 activities towards macroalgal biomass degradation.

35 **Keywords:** (5 to 10)

36 Stable isotope enrichment, alginate, fucose-containing sulphated polysaccharides (FCSPs),
37 macroalgae, degradation, microbial pathway

38

39 **Introduction**

40 The total biomass of macroalgae on Earth is estimated at 280 million tons (Carpenter &
41 Liss, 2000), covering an area of ca. 3.5 million km² for a net primary production of up to
42 1,500 TgC.year⁻¹ (Krause-Jensen & Duarte, 2016). Macroalgae are thus considered as major
43 primary producers in coastal areas, acting as a carbon sink and playing a crucial role in
44 biodiversity and in global carbon flows (Dayton, 1985). The algal chemical composition
45 differs drastically from that of plants and animals. It consists primarily of complex cell wall
46 and storage polysaccharides (more than 50% of the dry weight), with additional lipids,
47 proteins, pigments and phlorotannins (Deniaud-Bouët et al., 2014). In particular, macroalgae
48 synthesize unique polysaccharides that are absent from other organisms and specific to each
49 phylum, e.g sulfated agars and carrageenans in red algae, alginate and sulfated fucans in
50 brown algae, and ulvan in green algae (Popper et al., 2011). This specific composition of the
51 algal biomass can influence the processes mediating its trophic use.

52 In marine environments, the availability of organic matter derived from macrophytes for
53 consumers depends on trophic mediation by associated microorganisms (Adin & Riera, 2003;
54 Crosby, Newell, & Langdon, 1990; Langdon & Newell, 1990). Several studies highlighted
55 that bacteria growing on marine phanerogam detritus can facilitate the transfer of carbon to
56 consumers (Benner, Lay, K'nees, & Hodson, 1988; Tenore, 1977). Laboratory experiments
57 showed that 6-60% of oyster C requirements could be met by utilization of detrital complexes
58 comprising senescing tissues and associated bacteria (Crosby et al., 1990; Langdon & Newell,
59 1990). Previous studies have pointed out that only about 10% of the net macroalgal
60 production is consumed directly by grazers (Mann, 1982; Pomeroy, 1980). This low direct
61 grazing pressure on living macroalgal tissues is mostly due to the presence of recalcitrant
62 components including lignocellulosic compounds, and polyphenols like phlorotannins in
63 brown algae (Buchsbaum, Valiela, Swain, Dzierzeski, & Allen, 1991; Duggins & Eckman,

64 1997). On the other hand, about 90% of the net macroalgal production enter detritus food
65 chains mostly *via* microbial degradation (Mann, 1982; Pomeroy, 1980). The pool of detritus
66 derived from macroalgae can be predominant within the total suspended particulate organic
67 matter and represents a readily available food source for many consumers inhabiting kelp
68 forests or nearby areas (Duggins & Eckman, 1997; Kaehler, Pakhomov, Kalin, & Davis,
69 2006). This microbial reinjection of macroalgal biomass into marine food webs plays a
70 quantitative role of prime importance, especially during winter when the phytoplankton
71 production can be very low (Bustamante & Branch, 1996; Duggins, Simenstad, & Estes,
72 1989).

73

74 Stable isotopes have been successfully used to trace transformations of organic matter
75 during trophic flows and discriminate among various primary producers as food sources in
76 consumers' diets (Bustamante & Branch, 1996). Indeed, the isotopic composition of a
77 consumer depends on the diet isotopic composition, and the isotopic fractionation during food
78 processing. Many natural isotopic signatures have been determined for macroalgae during the
79 last decades (Mercado, de los Santos, Lucas Pérez-Lloréns, & Vergara, 2009; Pinnegar &
80 Polunin, 2000; Schaal, Riera, & Leroux, 2010) indicating a wide range of $\delta^{13}\text{C}$ values. In
81 general, average $\delta^{13}\text{C}$ are more ^{13}C -enriched in Chlorophyta, intermediate in Phaeophyta and
82 lowest in Rhodophyta (Maberly, Raven, & Johnston, 1992; Marconi, Giordano, & Raven,
83 2011; Mercado et al., 2009). In a kelp forest in western Brittany (France), $\delta^{13}\text{C}$ were more
84 negative for red algae (-38.0 to -15.5‰) compared to brown algae (-24.9 to -13.5‰) (Leclerc
85 et al., 2013). Significant differences among species in $\delta^{13}\text{C}$ and/or $\delta^{15}\text{N}$ were also observed
86 for living macroalgae or strandline algal species (Adin & Riera, 2003; Dauby, 1989). The δ
87 ^{13}C of marine macroalgae depends largely on their ability to use HCO_3^- rather than dissolved
88 CO_2 as carbon source (reviewed in (Raven et al., 2002)). Macroalgae displaying very low δ

89 ^{13}C likely use dissolved CO_2 as their main source of inorganic carbon, while $\delta^{13}\text{C}$ close to -
90 10‰ corresponds to a preferential fixation of HCO_3^- during primary production. Other factors
91 influencing $\delta^{13}\text{C}$ of marine macroalgae include the photosynthesis rates and the molecular
92 composition of algal tissues. These distinct natural isotopic values can be used to assess the
93 contribution of different macroalgal species as food sources for associated consumers.
94 Analyses of stable isotope signatures in kelp forest trophic webs have evidenced the relevance
95 of detrital material as a major food source for the associated benthic communities (Fredriksen,
96 2003). For example, in a kelp forest dominated by *Laminaria digitata*, filter-feeders derived
97 35% to 50% of their diet from macroalgae-derived detritus (Schaal et al., 2010). Overall,
98 approaches based on natural stable isotopes confirmed the importance of the microbial detrital
99 pathway to re-inject macroalgal primary production into marine food webs. However, such
100 bulk isotopic measurements of natural abundances cannot inform precisely on the specific
101 contribution of selected algal molecules and on the microbial processes involved in their
102 degradation. A thorough knowledge of these bacterial processes is essential because they
103 directly condition the fate of macroalgal organic matter in marine ecosystems.

104 A powerful approach to overcome these limitations is to use isotopic enrichment
105 experiments. Experiments based on isotopically labelled biomass have allowed to test specific
106 hypotheses on the fate of distinct food sources and to quantify the trophic fluxes in a variety
107 of marine ecosystems. Stable isotopes enrichments were carried out to selectively label
108 various food sources such as benthic diatoms or bacteria and to determine their relative
109 contribution to the diet of macroinvertebrates (Herman, Middelburg, Widdows, Lucas, &
110 Heip, 2000; Middelburg et al., 2000). Similarly, the specific uptake of microalgae-derived
111 carbon by the Polychaeta *Lanice conchilega* or the Echinodermata *Echinocardium cordatum*
112 was quantified through *in situ* experiments using ^{13}C -labelled diatoms (Kamp & Witte, 2005).
113 In addition, differential labelling experiments allowed to assess the preferential uptake of ^{15}N -

114 labelled bacteria or ^{13}C -labelled diatoms by pelagic larvae and foraminiferans (Leroy et al.,
115 2012; Moodley et al., 2000; Pascal, Dupuy, Richard, & Niquil, 2008). So far, stable isotope
116 enrichment studies in marine food webs almost exclusively focused on trophic transfers
117 within planktonic communities or between microorganisms (e.g. microalgae, bacteria) and
118 benthic grazers. By contrast, such approaches are currently lagging behind for macroalgae.
119 Few studies have implemented stable isotope labelling techniques on adult specimens of the
120 red algae *Gracilaria* spp. and *Solieria chordalis* to assess their fate in benthic systems
121 (Hardison, Canuel, Anderson, & Veuger, 2010; Legrand, Martin, Leroux, & Riera, 2018).
122 However, they used the labelled tissues as bulk biomass and did not investigate the allocation
123 of ^{13}C to specific algal compounds. Some high molecular weight compounds such as cell wall
124 structural polysaccharides can have slower turnover time compared to soluble molecules (e.g.
125 osmolytes, carbon storage compounds) (Brinkhuis, 1977; Macler, 1986) and thus require
126 active growth and longer incubation times to incorporate significant amounts of stable
127 isotopes. Given their high proportion in macroalgal tissues, it appears essential to ensure
128 sufficient labelling of cell wall polysaccharides to fully decipher the fate of macroalgal
129 biomass in marine food webs. In the present study, we developed an adapted protocol to
130 enrich actively growing macroalgae in ^{13}C and obtain isotopically labelled cell wall
131 polysaccharide fractions.

132

133

134 **Material and Methods**

135 *Materials*

136 Natural seawater for algal cultures was collected in opaque containers from the Astan site
137 (GPS coordinates 48°46'40", -2°56'15") offshore Roscoff (France), filtered on a 100 μm

138 cartridge and autoclaved before use. ^{13}C -enriched sodium bicarbonate $\text{NaH}^{13}\text{CO}_3$ (99% ^{13}C)
139 was purchased from Eurisotop (Saarbrücken, Germany). Unless otherwise stated, all other
140 chemicals were from Sigma-Aldrich.

141 *Algal culture procedures*

142 *Laminaria digitata* life cycle consists of microscopic haploid gametophyte phase, alternating
143 with macroscopic diploid sporophytes. In this study, all experiments were done on the
144 macroscopic diploid individuals. To obtain young laboratory-grown sporophytes, mature *L.*
145 *digitata* sporophytes showing sorus reproductive tissues were harvested at the "Roches Duon"
146 site (GPS coordinates 48°43'32", -3°55'20"). Spores were released from sorus pieces in
147 seawater for 3 h at 18°C with an irradiance of 20 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ and further kept at 13°C at an
148 irradiance of 5 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ to allow the development of gametophytes and random crosses.
149 The developing young *L. digitata* sporophytes (ca. 5 mm) were cultured in flasks containing
150 10 L of autoclaved seawater enriched with 1.5 ml of modified Provasoli solution (92 mM
151 H_3BO_3 , 1.5 mM FeCl_3 , 4.9 mM MnSO_4 , 0.4 mM ZnSO_4 , 0.09 mM CoSO_4 , 27 mM EDTA, 9
152 mM $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2$, 824.6 mM NaNO_3 , 46.3 mM $\text{C}_3\text{H}_7\text{Na}_{20}\text{P}$, 0.001 mM cyanocobalamine,
153 0.3 mM thiamine, 0.004 mM biotine, 413 mM Tris) (Starr & Zeikus, 1993), with continuous
154 bubbling of 0.2 μm -filtered air in a culture cabinet at 13°C illuminated with daylight-type
155 fluorescent lamps at an irradiance of 20 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ for 12 hours per day. Two separate
156 batches were prepared (^{12}C -control and ^{13}C -enriched) and were regularly amended with 1 g.L⁻¹
157 solutions of either NaHCO_3 with natural isotopic composition (98.9 $^{12}\text{C}\%$) or labelled
158 NaHCO_3 (99 $^{13}\text{C}\%$) previously sterilized on 0.2 μm filters. To favour the growth of larger
159 sporophytes, each batch was subdivided in separate 10L-flasks after 42 and 66 days of
160 cultures. Culture media were renewed biweekly during the first month and weekly afterwards.
161 At each medium renewal, sporophytes were visually examined to eliminate individuals
162 showing signs of necrosis or microbial contamination. All manipulations were performed

163 under a sterile laminar flow hood with ethanol-sterilized instruments. At each sampling time
164 (0, 10, 18, 28, 35, 52, 66, 95 days), three sporophytes were sampled from each batch and
165 frozen at -20°C for isotopic analysis. After 95 days of cultures, the total algal biomass was
166 collected on a strainer and excess water was removed by spinning for 1 min in a salad tosser.
167 Algae were weighed to estimate the fresh mass, dried at 40°C in a ventilated oven for 2 days
168 and stored at room temperature until polysaccharide extraction.

169 *Polysaccharide extraction*

170 Cell wall polysaccharides were retrieved sequentially using a modified chemical extraction
171 (Deniaud-Bouët et al., 2014; Hogsett & Quatrano, 1975; Vauchel, Kaas, Arhaliass, Baron, &
172 Legrand, 2008). Dried algae were pulverized in a MM200 mixer mill (Retsch) at 30 Hz for 90
173 seconds. Seven to ten grams of algal powder were homogenized in 300 mL of 70% ethanol
174 and filtered on a 100 µm nylon mesh placed on a G3 glass filter funnel. The powder was
175 repeatedly rinsed with sequential baths (250 mL, 10 min each) in 70% ethanol (3 L total),
176 80% ethanol (3 L total), 96% ethanol (3 L total), methanol / chloroform 50:50 (v/v, 2 L total)
177 and acetone (0.5 L total) until colourless and finally dried overnight in a ventilated oven at
178 40°C to yield alcohol-insoluble residues (AIR). AIRs were resuspended in 0.5 L of 2% (w/v)
179 aqueous CaCl₂ and mixed for 4 h at 80°C, followed by centrifugation for 15 min at 10000
180 rpm to separate the calcium-soluble supernatant that contains fucose-containing sulphated
181 polysaccharides (FCSP) from the calcium-insoluble pellet containing alginate. The calcium-
182 insoluble fraction was resuspended in 0.5 L of 4% (w/v) aqueous Na₂CO₃ for 2 h at 80°C
183 followed by centrifugation for 15 min at 10000 rpm. The supernatant was adjusted to pH 2
184 with slow addition of 10 mL H₂SO₄ to eliminate carbonates and precipitate alginic acid,
185 which was retrieved by pressing in a 100 µm nylon mesh. The precipitate was dissolved in
186 200 mL distilled water and neutralized with NaOH. FCSPs and alginate were retrieved from
187 their respective fraction by precipitating with 6 vol. of 96% ethanol. For recovery, alginate

188 was pressed on a 100 µm nylon mesh and FCSPs were centrifuged for 20 min at 9000 rpm.
189 Both fractions were lyophilized for long-term storage.

190 *Isotopic analysis*

191 Triplicate algal samples were dried at 50°C for two days in a ventilated oven, pulverized
192 using a mortar and pestle and inserted in tin capsules (1-2 mg per sample). Carbon isotopic
193 ratios were measured on an elemental analyser (Flash EA1112, ThermoScientific) coupled to
194 an isotope ratio mass spectrometer (IRMS Delta plus, ThermoScientific) *via* a gas interface
195 (Conflo III, ThermoScientific). To measure carbon quantities, expressed in micrograms, a
196 calibration was performed for each run with a certified standard compound (acetanilide,
197 Sylab), a correction for linearity using casein (SyLab) and a two-point linear normalisation
198 (Coplen et al., 2006; Paul, Skrzypek, & Fórizs, 2007) using the international standards IAEA-
199 600 and IAEA-CH6. Data were expressed in the standard δ notation in ‰ according to the
200 following equation:

$$201 \quad \delta^{13}\text{C} = \left(\frac{R_{\text{sample}} - R_{\text{reference}}}{R_{\text{reference}}} \right) \times 1000, \text{ where } R = {}^{13}\text{C} / {}^{12}\text{C}.$$

202 These abundances were calculated in relation to the certified reference materials Vienna Pee
203 Dee Belemnite-limestone (V-PDB), with $R_{\text{reference}} = 0.0112372$. The V-PDB was achieved
204 using in-house casein standards, calibrated against IAEA-600 and IAEA-CH6 reference
205 materials. The standard deviation of repeated measurements of $\delta^{13}\text{C}$ of nBS-19 was 0.10 ‰
206 versus V-PDB.

207 ^{13}C atom percent were calculated as follows:

$$208 \quad \text{At\% } {}^{13}\text{C} = \frac{R_{\text{sample}}}{R_{\text{sample}} + 1} \times 100$$

209

210 ¹³C uptake was calculated in two different ways. Cumulative ¹³C uptakes (cUp¹³C) were
 211 calculated for each time point (t) by comparing algae from ¹³C-enriched batch to algae from
 212 control batch at the same time point, as follows:

$$cUp^{13}C_t = \frac{At\%^{13}C_{labelled,t} - At\%^{13}C_{control,t}}{100} \times C_{conc}$$

213 The first term of the equation represents the ¹³C atom excess, where At%¹³C_{labelled,t} and
 214 At%¹³C_{control,t} are the ¹³C atom percent values at time t calculated for algae from batches
 215 amended with labelled or natural NaHCO₃, respectively. C_{conc} is the carbon concentration in
 216 dry algal biomass measured by the elemental analyser.

217 In addition, time-resolved ¹³C uptakes (tUp¹³C) were calculated for each time point (t) by
 218 comparing algae from ¹³C-enriched batch at time t to algae from the same batch at time t-1
 219 and expressed as daily uptake, as follows:

$$tUp^{13}C_t = \frac{At\%^{13}C_{labeled,t} - At\%^{13}C_{labeled,t-1}}{100} \times C_{conc} \times \frac{1}{n_{days}}$$

220 The first term of the equation represents the ¹³C atom excess compared to previous time,
 221 where At%¹³C_{labeled,t} and At%¹³C_{labeled,t-1} are the ¹³C atom percent values at time t and t-1,
 222 respectively. C_{conc} is the carbon concentration in dry algal biomass measured by the elemental
 223 analyser, and n_{days} is the number of days between sampling times t and t-1.

224 The percentage of ¹³C from sodium bicarbonate incorporated into algal biomass was
 225 calculated for the final time point as follows:

$$226 \quad {}^{13}C_{incorp\%} = \frac{M_{tot} \times cUp^{13}C_{tfinal}}{0.99 \times {}^{13}C_{bicarb}}$$

227 the cultivation period, cUp¹³C_{tfinal} is the cumulative ¹³C uptake after 95 days of culture, and

228 $^{13}\text{C}_{\text{bicarb}}$ is the total amount of carbon from sodium bicarbonate added during the cultivation
229 period.

230

231 **Results**

232 The protocol developed to obtain stable isotope-labelled brown algal biomass is summarized
233 in Figure 1. Labelling of cultured *L. digitata* sporophytes was tested using periodic additions
234 of ^{13}C -labelled sodium bicarbonate into seawater, over a 3-months period. In parallel, a
235 control batch of sporophytes from the same fecundation event was prepared with addition of
236 natural abundance sodium bicarbonate.

237 [Insert Figure 1 here]

238 In total, each batch was amended with 7.2 g of sodium bicarbonate, corresponding to 1.1 g of
239 carbon. Sporophytes grew from 5 mm to ca. 5-10 cm in length in 3 months, yielding 123 g
240 and 95 g wet biomass in the ^{12}C -control and ^{13}C -enriched batch, respectively. The water
241 content estimated from the dry biomass was 85%. The ^{13}C isotopic composition and ^{13}C
242 uptake of algal specimens collected at different time points are reported in Figure 2.

243 [insert Figure 2 here]

244 At the beginning of the culture, juvenile sporophytes showed a $\delta^{13}\text{C}$ of -0.50 ± 2.96 ‰.
245 Typical values obtained from wild adult populations of *L. digitata* in the vicinity of Roscoff
246 range from $\delta^{13}\text{C} = -17$ ‰ to -12 ‰ (Schaal, Riera, & Leroux, 2009; Schaal et al., 2010). The
247 higher ^{13}C -enrichment shown here might reflect differences due to the age of the algal tissues
248 or the specific conditions of cultivation in the laboratory (e.g. amendments with Provasoli
249 solution, air bubbling, etc.). In the control batch, stable isotope compositions remained stable
250 over the 3 months cultivation period (Figure 2A), ranging from $\text{At}\%^{13}\text{C} = 1.0855$ to 1.1416 ,

251 with an overall average of $\text{At}\%^{13}\text{C} = 1.1116 \pm 0.0201$ (n=27). By contrast, we observed a
252 rapid enrichment of algal biomass in the ^{13}C -enriched batch (Figure 2), already showing
253 $\text{At}\%^{13}\text{C} = 1.9735 \pm 0.0987$ after 10 days of culture. The ^{13}C content kept increasing until 80
254 days of culture, when it reached a plateau. The final isotopic composition of labelled algae
255 after 95 days was $\text{At}\%^{13}\text{C} = 3.3381 \pm 0.3490\%$ (Figure 2A). Cumulative ^{13}C uptakes were
256 calculated at each time point (Figure 2B), and showed a final value of $5.59 \text{ mg }^{13}\text{C.g}^{-1}$ dry
257 algal biomass after 95 days of culture. When extrapolated to the total dry mass of ^{13}C -
258 enriched sporophytes harvested at the end of the experiment (14.14 g), this corresponds to an
259 incorporation of $7.26 \pm 1.76\%$ of ^{13}C atoms from sodium bicarbonate into algal biomass.
260 Furthermore, we calculated daily ^{13}C uptakes between each sampling time point (Figure 2B).
261 This showed that most of the enrichment occurred during the first 40 days, before the plateau.
262 Daily uptakes became close to zero after 80 days of culture.

263 A sequential chemical extraction was used to purify cell wall polysaccharides from control
264 and ^{13}C -enriched sporophytes (Figure 3) obtained after 95 days.

265 [insert Figure 3 here]

266 Alginate was retrieved as the calcium-insoluble fraction of alcohol-insoluble residues whereas
267 the calcium-soluble fraction contained FCSPs, with final yields of ca. 20% and 7.5% of the
268 dry weight, respectively (Table 1).

269 [insert Table 1 here]

270 Isotopic analysis revealed strongly significant enrichment of polysaccharide fractions
271 extracted from ^{13}C -enriched algal biomass compared to controls (Table 2). Alginate and
272 FCSPs showed stable isotope content of $\text{At}\%^{13}\text{C} = 3.6279 \pm 0.0009 \%$ and 3.5868 ± 0.0064
273 $\%$, respectively, corresponding to ^{13}C uptakes of 7.02 ± 0.07 and $7.07 \pm 0.03 \text{ mg }^{13}\text{C.g}^{-1}$ dry
274 polysaccharide, respectively. This shows that carbon from sodium bicarbonate was allocated

275 to cell wall synthesis. In particular, 26% and 9% of ^{13}C incorporated into algal biomass was
276 allocated to alginate and FCSPs, respectively.

277 [insert Table 2 here]

278

279 **Discussion**

280 Several studies have shown that it is possible to selectively label different food sources
281 and follow their fate within food chains. The ^{13}C -enriched labelling can be focused on a
282 source of organic matter considered in its entirety (e.g., whole macroalgae). Experiments
283 based on the ^{13}C -labelled red macroalga *Gracilaria* spp (9 At% ^{13}C) under decomposition in
284 sediments revealed transfers of isotopic label to bacterial biomass through the ^{13}C enrichment
285 of specific bacterial amino acids or fatty acids (Hardison et al., 2010). Recently, a dual stable
286 isotope labelling experiment was performed on adult specimens of the red alga *Solieria*
287 *chordalis* using both $\text{H}^{13}\text{CO}_3^-$ and $^{15}\text{NH}_4$ additions to investigate the uptake of whole algal
288 tissues by the sea urchin *Psammechinus miliaris* under different temperature regimes
289 (Legrand et al., 2018). However, stable isotope labelling studies considering macroalgae as a
290 bulk biomass do not inform on (1) the labelling of different molecular fractions of the algae,
291 (2) quantitative transfers of specific algal compounds to different consumers and, (3) the
292 specialization of consumers towards the degradation of specific compounds. When
293 considering the trophic fate of algal constituents, cell wall polysaccharides are of particular
294 interest since they can represent up to 45% of the dry weight (Mabeau & Kloareg, 1987), are
295 chemically distinct from those found in other primary producers (Popper et al., 2011),
296 constitute one of the first physical barrier to be attacked by consumers and are generally
297 recognized as more recalcitrant than other small (e.g. simple sugars) or high molecular weight
298 compounds (e.g. proteins). A deeper understanding of the trophic impact of cell wall

299 polysaccharides and on the microorganisms specialized in their turnover is therefore crucial to
300 fully understand macroalgal biomass recycling.

301 In the present study, stable isotope labelling was performed on actively growing
302 juvenile brown algae for 3 months, contrasting with the shorter incubation periods (1-2
303 weeks) previously used on red algae (Hardison et al., 2010; Legrand et al., 2018). This was
304 required to ensure incorporation of ^{13}C -label into cell wall polysaccharides, which can have a
305 long turnover time compared to smaller compounds (Macler, 1986). Indeed, seminal
306 incubation studies of brown algae with $^{14}\text{CO}_2$ or $\text{H}^{14}\text{CO}_3^-$ showed that among sugar
307 constituents, alginate has a slow rate of radioactive carbon incorporation, whereas mannitol is
308 the most rapidly and strongly labelled (Bidwell, 1958; Hellebust & Haug, 1972; Yamaguchi,
309 Ikawa, & Nisizawa, 1966). Isotopic ^{13}C enrichments obtained on brown algae in the present
310 study are consistent with levels previously observed on sources characterized by high
311 polysaccharide contents. Recent labelling experiments were performed on fresh leaves of
312 terrestrial source (i.e. alders) with an isotopic ^{13}C enrichment of about 2.5 At% ^{13}C to assess
313 their assimilation by isopods in groundwater (Francois et al., 2016). A mean ^{15}N labelling of
314 the seagrass *Posidonia oceanica* around 4.5 At% ^{15}N was also obtained for a quantitative
315 experimental study (Lepoint, Millet, Dauby, Gobert, & Bouquegneau, 2002). Our detailed
316 time course of ^{13}C -incorporation showed that the extent of labelling reached a plateau at the
317 end of the 3-months period and that most of the ^{13}C uptake occurred within 40 days. This
318 suggests that longer incubations in the present experimental setup may not yield a greater
319 incorporation. If even higher ^{13}C enrichments are needed for specific applications, additional
320 ventilation with $^{13}\text{CO}_2$ could be tested.

321 The present results appear very promising for future experimental studies related to
322 the fate and trophic transfers of macroalgal biomass in general and of cell wall
323 polysaccharides in particular. The protocol developed here for the kelp *L. digitata* can be

324 applied to other species of brown, red or green algae for which laboratory cultivation is
325 available. Therefore, the present method complements the toolbox for quantitative trophic
326 ecology approaches to trace ^{13}C -labelled macroalgal biomass within complex pelagic or
327 benthic communities of marine ecosystems. Furthermore, this study is to our knowledge the
328 first to report the purification of ^{13}C -labelled cell wall polysaccharide fractions containing
329 alginate and FCSPs. Macroalgae are generally considered as a unique pure source in trophic
330 models, although they in fact comprise diverse molecular compounds potentially at the base
331 of different food chains due to their specific utilization by micro- and macro-organisms in
332 aquatic ecosystems. By giving access to specific ^{13}C -labelled polysaccharide fractions, the
333 present protocol will help improving the definition of these distinct trophic niches and paves
334 the way for detailed investigations of the diversity and functional processes of uncultured
335 specialized microorganisms responsible for the reinjection of algal polysaccharides into
336 trophic chains *via* the detrital pathway. To this end, various stable-isotope-enabled microbial
337 ecology techniques are available, such as Stable Isotope Probing (SIP) combined to high-
338 throughput sequencing (Dumont & Murrell, 2005), Raman single-cell microspectroscopy
339 (Wagner, 2009) or nano-scale chemical imaging by secondary ion mass spectrometry
340 (nanoSIMS) (Musat, Foster, Vagner, Adam, & Kuypers, 2012). Such studies using ^{13}C -
341 labelled polysaccharides as substrates would allow linking specific members of natural
342 microbial communities to their function as degraders of the macroalgal biomass *in situ*,
343 greatly enhancing our current knowledge on the carbon cycle in marine regions.

344

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517 **Tables**

518 **Table 1:** Yields of cell wall polysaccharide extraction from *L. digitata* cultured sporophytes
 519 from the ¹²C-control and ¹³C-enriched batch. FCSP: fucose-containing sulphated
 520 polysaccharides

	¹² C-control	¹³ C-enriched
Starting dry biomass	10 g	7.5 g
Alcohol-insoluble residues ^a	5.73 g (57.3%)	4.41 g (58.8%)
Alginate ^a	1.92 g (19.2%)	1.57 g (20.9%)
FCSP ^a	0.83 g (8.3%)	0.53 g (7.1%)

^a Values in brackets are yields based on starting dry biomass

521

522

523 **Table 2:** Stable isotope ratios of polysaccharide fractions extracted from ¹²C-control and ¹³C-
 524 enriched *L. digitata* sporophytes. Values are mean ± standard deviation of technical
 525 triplicates.

Sample	At% ¹³ C	
	Alginate	FCSP
¹² C-control	1.1337 ± 0.0004	1.1454 ± 0.0008
¹³ C-enriched	3.6279 ± 0.0009	3.5868 ± 0.0064

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527

528 **Figure legends**

529 **Figure 1: A.** Schematic overview of the algal cultivation protocol for stable isotope labelling.

530 **B.** Photograph of the experimental setup for cultivation of *L. digitata* sporophytes.

531 **Figure 2: A.** ¹³C atom percent of algal specimens collected at different time points in the
 532 control (blue) and ¹³C-enriched culture batches. Values are mean ± standard deviation (n=3).

533 **B.** Uptake in ¹³C calculated for algae in the ¹³C-enriched culture batch, calculated either in a

534 cumulative way compared to algae from control batch (black, left axis) or as a daily uptake
535 calculated for each time point compared to previous time point (white, right axis).

536 **Figure 3:** Schematic overview of the polysaccharide extraction process. EtOH, ethanol.
537 MeOH, methanol. FCSP, fucose-containing sulphated polysaccharides.

538