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Isotopic labelling of cultured macroalgae and isolation of ^{13}C -labelled cell wall polysaccharides for trophic investigations

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Abstract

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

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

Keywords: (5 to 10)

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

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,

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

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

¹³C likely use dissolved CO₂ as their main source of inorganic carbon, while $\delta^{13}\text{C}$ close to -10‰ corresponds to a preferential fixation of HCO₃⁻ during primary production. Other factors influencing $\delta^{13}\text{C}$ of marine macroalgae include the photosynthesis rates and the molecular composition of algal tissues. These distinct natural isotopic values can be used to assess the contribution of different macroalgal species as food sources for associated consumers. Analyses of stable isotope signatures in kelp forest trophic webs have evidenced the relevance of detrital material as a major food source for the associated benthic communities (Fredriksen, 2003). For example, in a kelp forest dominated by *Laminaria digitata*, filter-feeders derived 35% to 50% of their diet from macroalgae-derived detritus (Schaal et al., 2010). Overall, approaches based on natural stable isotopes confirmed the importance of the microbial detrital pathway to re-inject macroalgal primary production into marine food webs. However, such bulk isotopic measurements of natural abundances cannot inform precisely on the specific contribution of selected algal molecules and on the microbial processes involved in their degradation. A thorough knowledge of these bacterial processes is essential because they directly condition the fate of macroalgal organic matter in marine ecosystems.

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

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

Material and Methods

Materials

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

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

Algal culture procedures

Laminaria digitata life cycle consists of microscopic haploid gametophyte phase, alternating with macroscopic diploid sporophytes. In this study, all experiments were done on the macroscopic diploid individuals. To obtain young laboratory-grown sporophytes, mature *L. digitata* sporophytes showing sorus reproductive tissues were harvested at the "Roches Duon" site (GPS coordinates 48°43'32", -3°55'20"). Spores were released from sorus pieces in seawater for 3 h at 18°C with an irradiance of $20 \mu\text{mol.s}^{-1}.\text{m}^{-2}$ and further kept at 13°C at an irradiance of $5 \mu\text{mol.s}^{-1}.\text{m}^{-2}$ to allow the development of gametophytes and random crosses. The developing young *L. digitata* sporophytes (ca. 5 mm) were cultured in flasks containing 10 L of autoclaved seawater enriched with 1.5 ml of modified Provasoli solution (92 mM 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 mM $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2$, 824.6 mM NaNO_3 , 46.3 mM $\text{C}_3\text{H}_7\text{Na}_{206}\text{P}$, 0.001 mM cyanocobalamine, 0.3 mM thiamine, 0.004 mM biotine, 413 mM Tris) (Starr & Zeikus, 1993), with continuous bubbling of 0.2 μm -filtered air in a culture cabinet at 13°C illuminated with daylight-type fluorescent lamps at an irradiance of $20 \mu\text{mol.s}^{-1}.\text{m}^{-2}$ for 12 hours per day. Two separate batches were prepared (^{12}C -control and ^{13}C -enriched) and were regularly amended with 1 g.L^{-1} solutions of either NaHCO_3 with natural isotopic composition (98.9 $^{12}\text{C}\%$) or labelled NaHCO_3 (99 $^{13}\text{C}\%$) previously sterilized on 0.2 μm filters. To favour the growth of larger sporophytes, each batch was subdivided in separate 10L-flasks after 42 and 66 days of cultures. Culture media were renewed biweekly during the first month and weekly afterwards. At each medium renewal, sporophytes were visually examined to eliminate individuals showing signs of necrosis or microbial contamination. All manipulations were performed

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

Polysaccharide extraction

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

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

Isotopic analysis

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

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

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

${}^{13}\text{C}$ atom percent were calculated as follows:

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

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

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

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

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

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

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

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

¹³C_{incorp%} = $\frac{M_{tot} \times cUp^{13}C_{tfinal}}{0.99 \times ^{13}C_{bicarb}}$, where M_{tot} is the total dry algal biomass harvested at the end of the cultivation period, cUp¹³C_{tfinal} is the cumulative ¹³C uptake after 95 days of culture, and

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

Results

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

[Insert Figure 1 here]

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

[insert Figure 2 here]

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

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

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

[insert Figure 3 here]

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

[insert Table 1 here]

Isotopic analysis revealed strongly significant enrichment of polysaccharide fractions extracted from ^{13}C -enriched algal biomass compared to controls (Table 2). Alginate and FCSPs showed stable isotope content of $\text{At}\%^{13}\text{C} = 3.6279 \pm 0.0009 \%$ and $3.5868 \pm 0.0064 \%$, 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 polysaccharide, respectively. This shows that carbon from sodium bicarbonate was allocated

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

[insert Table 2 here]

Discussion

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

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

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

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

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

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References

- Adin, R., & Riera, P. (2003). Preferential food source utilization among stranded macroalgae by *Talitrus saltator* (Amphipod, Talitridae): a stable isotopes study in the northern coast of Brittany (France). *Estuarine, Coastal and Shelf Science*, 56(1), 91–98.
[https://doi.org/10.1016/S0272-7714\(02\)00124-5](https://doi.org/10.1016/S0272-7714(02)00124-5)
- Benner, R., Lay, J., K'nees, E., & Hodson, R. E. (1988). Carbon conversion efficiency for bacterial growth on lignocellulose: Implications for detritus-based food webs. *Limnology and Oceanography*, 33(6, part2), 1514–1526.
<https://doi.org/10.4319/lo.1988.33.6part2.1514>
- Bidwell, R. G. S. (1958). Photosynthesis and metabolism of marine algae: II. A survey of rates and products of photosynthesis in $C^{14}O_2$. *Canadian Journal of Botany*, 36(3), 337–349. <https://doi.org/10.1139/b58-029>
- Brinkhuis, B. H. (1977). Seasonal variations in salt-marsh macroalgae photosynthesis. I. *Ascophyllum nodosum* ecad *scorpioides*. *Marine Biology*, 44(2), 165–175.
<https://doi.org/10.1007/BF00386956>
- Buchsbaum, R., Valiela, I., Swain, T., Dzierzeski, M., & Allen, S. (1991). Available and refractory nitrogen in detritus of coastal vascular plants and macroalgae. *Marine Ecology Progress Series*, 72(1/2), 131–143. <https://doi.org/10.2307/24825436>
- Bustamante, R. H., & Branch, G. M. (1996). The dependence of intertidal consumers on kelp-

372 derived organic matter on the west coast of South Africa. *Journal of Experimental*
373 *Marine Biology and Ecology*, 196, 1–28. [https://doi.org/10.1016/0022-0981\(95\)00093-3](https://doi.org/10.1016/0022-0981(95)00093-3)

374 Carpenter, L. J., & Liss, P. S. (2000). On temperate sources of bromoform and other reactive
375 organic bromine gases. *Journal of Geophysical Research*, 105(D16), 20539–20547.
376 <https://doi.org/10.1029/2000JD900242>

377 Coplen, T. B., Brand, W. A., Gehre, M., Gröning, M., Meijer, H. A. J., Toman, B., &
378 Verkouteren, R. M. (2006). New Guidelines for $\delta^{13}\text{C}$ Measurements. *Analytical*
379 *Chemistry*, 78(7), 2439–2441. <https://doi.org/10.1021/ac052027c>

380 Crosby, M. P., Newell, R. I. E., & Langdon, C. J. (1990). Bacterial mediation in the
381 utilization of carbon and nitrogen from detrital complexes by *Crassostrea virginica*.
382 *Limnology and Oceanography*, 35(3), 625–639.
383 <https://doi.org/10.4319/lo.1990.35.3.0625>

384 Dauby, P. (1989). The stable carbon isotope ratios in benthic food webs of the Gulf of Calvi,
385 Corsica. *Continental Shelf Research*, 9(2), 181–195.

386 Dayton, P. K. (1985). Ecology of kelp communities. *Annual Review of Ecology and*
387 *Systematics*, 16(1), 215–245. <https://doi.org/10.1146/annurev.es.16.110185.001243>

388 Deniaud-Bouët, E., Kervarec, N., Michel, G., Tonon, T., Kloareg, B., & Hervé, C. (2014).
389 Chemical and enzymatic fractionation of cell walls from Fucales: insights into the
390 structure of the extracellular matrix of brown algae. *Annals of Botany*, 114(6), 1203–
391 1216. <https://doi.org/10.1093/aob/mcu096>

392 Duggins, D. O., & Eckman, J. E. (1997). Is kelp detritus a good food for suspension feeders?
393 Effects of kelp species, age and secondary metabolites. *Marine Biology*, 128(3), 489–
394 495. <https://doi.org/10.1007/s002270050115>

395 Duggins, D. O., Simenstad, C. A., & Estes, J. A. (1989). Magnification of secondary
396 production by kelp detritus in coastal marine ecosystems. *Science*, 245(4914), 170–173.

397 Dumont, M. G., & Murrell, J. C. (2005). Stable isotope probing — linking microbial identity
398 to function. *Nature Reviews Microbiology*, 3(June), 499–504.

399 Francois, C. M., Mermillod-Blondin, F., Malard, F., Fourel, F., Lécuyer, C., Douady, C. J., &
400 Simon, L. (2016). Trophic ecology of groundwater species reveals specialization in a
401 low-productivity environment. *Functional Ecology*, 30(2), 262–273.
402 <https://doi.org/10.1111/1365-2435.12484>

403 Fredriksen, S. (2003). Food web studies in a Norwegian kelp forest based on stable isotope
404 ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) analysis. *Marine Ecology Progress Series*, 260, 71–81.
405 <https://doi.org/10.2307/24867089>

406 Hardison, A. K., Canuel, E. A., Anderson, I. C., & Veuger, B. (2010). Fate of macroalgae in
407 benthic systems: Carbon and nitrogen cycling within the microbial community. *Marine*
408 *Ecology Progress Series*, 414, 41–55. <https://doi.org/10.3354/meps08720>

409 Hellebust, J. A., & Haug, A. (1972). Photosynthesis, translocation, and alginic acid synthesis
410 in *Laminaria digitata* and *Laminaria hyperborea*. *Canadian Journal of Botany*, 50(1),
411 169–176. <https://doi.org/10.1139/b72-022>

412 Herman, P. M. J., Middelburg, J. J., Widdows, J., Lucas, C. H., & Heip, C. H. R. (2000).
413 Stable isotopes as trophic tracers: combining field sampling and manipulative labelling
414 of food resources for macrobenthos. *Marine Ecology Progress Series*, 204, 79–92.
415 <https://doi.org/10.2307/24863624>

416 Hogsett, W. E., & Quatrano, R. S. (1975). Isolation of polysaccharides sulfated during early
417 embryogenesis in *Fucus*. *Plant Physiology*, 55, 25–29.

<https://doi.org/10.1104/pp.55.1.25>

Kaehler, S., Pakhomov, E. A., Kalin, R. M., & Davis, S. (2006). Trophic importance of kelp-derived suspended particulate matter in a through-flow sub-Antarctic system. *Marine Ecology Progress Series*, 316, 17–22. <https://doi.org/10.3354/meps316017>

Kamp, A., & Witte, U. (2005). Processing of ^{13}C -labelled phytoplankton in a fine-grained sandy-shelf sediment (North Sea): Relative importance of different macrofauna species. *Marine Ecology Progress Series*, 297, 61–70. <https://doi.org/10.3354/meps297061>

Krause-Jensen, D., & Duarte, C. M. (2016). Substantial role of macroalgae in marine carbon sequestration. *Nature Geoscience*, 9(10), 737–742. <https://doi.org/10.1038/ngeo2790>

Langdon, C. J., & Newell, R. I. E. (1990). Utilization of detritus and bacteria as food sources by two bivalve suspension-feeders, the oyster *Crassostrea virginica* and the mussel *Geukensia demissa*. *Marine Ecology Progress Series*. Inter-Research Science Center. <https://doi.org/10.2307/24842204>

Leclerc, J.-C., Riera, P., Leroux, C., Lvque, L., Laurans, M., Schaal, G., & Davoult, D. (2013). Trophic significance of kelps in kelp communities in Brittany (France) inferred from isotopic comparisons. *Marine Biology*, 160(12), 3249–3258. <https://doi.org/10.1007/s00227-013-2306-5>

Legrand, E., Martin, S., Leroux, C., & Riera, P. (2018). Effect of temperature on an alga-grazer trophic transfer: A dual stable isotope (^{13}C , ^{15}N) labeling experiment. *Marine Ecology*, 39(2), 1–6. <https://doi.org/10.1111/maec.12495>

Lepoint, G., Millet, S., Dauby, P., Gobert, S., & Bouqueneau, J. M. (2002). Annual nitrogen budget of the seagrass *Posidonia oceanica* as determined by in situ uptake experiments. *Marine Ecology Progress Series*, 237, 87–96. <https://doi.org/10.3354/meps237087>

441 Leroy, F., Riera, P., Jeanthon, C., Edmond, F., Leroux, C., & Comtet, T. (2012). Importance
 442 of bacterivory and preferential selection toward diatoms in larvae of *Crepidula fornicata*
 443 (L.) assessed by a dual stable isotope (^{13}C , ^{15}N) labeling approach. *Journal of Sea*
 444 *Research*, 70, 23–31. <https://doi.org/10.1016/j.seares.2012.02.006>

445 Mabeau, S., & Kloareg, B. (1987). Isolation and analysis of the cell walls of brown algae :
 446 *Fucus spiralis*, *F. ceranoides*, *F. vesiculosus*, *F. serratus*, *Bifurcaria bifurcata* and
 447 *Laminaria digitata*. *J. Exp. Bot.*, 38(194), 1573–1580.

448 Maberly, S. C., Raven, J. A., & Johnston, A. M. (1992). Discrimination between ^{12}C and ^{13}C
 449 by marine plants. *Oecologia*, 91(4), 481–492.

450 Macler, B. A. (1986). Regulation of carbon flow by nitrogen and light in the red alga,
 451 *Gelidium coulteri*. *Plant Physiology*, 82(1), 136–141.
 452 <https://doi.org/10.1104/pp.82.1.136>

453 Mann, K. H. (Kenneth H. (1982). *Ecology of coastal waters : a systems approach*. University
 454 of California Press.

455 Marconi, M., Giordano, M., & Raven, J. A. (2011). Impact of taxonomy, geography, and
 456 depth on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ variation in a large collection of macroalgae. *Journal of*
 457 *Phycology*, 47(5), 1023–1035. <https://doi.org/10.1111/j.1529-8817.2011.01045.x>

458 Mercado, J. M., de los Santos, C. B., Lucas Pérez-Lloréns, J., & Vergara, J. J. (2009). Carbon
 459 isotopic fractionation in macroalgae from Cádiz Bay (Southern Spain): Comparison with
 460 other bio-geographic regions. *Estuarine, Coastal and Shelf Science*, 85(3), 449–458.
 461 <https://doi.org/10.1016/j.ecss.2009.09.005>

462 Middelburg, J. J., Barranguet, C., Boschker, H. T. S., Herman, P. M. J., Moens, T., & Heip,
 463 C. H. R. (2000). The fate of intertidal microphytobenthos carbon: An in situ ^{13}C -

464 labeling study. *Limnology and Oceanography*, 45(6), 1224–1234.
 465 <https://doi.org/10.4319/lo.2000.45.6.1224>

466 Moodley, L., Boschker, H. T. S., Middelburg, J. J., Pel, R., Herman, P. M. J., De Deckere, E.,
 467 & Heip, C. H. R. (2000). Ecological significance of benthic foraminifera: ¹³C labelling
 468 experiments. *Marine Ecology Progress Series*, 202, 289–295.
 469 <https://doi.org/10.3354/meps202289>

470 Musat, N., Foster, R., Vagner, T., Adam, B., & Kuypers, M. M. M. (2012). Detecting
 471 metabolic activities in single cells, with emphasis on nanoSIMS. *FEMS Microbiology*
 472 *Reviews*, 36(2), 486–511. <https://doi.org/10.1111/j.1574-6976.2011.00303.x>

473 Pascal, P.-Y., Dupuy, C., Richard, P., & Niquil, N. (2008). Bacterivory in the common
 474 foraminifer *Ammonia tepida*: Isotope tracer experiment and the controlling factors.
 475 *Journal of Experimental Marine Biology and Ecology*, 359(1), 55–61.
 476 <https://doi.org/10.1016/J.JEMBE.2008.02.018>

477 Paul, D., Skrzypek, G., & Fórizs, I. (2007). Normalization of measured stable isotopic
 478 compositions to isotope reference scales – a review. *Rapid Communications in Mass*
 479 *Spectrometry*, 21, 3006–3014. <https://doi.org/10.1002/rcm>

480 Pinnegar, J. K., & Polunin, N. V. C. (2000). Contributions of stable-isotope data to
 481 elucidating food webs of Mediterranean rocky littoral fishes. *Oecologia*, 122(3), 399–
 482 409. <https://doi.org/10.1007/s004420050046>

483 Pomeroy, L. R. (1980). Detritus and its role as a food source. In R. S. K. Barnes & K. H.
 484 Mann (Eds.), *Fundamentals of aquatic ecosystems* (pp. 84–102). Blackwell Scientific
 485 Publications, Oxford, UK.

486 Popper, Z. a, Michel, G., Hervé, C., Domozych, D. S., Willats, W. G. T., Tuohy, M. G., ...

487 Stengel, D. B. (2011). Evolution and diversity of plant cell walls: from algae to
 488 flowering plants. *Annual Review of Plant Biology*, 62, 567–590.
 489 <https://doi.org/10.1146/annurev-arplant-042110-103809>

490 Raven, J. A., Johnston, A. M., Kübler, J. E., Korb, R., McInroy, S. G., Handley, L. L., ...
 491 Dunton, K. H. (2002). Mechanistic interpretation of carbon isotope discrimination by
 492 marine macroalgae and seagrasses. *Functional Plant Biology*, 29(3), 355–378.
 493 <https://doi.org/10.1071/PP01201>

494 Schaal, G., Riera, P., & Leroux, C. (2009). Trophic significance of the kelp *Laminaria*
 495 *digitata* (Lamour.) for the associated food web: a between-sites comparison. *Estuarine,*
 496 *Coastal and Shelf Science*, 85(4), 565–572. <https://doi.org/10.1016/J.ECSS.2009.09.027>

497 Schaal, G., Riera, P., & Leroux, C. (2010). Trophic ecology in a Northern Brittany (Batz
 498 Island, France) kelp (*Laminaria digitata*) forest, as investigated through stable isotopes
 499 and chemical assays. *Journal of Sea Research*, 63, 24–35.

500 Starr, R. C., & Zeikus, J. A. (1993). UTEX – the culture collection of algae at the University
 501 of Texas at Austin. *Journal of Phycology*, 29(s2), 1–106.

502 Tenore, K. R. (1977). Growth of *Capitella capitata* cultured on various levels of detritus
 503 derived from different sources. *Limnology and Oceanography*, 22(5), 936–941.

504 Vauchel, P., Kaas, R., Arhaliass, A., Baron, R., & Legrand, J. (2008). A new process for
 505 extracting alginates from *Laminaria digitata*: Reactive extrusion. *Food and Bioprocess*
 506 *Technology*, 1(3), 297–300. <https://doi.org/10.1007/s11947-008-0082-x>

507 Wagner, M. (2009). Single-cell ecophysiology of microbes as revealed by Raman
 508 microspectroscopy or secondary ion mass spectrometry imaging. *Annual Review of*
 509 *Microbiology*, 63, 411–429. <https://doi.org/10.1146/annurev.micro.091208.073233>

510 Yamaguchi, T., Ikawa, T., & Nisizawa, K. (1966). Incorporation of radioactive carbon from
511 $\text{H}^{14}\text{CO}_3^-$ into sugar constituents by a brown alga, *Eisenia bicyclis*, during photosynthesis
512 and its fate in the dark. *Plant and Cell Physiology*, 7(2), 217–229.
513 <https://doi.org/10.1093/oxfordjournals.pcp.a079175>

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Tables

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

Table 2: Stable isotope ratios of polysaccharide fractions extracted from ^{12}C -control and ^{13}C -enriched *L. digitata* sporophytes. Values are mean $\pm$ standard deviation of technical triplicates.

Sample	At% ^{13}C	
	Alginate	FCSP
^{12}C -control	1.1337 $\pm$ 0.0004	1.1454 $\pm$ 0.0008
^{13}C -enriched	3.6279 $\pm$ 0.0009	3.5868 $\pm$ 0.0064

Figure legends

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

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

Figure 2: A. ^{13}C atom percent of algal specimens collected at different time points in the control (blue) and ^{13}C -enriched culture batches. Values are mean $\pm$ standard deviation (n=3).

B. Uptake in ^{13}C calculated for algae in the ^{13}C -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.

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