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Food sources used by sediment meiofauna in an intertidal *Zostera noltii* seagrass bed: a seasonal stable isotope study

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19 **ABSTRACT**

20 In an intertidal *Zostera noltii* Hornem. seagrass bed, food sources used by sediment
21 meiofauna were determined seasonally by comparing stable isotope signatures ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) of
22 sources with those of nematodes and copepods. Proportions of different carbon sources used
23 by consumers were estimated using the SIAR mixing model on $\delta^{13}\text{C}$ values. Contrary to $\delta^{15}\text{N}$
24 values, food source mean $\delta^{13}\text{C}$ values encompassed a large range, from -22.1‰ (suspended
25 particulate organic matter) to -10.0‰ (*Z. noltii* roots). $\delta^{13}\text{C}$ values of copepods (from -22.3 to
26 -12.3‰) showed that they use many food sources (benthic and phytoplanktonic microalgae, *Z.*
27 *noltii* matter). Nematode $\delta^{13}\text{C}$ values ranged from -14.6 to -11.4‰, indicating a strong role of
28 microphytobenthos and/or *Z. noltii* matter as carbon sources. The difference of food source
29 uses between copepods and nematodes is discussed in the light of source accessibility and
30 availability.

31

32

33 INTRODUCTION

34 Seagrass beds are widespread in shallow coastal waters and are considered one of the
35 most productive marine ecosystems in the world (Duarte and Chiscano 1999). Seagrass beds
36 support a high diversity of consumers because they provide a wide range of potential food
37 sources including seagrass leaves, roots and detrital matter, epiphytes, microphytobenthos,
38 bacteria and allochthonous inputs of organic matter (Valentine and Duffy 2006). These food
39 sources have different levels of digestibility (Cebrián 1999) and some of them (*e.g.* seagrass,
40 phytoplankton) have a large seasonal pattern of production (Duarte 1989; Borowitzka et al.
41 2006), affecting consumer population structure and diets (Escavara et al. 1989; Danovaro
42 1996; Danovaro and Gambi 2002).

43 Meiofauna are often characterized by high densities on seagrass leaves (*i.e.* harpacticoid
44 copepods) (Bell et al. 1984; De Troch et al. 2001) and in surface sediments (Escavara et al.
45 1989; Danovaro et al. 2002). Meiofaunal biomass is often high in seagrass sediments, from
46 1.0 to 2.4 g C.m⁻² (Escavara et al. 1989; Danovaro et al. 2002), oftentimes higher than in
47 unvegetated areas (Castel et al. 1989; Fonseca et al. 2011; Leduc and Probert 2011). Because
48 of their short life cycle and high turnover rates (Hicks and Coull 1983; Heip et al. 1985),
49 meiofaunal communities are thought to respond rapidly to organic matter inputs and may be
50 closely coupled with primary production inputs (Escavara et al. 1989). Meiofauna have
51 high estimated secondary production rates, from 9.05 to 29.40 g C.m⁻².yr⁻¹ (Escavara et al.
52 1989; Danovaro et al. 2002), and may play a key role in benthic energy flows. Meiofauna also
53 represent a direct link between primary producers and higher trophic levels because
54 meiofauna (harpacticoid copepods in particular) are a common prey item of fish and shrimps
55 (Coull 1999; Hyndes and Lavery 2005).

56 Food sources of meiofauna are not well characterized, despite their potentially important
57 role in the trophic dynamics of seagrass beds. This is due to the small size of these animals,
58 rendering their sorting and study complex. At the species level harpacticoid copepods and
59 nematodes often have specialized diets (Rieper 1982; Romeyn and Bouwman 1983; Buffan-
60 Dubau et al. 1996; Moens and Vincx 1997; Rzeznik-Orignac et al. 2008). Nevertheless,
61 changes of feeding strategies can be observed depending on the availability of food sources,
62 both at community (Riera and Hubas 2003; Hyndes and Lavery 2005) and species level
63 (Moens and Vincx 1997). Variations of food resource availability may thus influence
64 meiofaunal community structure (Escavara et al. 1989; Danovaro 1996; Danovaro and
65 Gambi 2002).

66 Seagrass beds are ecosystems where food sources typically exhibit large variations of
67 quality, quantity and availability throughout the year (Duarte 1989; Lebreton et al. 2009).
68 Production of seagrass beds is usually high (Cebrián 1999) but follows strong temporal
69 patterns of growth (Duarte 1989; Lebreton et al. 2009). Some vertebrate or invertebrate
70 consumers directly use seagrass organic matter (Valentine and Heck, 1999) but a large part of
71 it becomes detritus (Cebrián, 1999). Detrital organic matter constitutes a substrate for the
72 development of bacteria (Anesio et al. 2003; Holmer et al. 2004), which may represent a food
73 source for meiofauna (Danovaro 1996). Another food source is microphytobenthos. It often
74 exhibits high production rates in seagrass beds (Asmus and Asmus 1985; Daehnick et al.
75 1992; Kaldy et al. 2002), it is available to consumers and is easily digestible (Duarte and
76 Cebrián 1996). Microphytobenthos can also be a major carbon source for bacteria in seagrass
77 beds (Boschker et al. 2000). Very few studies have addressed microphytobenthos fate in
78 seagrass bed food webs and *a fortiori* the contribution of microphytobenthos as a food
79 resource to meiofauna (Leduc et al. 2009).

80 Stable isotopes are commonly used to study trophodynamics in ecosystems (Fry 2006).
81 Configurations for isotopic composition determination, requiring around 100 µg of matter or
82 less (Carman and Fry 2002), now enable the measurement of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of
83 meiofaunal communities and the ability to determine their food resources (Riera et al. 1996;
84 Riera and Hubas 2003). Contrary to gut content analyses, stable isotope analyses allow
85 determination of food sources actually assimilated in the tissues of consumers over time,
86 properly reflecting their trophodynamics depending on food source availability (Fry 2006). A
87 limit of this technique is that stable isotope signatures sometimes overlap, making data
88 interpretation difficult.

89 The aim of this study is to describe, using natural abundances of ^{13}C and ^{15}N , the food
90 sources used by nematodes and copepods in the sediment of an intertidal *Zostera noltii*
91 Hornem. seagrass bed in Marennes-Oléron Bay over an annual cycle. Copepod and nematode
92 food resources are characterized by comparisons between their stable isotope ratios and those
93 of the available food sources.

94

95 **MATERIAL AND METHODS**

96 *Study area*

97 The study was carried out in a *Zostera noltii* meadow in Marennes-Oléron Bay, a semi-
98 enclosed system along the French Atlantic coast. This macrotidal bay (tidal range 0.9-6.5 m),

99 located between Oléron Island and the mainland, receives continental water mainly from the
100 Charente River, which flows out into the eastern side of the bay. Tidal current speeds range
101 from 0.04 to 0.27 m.s⁻¹ (Struski, unpublished data). The studied seagrass bed is located on the
102 western side of the bay, along Oléron Island, where the bay is more strongly influenced by
103 offshore water (Dechambenoy et al. 1977). The *Z. noltii* bed extends over 15 km along the
104 shore and is 1.5 km wide in the upper part of the flat, limited in its lower part by extensive
105 oyster farm structures (Guillaumont 1991).

106 The sampling station (45°54'32.0'' N, 1°12'50.3'' W) was located at about 250 m from
107 the upper limit of the intertidal seagrass bed. At this station, sediment is composed of silty
108 fine sand (Weber, 2003). Mean emersion time is about 5 hours per tide and the mean water
109 level is about 1.80 m during immersion. Biomass fluctuations of food resources, meiofauna
110 and macrofauna were studied in 2006 and 2007 (Lebreton et al. 2009; Lebreton unpublished
111 data).

112

113 ***Sampling and preparation of primary producers and composite food sources***

114 Seagrass roots and leaves, suspended particulate organic matter (SPOM) and surface
115 sediment fine organic matter (SSOM) were sampled seasonally from winter 2006 to summer
116 2007 (winter 2006: 02/16, spring 2006: 05/22, summer 2006: 08/09, fall 2006: 11/09, winter
117 2007: 02/21, spring 2007: 05/29, summer 2007: 07/31). Microphytobenthos was sampled
118 from winter 2007 to fall 2007 (winter 2007: 02/21, spring 2007: 05/29, summer 2007: 07/31,
119 fall 2007: 11/28), and a complementary sampling was carried out in winter 2010 (02/21).
120 Epiphytes were sampled in spring (05/29) and summer 2007 (08/02). Data from food sources
121 sampled in winter and summer 2006 and 2007 are given by Lebreton et al. (2011).

122 Below-ground and above-ground parts (roots and leaves, respectively) of *Zostera noltii*
123 were rinsed with tap water to remove detrital fragments. Detrital matter from *Z. noltii* – made
124 up of pieces of leaves or roots dark brown to black colored - was collected by sieving
125 sediment on a 500-µm sieve and then washed. Samples were freeze-dried then ground to a
126 fine and homogeneous powder using a ball mill. Microphytobenthos samples were collected
127 by scraping surficial sediment on the field and then by extracting microalgae in the laboratory
128 following the method of Riera and Richard (1996), slightly modified by Herlory et al. (2007).
129 Extracted samples were checked under a microscope for purity, then concentrated by
130 centrifugation (10 min, 1000 ×g) and freeze-dried. Microphytobenthos was mainly made up
131 of a large diversity of small pennate diatoms either epipellic, like *Amphora acutiuscula* and
132 *Navicula heterovalvata*, or epipsammic, like *Achnanthes minuscula*, *Plagiogramma*

133 *staurophorum* and *Plagiogrammopsis vanheurckii*, as observed by Bogaczewicz-Adamczak
134 (unpublished data). Epiphytes samples were composed of two species of diatoms (*Cocconeis*
135 *scutellum* and *C. placentula*) (Lebreton et al. 2009). These diatoms were separated from
136 leaves by agitation following the procedure described by Lebreton et al. (2011).

137 Stable isotope analyses were also carried out on composite food sources - *i. e.* SSOM and
138 SPOM – which composition results from a mix of alive or detrital primary producers. For
139 SSOM analyses, surface sediment (top first cm) was sieved wet on a 315- μ m sieve to remove
140 large detritus and macrofauna. Sediment was freeze-dried, ground using a mortar and pestle
141 then acidified to remove carbonates using 1 mol.L⁻¹ HCl. HCl was added drop-by-drop until
142 cessation of bubbling. Samples were then dried at 60°C using a dry bath under air flow. Dried
143 samples were re-homogenized into ultrapure water using an ultrasonic bath. Sediment
144 samples were then freeze-dried again and re-grinded. SPOM from surface water was sampled
145 close to the seagrass bed (45°55'50.4'' N, 1°10'12.0'' W) at mid-tide, biweekly to monthly.
146 A volume of seawater from 50 to 60 mL was pre-filtered on a 200- μ m sieve to eliminate large
147 zooplankton and detrital particles. Then water was filtered on precombusted Whatman GF/F
148 fiber glass filters (0.7 μ m porosity) under moderate vacuum. Filters were freeze dried then
149 acidified using HCl fumes to remove carbonates. All samples were stored at -20°C before
150 analysis.

151

152 ***Sampling and preparation of meiofauna for isotope analyses***

153 Sampling was carried out following the seasonal cycle (spring 2007: 05/05, summer 2007:
154 09/10, fall 2007: 11/28, winter 2008: 03/18). Meiofauna were collected by scraping about
155 0.25 m² of surficial sediment. A surface sediment layer, upper first cm of sediment, and a
156 subsurface sediment layer, from 1 to 4 cm deep, were sampled separately in order to study
157 both meiofauna communities. Surface sediment layer was light brown colored whereas
158 subsurface sediment layer was dark brown to black colored, which was the evidence of anoxic
159 conditions in this last layer. Surface sediment layer was scrapped using a spatula until the
160 required depth (1 cm) was reached. Depth was measured using steel rulers pushed in sediment
161 and regularly disposed on the scrapped area. The same procedure was applied to sample the
162 subsurface sediment layer on the previously scrapped area. In the laboratory, sediment
163 samples were sieved on a 1 mm-mesh sieve to eliminate fresh and detrital *Zostera noltii*
164 matter, macrofauna and shells. Sieved sediment was then stored at 18°C and meiofauna were
165 extracted within 24 hours following field sampling.

166 Some conditions are necessary to determine the isotopic composition of meiofauna. Large
167 numbers of individuals must be extracted from the sediment to get enough material for
168 analyses, and the extracted population must be representative of the whole community.
169 Moreover, meiofauna must be extracted alive and kept in filtered seawater to allow
170 evacuation of gut contents. Some authors used methods based on downward migration of
171 nematodes under permanent light, taking advantage of the negatively phototactic behavior of
172 nematodes (Riera et al. 1996; Rzeznik-Orignac et al. 2008). However these methods do not
173 allow a complete and representative extraction of the community (Rzeznik-Orignac et al.
174 2004), particularly for copepods, that may have some influence on isotopic signatures. Other
175 protocols are based on successive elutriation and centrifugation procedures using the colloidal
176 silica Ludox™ HS 40 (Heip et al. 1985; Rzeznik-Orignac et al. 2004) or MgSO₄, adjusted
177 with distilled water to the meiofauna density of 1.130 (Somerfield et al. 2005). These methods
178 give more representative samples of meiofauna (nematodes and copepods) but are performed
179 on fixed or frozen samples (Giere 2009).

180 We used a similar Ludox-based protocol but slightly modified as to keep meiofauna alive.
181 The usual procedure involves a thorough rinse of the sediment with distilled water to remove
182 the interstitial seawater containing some ions, such as Ca²⁺ and Mg²⁺, which turn the Ludox™
183 solution to a gel (de Jonge 1979). This procedure gave low recovery of live meiofauna. We
184 therefore tested extractions with solutions of higher osmolarity, *i.e.* NaCl solutions at 20 g L⁻¹
185 and at 30 g L⁻¹, both for the Ludox™ dilution and the sediment rinse. Both protocols gave a
186 recovery of fully alive meiofauna. Since the NaCl 20 g L⁻¹ solution method gave clean
187 samples (*i.e.* absence of detrital matter, diatoms...), it was used throughout this study.

188 Fifty ml of sediment were mixed with 200 ml of a 20 g L⁻¹ NaCl solution, vigorously
189 shaken then centrifuged at 2500 ×g during 1 min at 10°C. The supernatant was discarded and
190 this step was repeated once. Meiofauna were extracted by addition of 200 ml of Ludox™ HS
191 40 adjusted to a density of 1.130 with a 20 g L⁻¹ NaCl solution. Samples were vigorously
192 shaken then centrifuged at 2500 ×g during 4 min at 10°C. The supernatant, containing
193 meiofauna, was collected and the resultant pellet was processed once again following the
194 same procedure. Burgess (2001) observed very high extraction efficiencies of meiofauna taxa
195 using this technique after one extraction (nematodes: 97.4%, copepods: 96.0%). Repeating the
196 procedure yielded very few individuals, and increased mortality (Lebreton pers obs).
197 Supernatants with meiofauna were thoroughly rinsed on a 40-µm sieve, firstly with a solution
198 of NaCl at 20 g L⁻¹ in order to wash the Ludox™ HS 40 from samples, then with filtered sea
199 water. Sample rinsing was always performed just after supernatant collection in order to

200 quickly remove meiofauna from Ludox™ HS 40. Both supernatants from same samples were
201 pooled then their quality (state of meiofauna, absence of detrital matter) was checked under
202 binocular. Meiofauna were kept alive in Petri dishes during 12 hours at 18°C in filtered
203 seawater to allow evacuation of gut contents (Buffan-Dubau et al. 1996; Riera et al. 1996),
204 before storage at -20°C without preliminary sorting.

205 After thawing, sorting of samples was done in a Dollfus counting chamber in which
206 nematodes and copepods were picked with fine forceps (Dumont #55). They were then
207 washed in distilled water (Milli Q), counted and brought together in a tin capsule for stable
208 isotope analysis, which had been previously weighed (Microbalance Sartorius ME5, ± 0.001
209 mg) and filled with 300 µl of ultrapure water. Water was then evaporated under vacuum in a
210 desiccator containing P₂O₅ and capsules were precisely weighted (± 0.001 mg). When
211 possible, three replicates of 300 nematodes or copepods (about 100 µg DW) were done per
212 season and per sediment layer (surface or subsurface). Due to lack of material, only one
213 sample per season was analyzed for subsurface copepod communities.

214

215 ***Stable isotope ratios and data analyses***

216 Samples were analyzed using an elemental analyzer (Flash EA 1112, Thermo Scientific,
217 Milan, Italy) coupled to an isotope ratio mass spectrometer (Delta V Advantage with a Conflo
218 IV interface, Thermo Scientific, Bremen, Germany). Results are expressed in the δ unit
219 notation as deviations from standards (Vienna Pee Dee Belemnite for δ¹³C and N₂ in air for
220 δ¹⁵N) following the formula: δ¹³C or δ¹⁵N = [(R_{sample}/R_{standard})-1] x 10³, where R is ¹³C/¹²C or
221 ¹⁵N/¹⁴N. Calibration was done using reference materials (USGS-24, IAEA-CH6, IAEA-600
222 for carbon; IAEA-N1, -N2, -N3, -600 for nitrogen). Analytical precision based on analyses of
223 acetanilide (Thermo Scientific) used as laboratory internal standard was < 0.06‰ and < 0.1‰
224 for carbon and nitrogen, respectively.

225 Comparisons between stable isotope values were conducted using non-parametric
226 procedures, which are more powerful than parametric statistics for small sized samples
227 (replicate numbers almost always < 10) (Zar 2011). Kruskal-Wallis tests were used: 1. to
228 compare stable isotope signatures of the different food sources (all seasons merged), 2. to
229 study seasonal variations of food source and consumer signatures among seasons. Kruskal-
230 Wallis tests were followed by multiple comparisons of means by using the pgirmess package
231 (Giraudoux 2011) of the R software (R Development Core Team 2008). Mann-Whitney-
232 Wilcoxon tests were applied: 1. to compare stable isotope signatures of nematodes between
233 surface and subsurface sediment samples, 2. to compare nematode and copepod signatures.

234 Seasonal composition of SSOM was defined by using the mixing model developed in the R
235 package SIAR (Parnell et al 2010). Only $\delta^{13}\text{C}$ values were used for computations and no
236 trophic enrichment was weighted into computations. Only SPOM, microphytobenthos and *Z.*
237 *noltii* detrital matter were taken into account because *Z. noltii* (leaves and roots) and epiphytes
238 were considered as not being part of SSOM (Lebreton et al 2009, 2011). Models were run for
239 500,000 iterations and the first 50,000 iterations were discarded. Credibility intervals (CI) of
240 0.95, 0.75 and 0.25 were computed and displayed on figures. Only the lowest and highest
241 limits of 0.95 CI were detailed in the manuscript. Credibility intervals are used in Bayesian
242 statistics to define the domain of *a posteriori* probability distribution used for interval
243 estimation (*e. g.* if the 0.95 CI of a contribution value ranges from A to B, it means that there
244 is a 95% chance that the contribution value lies between A and B) (Edwards et al. 1963).

245 Isotopic ratios of consumers and food sources were compared considering a trophic
246 enrichment of 0.3‰ for $\delta^{13}\text{C}$ values and of 2.3‰ for $\delta^{15}\text{N}$ values (Vander Zanden and
247 Rasmussen 2001). Contributions of carbon food sources for nematodes and copepods were
248 estimated by running SIAR mixing model on $\delta^{13}\text{C}$ values. SIAR does not accept missing data.
249 $\delta^{15}\text{N}$ values were not included in the model due the lack of few $\delta^{15}\text{N}$ data of some food
250 sources. In order to reduce the number of food sources in calculations, *Z. noltii* leaves and
251 roots were considered as a single group called *Z. noltii* fresh matter. SSOM was not taken into
252 account because its composition was primarily based on three other food sources already
253 included: *Z. noltii* detrital matter, microphytobenthos and pelagic algae (Lebreton et al 2011),
254 signatures of which were assumed to be close to those of SPOM. Due to the lack of epiphyte
255 $\delta^{13}\text{C}$ value in winter, the winter value used in the model was the overall mean value of the
256 three other seasons. Trophic enrichment used for computations was 0.3 ± 1.3 ‰ (mean \pm
257 standard deviation) (Vander Zanden and Rasmussen 2001). Running model parameters were
258 the same as for SSOM.

259

260 **RESULTS**

261 *Stable isotope signatures of primary producers*

262 Mean $\delta^{13}\text{C}$ values ranged from -15.5‰ (microphytobenthos, winter 2007) to -9.3‰ (roots,
263 spring 2006) (Table 1). Microphytobenthos was characterized by the most depleted mean
264 annual $\delta^{13}\text{C}$ value (-14.1‰), followed by *Zostera noltii* detrital matter (-12.7‰), epiphytes (-
265 11.6‰), *Z. noltii* leaves (-10.3‰) and roots (-10.0‰). $\delta^{13}\text{C}$ values of *Z. noltii* (roots, leaves,
266 detrital matter) showed no clear seasonal pattern (Table 1). Due to analytical issues, $\delta^{15}\text{N}$

267 values of microphytobenthos samples could not be measured in winter 2007. Mean $\delta^{15}\text{N}$
268 values ranged from 4.9 (leaves, winter 2006) to 11.0‰ (roots, fall 2006) (Table 1). No clear
269 seasonal pattern was observed among $\delta^{15}\text{N}$ values of primary producers.

270

271 *Stable isotope signatures of composite food sources*

272 Mean $\delta^{13}\text{C}$ values of SPOM ranged from -23.5‰ (spring 2006) to -21.1‰ (spring 2007)
273 (Table 1). SPOM presented significantly lower $\delta^{13}\text{C}$ values than SSOM, microphytobenthos,
274 epiphytes, *Z. noltii* roots, leaves and detrital matter (Kruskal-Wallis test, $P < 0.001$). Due to
275 analytical issues, $\delta^{15}\text{N}$ values of SPOM samples could not be determined.

276 Stable isotope signatures of SSOM ranged from -19.1‰ (spring 2007) to -17.4‰ (winter
277 2007) and from 5.0 (spring 2006) to 6.8‰ (summer 2006) for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively
278 (Table 1). Stable isotope composition of SSOM showed no clear seasonal pattern (Table 1).
279 SSOM was significantly more depleted in ^{15}N than *Z. noltii* roots, leaves and detrital matter
280 (Kruskal-Wallis tests, $P < 0.001$).

281 Mixing model estimations of contributions showed that SSOM was primarily composed
282 of settled SPOM and secondarily of microphytobenthos and *Z. noltii* detrital matter (Fig. 1).
283 All seasons put together, 0.95 CI ranged in fact from 15 to 81%, from 0 to 60% and from 0 to
284 54% for SPOM, microphytobenthos and *Z. noltii* detrital matter, respectively. Contribution of
285 SPOM to SSOM was particularly high in fall, with 0.95 CI of SPOM ranging from 44 to 81%.

286

287 *Copepods: stable isotope signatures and mixing model estimations of contributions*

288 Copepod abundance was very low in subsurface (1-4 cm) sediment layer (Lebreton
289 unpubl data). As a result, none or only one sample of copepods was collected per season in
290 this sediment layer. When they were determined, isotopic signatures of subsurface samples
291 were always in the range of those of surface (0-1 cm) samples. Surface and subsurface
292 communities have thus been considered as coming from the same community thereafter.

293 Copepods presented a wide range of $\delta^{13}\text{C}$ values (-22.3 to -12.3‰). Range of $\delta^{15}\text{N}$ values
294 was smaller, with values from 6.6 to 8.9‰ (Fig. 2). No significant seasonal variations of
295 copepod signatures were observed (Table 2). For $\delta^{13}\text{C}$ signatures, this absence of difference is
296 probably related with the large standard deviations observed at most seasons. The difference
297 between the average of food sources $\delta^{15}\text{N}$ values (SSOM, microphytobenthos and *Z. noltii*
298 detrital matter) and copepods $\delta^{15}\text{N}$ values was 1.1‰ on average (range from 0.0 to 2.2‰).

299 In summer, mixing model gives higher upper and lower limits of 0.95 CI (from 16 to
300 49%) for SPOM relative to epiphytes and *Z. noltii* fresh and detrital matter, for which 0.95 CI

301 ranged from 0 to 36% (Fig. 3). At other seasons, ranges of food source contributions are large
302 and relatively equal: SPOM: 0.95 CI from 1 to 45%, epiphytes: 0.95 CI from 0 to 37%, *Z.*
303 *noltii* detrital matter: 0.95 CI from 0 to 39%, *Z. noltii* fresh matter: 0.95 CI from 0 to 37%.
304 Contributions of microphytobenthos to copepod carbon sources are equal all year long (0.95
305 CI from 0 to 41%).

306

307 *Nematodes: stable isotope signatures and mixing model estimations of contributions*

308 Stable isotope signatures of nematodes from surface and subsurface samples were similar
309 at all sampling dates (Table 3). Thus, they have been considered as a single community
310 thereafter. Isotopic signatures ranged from -14.7 to -11.4‰ for $\delta^{13}\text{C}$ and from 8.5 to 11.6‰
311 for $\delta^{15}\text{N}$. Considering the trophic enrichment, the theoretical signatures of nematode's food
312 sources are close to those of *Z. noltii* detrital matter and of microphytobenthos (Fig. 2). The
313 difference between the average of food sources $\delta^{15}\text{N}$ values (microphytobenthos and *Z. noltii*
314 detrital matter) and nematodes $\delta^{15}\text{N}$ values is equal to 2.7‰ in average and ranged from 1.4 to
315 4.4‰. Nematode $\delta^{13}\text{C}$ values were more depleted in summer ($-13.6 \pm 0.6\text{‰}$) than in fall and
316 winter ($-12.2 \pm 0.5\text{‰}$ and $-12.5 \pm 0.2\text{‰}$, respectively; Kruskal-Wallis tests, $P < 0.001$) (Table
317 2). $\delta^{15}\text{N}$ values were higher in winter than in summer ($10.7 \pm 0.7\text{‰}$ vs. $8.9 \pm 0.3\text{‰}$, Kruskal-
318 Wallis tests, $P < 0.001$). Mixing model computations show relatively equal contributions of
319 microphytobenthos, epiphytes and fresh and detrital *Z. noltii* organic matter as carbon sources
320 for nematodes (0.95 CI ranging from 0 to 46%, Fig. 3). SPOM contributions are low, with
321 0.95 CI ranging from 1 to 24%.

322 Copepods and nematodes showed similar signatures for $\delta^{13}\text{C}$ in fall and winter (Mann-
323 Whitney-Wilcoxon tests, fall: $P = 0.095$, winter: $P = 0.095$) and for $\delta^{15}\text{N}$ in fall (Mann-
324 Whitney-Wilcoxon test, $P = 0.071$). Copepods presented lower $\delta^{13}\text{C}$ values than nematodes in
325 spring and summer (Mann-Whitney-Wilcoxon tests, spring: $P = 0.010$, summer: $P = 0.009$),
326 with $\delta^{13}\text{C}$ values 2.1‰ less enriched than nematodes. $\delta^{15}\text{N}$ values of copepods were lower
327 than these of nematodes in winter, spring and summer (Mann-Whitney-Wilcoxon tests,
328 winter: $P = 0.024$, spring: $P = 0.010$, summer: $P = 0.010$), with values 3.0 ‰ less enriched
329 than nematodes.

330

331 **DISCUSSION**

332 *Stable isotope signatures of primary producers*

333 $\delta^{13}\text{C}$ values well discriminate the potential food sources, except between *Z. noltii* leaves
334 and roots and between microphytobenthos and *Zostera noltii* detrital matter, mainly because
335 of the large seasonal variations of microphytobenthos $\delta^{13}\text{C}$ signatures. These food source
336 signatures are well within the range of previous observations already made in the same
337 seagrass bed (Kang et al. 1999) and in others (Boschker et al. 2000; Leduc et al. 2006;
338 Kharlamenko et al. 2008; Schaal et al. 2008). Microphytobenthos signatures are also similar
339 to those observed for this food source in adjacent bare mudflats (Riera and Richard 1996;
340 Rzeznik-Orignac et al. 2008). The ^{13}C -depletion observed between live and detrital *Z. noltii*
341 matter is probably due to the higher levels of lignin, which is a ^{13}C -depleted component, in
342 detrital material (Benner et al. 1987).

343

344 ***Origin and composition of composite food sources***

345 SPOM values (from -23.5 to -21.1‰) are ranging from those of marine (-19.1‰) and
346 estuarine phytoplankton (-23.5‰) observed by Riera and Richard (1996) in Marennes-Oléron
347 Bay. SPOM was thus composed of a mix of both these primary producers, possibly with a
348 different influence of the former or the latter depending on seasons.

349 SSOM $\delta^{13}\text{C}$ values were intermediate between those of SPOM and those of two benthic
350 sources: microphytobenthos and *Zostera noltii* detrital matter. Mixing model contributions
351 thus suggest that SSOM is clearly composed of a high amount of settled SPOM. SSOM is
352 also composed of microphytobenthos and *Z. noltii* detrital matter. Because stable isotope
353 signatures of these sources are close, mixing model results do not allow determining
354 contributions of benthic sources (*i. e.* microphytobenthos and *Z. noltii* detrital matter) to
355 SSOM. Nevertheless, Lebreton et al. (2011) ruled out the hypothesis of a high content of *Z.*
356 *noltii* fine particles ($<315\ \mu\text{m}$) in SSOM, because very low amounts of seagrass fatty acid
357 markers were observed in SSOM. This weak contribution of *Z. noltii* matter was unexpected
358 considering the high amount of large ($>500\ \mu\text{m}$) *Z. noltii* detrital particles in the sediment
359 (Lebreton et al. 2009). This high amount was however measured on 15 cm deep cores, so *Z.*
360 *noltii* detrital matter is likely stored as large particles in sediment or buried deeper than the
361 first top centimeter sediment layer.

362 This weak influence of *Z. noltii* to SSOM composition (Lebreton et al. 2009) thus
363 reinforces the role of microalgae, either benthic or pelagic when settled, in the functioning of
364 this intertidal seagrass bed. Food source contributions to SSOM originating from mixing
365 model computations are therefore probably a few overestimated for *Z. noltii* and a few
366 underestimated for pelagic and benthic microalgae. These algae were primarily originating

367 from phytoplankton and secondarily from microphytobenthos. Contribution of settled pelagic
368 microalgae was particularly high in fall, which was confirmed with relatively high amount of
369 flagellate fatty acid markers, particularly 22:6(*n*-3), observed in SSOM (Lebreton unpublished
370 data). Flagellate densities are generally low in sediment (Pascal et al. 2009) and high in water
371 column (Galois et al. 1996), indicating that flagellate organic matter from SSOM was mainly
372 issued from SPOM. The *Z. noltii* seagrass bed thus traps high quantities of SPOM into
373 sediment because seagrass canopy weakens hydrodynamics (Koch et al. 2006), making settled
374 SPOM available to benthic consumers.

375

376 ***Food sources used by benthic copepod communities***

377 Mixing model estimations of contributions and the very large range of copepod $\delta^{13}\text{C}$
378 values suggest that these consumers can use many food sources: SPOM (*i. e.* settled pelagic
379 microalgae), microphytobenthos, epiphytes and *Zostera noltii* matter. The small differences of
380 $\delta^{15}\text{N}$ values between these food sources and copepods (average equal to 1.1‰) suggest that
381 they are primary consumers.

382 The very low $\delta^{13}\text{C}$ value (-22.3‰) noticed in spring and the high contribution of SPOM to
383 copepod carbon sources observed in summer indicate an increased feeding on a mixture of
384 marine/estuarine phytoplankton, likely due its higher availability during blooms occurring at
385 these periods (Galois et al. 1996). Higher amounts of SPOM in SSOM during fall (see
386 previous section) did not clearly affect copepod isotopic composition, demonstrating that
387 copepod food sources were not directly depending on SSOM composition and suggesting that
388 copepods probably perform some selection on food sources in SSOM. Copepod community is
389 in fact probably composed of different feeding mode individuals, like surface-dwelling
390 consumers, which directly graze settled phytoplankton cells at sediment surface, and
391 endobenthic individuals, which performed filter-feeding, like Canuellidae (De Troch et al.
392 2003; Hicks and Coull 1983).

393 Copepods also rely on benthic ^{13}C -enriched food resources (*i. e.* microphytobenthos,
394 epiphytes and/or *Z. noltii* matter). The high contribution of epiphytes as a carbon source for
395 copepods is most probably an artifact of the mixing model in relation with epiphyte $\delta^{13}\text{C}$
396 values, which are intermediate between those of microphytobenthos and of *Z. noltii* matter.
397 Epiphytes are absent in winter, due to the absence of seagrass leaves, and epiphyte biomass is
398 very low during other seasons in Marennes-Oléron seagrass bed, with biomass at least 1000
399 times less than those of microphytobenthos or *Z. noltii* detrital matter (Lebreton et al, 2009).
400 Thus, even if epiphytes could be eaten by benthic copepods, they most probably represent a

401 very weak carbon source. The second main contribution for copepods is thus
402 microphytobenthos and/or *Z. noltii* matter. The relatively close stable isotope signatures
403 between these food sources do not allow clearly determining which of these food sources, or
404 if both of them, are used by copepods.

405 This large range of food source intakes (SPOM, microphytobenthos and *Z. noltii* matter)
406 and their seasonal variations well correspond with the opportunistic trophic behavior of
407 benthic copepods, which are known to feed on microalgae (Hicks and Coull 1983; De Troch
408 et al. 2006) and also on detritus (Rieper-Kirchner 1990). In seagrass beds, the studies
409 conducted by Leduc et al. (2009) and Hyndes and Lavery (2005) on copepods from sediment
410 also showed that they are opportunistic feeders, feeding either on macroalgae or seagrass
411 detrital matter, SPOM, microphytobenthos or bacteria, depending on resource availability.

412

413 ***Food resources of nematodes: microphytobenthos and seagrass matter***

414 Except in fall, mean $\delta^{15}\text{N}$ values of nematodes were higher than those of copepods.
415 Taking an average trophic-level increase in $\delta^{15}\text{N}$ values of 2.5‰ for first level consumers and
416 of 3.4‰ for higher level consumers (Vander Zanden and Rasmussen 2001; Moens et al.
417 2005), the difference between $\delta^{15}\text{N}$ values of food sources and of nematodes (ranging from
418 1.4 to 4.4‰) spans one or two trophic levels. This large range - with higher values than for
419 copepods - could be explained by two reasons: 1. Nematode may rely on higher quantities of
420 *Z. noltii* detrital matter than copepods. Detritivores are thought to derive their nutrition from
421 bacteria associated with detrital particles (*e.g.* *Z. noltii* detrital matter) and not from the non-
422 living plant substrate, particularly for nitrogen (Findlay and Tenore 1982). This mediation
423 increases $\delta^{15}\text{N}$ values in bacteria (Dijkstra et al. 2008) and then in nematodes due to trophic
424 isotopic enrichment. 2. Nematodes are characterized by different feeding modes: selective or
425 non-selective deposit-feeders, epistrate-feeders (*i.e.* protists and microalgae consumers) or
426 omnivore-carnivores (Wieser 1953; Romeyn and Bouwman 1983). These feeding modes
427 place them at different trophic levels and $\delta^{15}\text{N}$ values of nematodes are related to these trophic
428 levels (Carman & Fry, 2002; Moens et al. 2005; Rzeznick et al. 2008). Thus, the high range
429 observed for $\delta^{15}\text{N}$ values is probably representative of this range of feeding behaviors.

430 Mixing model results suggest relatively similar contributions of microphytobenthos,
431 epiphytes and *Zostera noltii* fresh and detrital matter to nematode carbon sources at all
432 sampling dates. The ^{13}C -enriched composition of nematodes and the low contributions of
433 SPOM computed with SIAR demonstrate a lower use of SPOM by nematodes in comparison
434 with copepods. As already detailed for copepods, we suggest that the high contribution of

435 epiphytes as nematode carbon source is an artifact of the mixing model (see previous section).
436 Moreover, accessibility of epiphytes to nematodes is probably very low, due to the
437 exclusively benthic behavior of these consumers. Contributions of other food sources are thus
438 probably slightly underestimated.

439 In the seagrass bed, nematodes thus mostly rely on microphytobenthos and *Zostera noltii*
440 matter. The close isotopic composition between microphytobenthos, fresh and detrital *Z. noltii*
441 matter does not clearly allow determining which one or if all these food resources are used by
442 nematodes. Nevertheless, none of the known marine nematode feeding modes has ever been
443 demonstrated to be capable of directly grazing living macrophyte tissue (Wieser, 1953;
444 Romeyn and Bouwman, 1983; Moens and Vincx, 1997), suggesting that *Z. noltii* matter is
445 only eaten as detrital by nematodes. When detrital, *Z. noltii* organic matter is probably
446 assimilated through bacteria mediation (Findlay and Tenore, 1982). Nematofauna community
447 presents a large diversity of feeding modes (Wieser, 1953; Romeyn and Bouwman, 1983;
448 Moens and Vincx, 1997). The range of nematode $\delta^{13}\text{C}$ values thus includes herbivores and
449 bacterivores, ^{13}C -depleted, but also carnivores, ^{13}C -enriched. These ^{13}C -enriched values can
450 explain the high contributions of fresh *Z. noltii* matter computed through SIAR, which may be
451 overestimated.

452 In another intertidal seagrass bed, Leduc et al. (2009) observed that nematodes also rely
453 mainly on microphytobenthos and on seagrass detrital matter. Improving upon this previous
454 study, our work follows seasonal variations of nematode food sources: on a temporal view,
455 nematode signatures are generally closer to those of microphytobenthos in summer and to
456 those of *Zostera noltii* matter from fall to winter. This seasonal change is probably related
457 with the higher quantities of *Z. noltii* detrital matter in sediment from fall to winter (Lebreton
458 et al. 2009), making this resource more available to nematodes. These variations probably
459 cause changes of food resources at two scales for nematodes. At the individual scale, the
460 nematodes characterized by opportunistic feeding behavior probably rely on availability of
461 sources (Riera and Hubas 2003). At the community scale, there is also probably an evolution
462 of the structure of the nematode community, depending on nematode trophic behaviors
463 (Escavara et al. 1989; Danovaro 1996; Danovaro and Gambi 2002).

464

465 ***Role of SPOM as meiofauna food source***

466 Even though nematodes weakly use SPOM, this food resource appeared to be largely
467 consumed by copepods. This difference between nematodes and copepods is probably related
468 with availability of food sources to these consumers in relation with their location in the

469 sediment. Nematodes are able to migrate through sediment layers (Heip et al. 1985) whereas
470 copepods are mostly located in first top millimeters in muddy sediment (Hicks and Coull
471 1983; Buffan-Dubau and Castel 1996) due to their sensitivity to anoxic conditions (Hicks and
472 Coull 1983). As a result, nematodes may have access to the whole range of sediment food
473 resources (*i. e. Z. noltii* detrital matter, microphytobenthos, trapped SPOM). Exploitation of
474 food sources by nematodes probably reflects the biomass of available resources in both
475 surface and subsurface sediment layers. On the contrary, copepods may mostly access to food
476 resources from the very top sediment layer, which may contain higher quantities of trapped
477 SPOM than underneath sediment layers. This assumption is confirmed by meiofauna densities
478 in this seagrass bed: $12 \cdot 10^6$ individuals m^{-2} and $2.3 \cdot 10^6$ individuals m^{-2} in surface and
479 subsurface sediment layers, respectively, for nematodes and $1.2 \cdot 10^6$ individuals m^{-2} and 8.2
480 10^4 individuals m^{-2} in surface and subsurface sediment layers, respectively, for copepods
481 (Lebreton unpubl data).

482 SPOM availability for the copepod community is also influenced by the location
483 (intertidal *vs.* subtidal) and topography of the seagrass bed. Harpacticoid copepods sampled
484 with nets in a Mediterranean subtidal seagrass bed were mainly relying on SPOM and of
485 benthic organic matter (*e.g.* epiphytes) (Vizzini et al. 2002; Vizzini and Mazzola 2003). In
486 addition, Leduc et al (2009) suggested that seagrass bed architecture (*e.g.* large *vs.* thin leaves
487 of *Posidonia vs. Zostera*, respectively) probably has a strong influence on particle trapping,
488 explaining why SPOM has more influence in *Posidonia* beds. On Marennes-Oléron *Zostera*
489 *noltii* seagrass bed, influence of marine and estuarine SPOM is probably weakened due to the
490 intertidal location of the seagrass bed and thinness of leaves. Phytoplankton is nevertheless an
491 important carbon source for copepods, probably thanks to the high quality of pelagic
492 microalgae as a food resource and to the influence of marine offshore water at this location
493 (Dechambenoy et al. 1977).

494 The influence of SPOM has been previously observed for suspension feeders in this
495 seagrass bed (Lebreton et al 2011), underlining the role of SPOM in this intertidal ecosystem.
496 Like suspension feeders, benthic copepods mediate a benthic-pelagic coupling by consuming
497 pelagic organic matter, increasing the flux of organic matter from pelagos to benthos.
498 Moreover, by using this organic matter, copepods increase its quality through its
499 transformation into animal tissue and make it more available for strictly benthic consumers.

500

501 ***Role of benthic food sources: microphytobenthos and Zostera noltii matter***

502 As previously observed in other seagrass beds, microalgae are an important food resource
503 for meiofauna; particularly microphytobenthos for nematodes (Vizzini et al. 2002; Vizzini
504 and Mazzola 2003; Leduc et al. 2009). This trophic scheme, in which meiofauna largely use
505 microphytobenthos, is very close to those observed in salt marshes (Riera et al. 1996; Moens
506 et al. 2002), where high production of detrital matter from vascular plants occurs. This
507 demonstrates that meiofauna can favor microphytobenthos even if high amounts of organic
508 matter are present (Moens et al. 2002). Few studies about meiofauna in seagrass beds take
509 microphytobenthos into account (Hyndes and Lavery 2005; Leduc et al. 2009) so the role of
510 this food resource remains poorly understood in this habitat.

511 Another food source exploited by meiofauna is *Zostera noltii* detrital matter. The use of
512 detrital matter is generally mediated by bacteria which colonize it (Findlay and Tenore 1982;
513 Holmer et al. 2004). Bacteria have $\delta^{13}\text{C}$ similar to their substrate (Boschker et al. 2000) but
514 higher $\delta^{15}\text{N}$ values (Dijkstra et al, 2008). The role of bacterial mediation in this transfer
515 between *Z. noltii* detrital matter and meiofauna could not be clearly determined in this study,
516 mainly because it has been carried out at the community level. About copepods, differences
517 between $\delta^{15}\text{N}$ values of food sources and these of copepods remains weak in this ecosystem.
518 This suggests that bacterial mediation is low even for *Z. noltii* detrital matter, or that fresh *Z.*
519 *noltii* could be eaten by copepods which is unlikely. About nematodes, the large range of $\delta^{15}\text{N}$
520 values does not allow determining if the higher $\delta^{15}\text{N}$ values of these consumers are related to
521 a bacterial activity or to their different feeding modes. Bacteria role remains unclear since
522 numerous studies on detritus based-food webs have determined that bacteria mediation can be
523 important (Findlay and Tenore, 1982; Anesio et al. 2003, Holmer et al. 2004) while others
524 showed that bacteria mainly use microalgae as a substrate instead of seagrass matter
525 (Boschker et al. 2000). This suggests that the knowledge about bacteria mediation in trophic
526 fluxes needs to be improved.

527 The use of microphytobenthos and *Z. noltii* detrital matter by meiofauna is probably
528 related with different properties of these resources: quality and availability for
529 microphytobenthos and quantity for *Z. noltii* detrital matter. Microphytobenthos presents high
530 nutritional quality and assimilation rates (Cebrián 1999). Moreover, microphytobenthos has
531 generally constant biomass all year long (Lebreton et al. 2009), particularly in surficial
532 sediment. It is thus constantly available for consumers. Microphytobenthos is also probably
533 more available for copepods than *Zostera noltii* detrital matter, which quantities are low in
534 surficial sediment (Lebreton et al. 2011). High biomass of *Z. noltii* detrital matter is stored in
535 deep sediment layers, that nematodes can reach thanks to their migration ability. The

536 utilization of this food source is probably more related with quantitative than with qualitative
537 issues because phanerogams are known to have low nutritional quality, particularly detrital
538 matter, in comparison with microphytobenthos (Cebrián 1999).

539 Nevertheless, the close signatures between microphytobenthos and seagrass detrital matter
540 makes that the role of each of these two resources needs to be clarified. The overlap of
541 signatures between different food sources is a common problem in stable isotope studies. The
542 combination of those results with other trophic markers studies (*i.e.* sulfur stable isotopes,
543 fatty acid analyses, labeled food source experiments) could be an interesting way to dispel
544 some uncertainties (Leduc et al. 2006; Leduc et al. 2009).

545

546 **CONCLUSION**

547 Three food sources are mostly used by meiofauna in the seagrass bed: SPOM, by
548 copepods, microphytobenthos and *Z. noltii* detrital matter, both by copepods and nematodes.
549 Food sources used by meiofauna appeared related to their accessibility (*i.e.* water column or
550 sediment location of food sources) and availability (*i.e.* seasonality of inputs). In this study,
551 carbon sources used by meiofauna were determined at the community scale. Thus, changes of
552 community stable isotope signatures can be related with a trophic plasticity of dominant
553 species but can also reflect changes of community structure. This shows limits of community
554 scale studies and suggests that studies about carbon sources used by meiofauna should now
555 focus on species or trophic groups.

556

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567

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762

763 **FIGURE CAPTIONS**

764

765 **Fig. 1** Seasonal comparison of contributions (%) of SPOM (suspended particulate organic
766 matter), microphytobenthos and *Z. noltii* detrital matter to surface sediment fine organic
767 matter resulting from the mixing model SIAR. 0.95, 0.75, 0.25 credibility intervals are in dark
768 grey, light gray and white, respectively

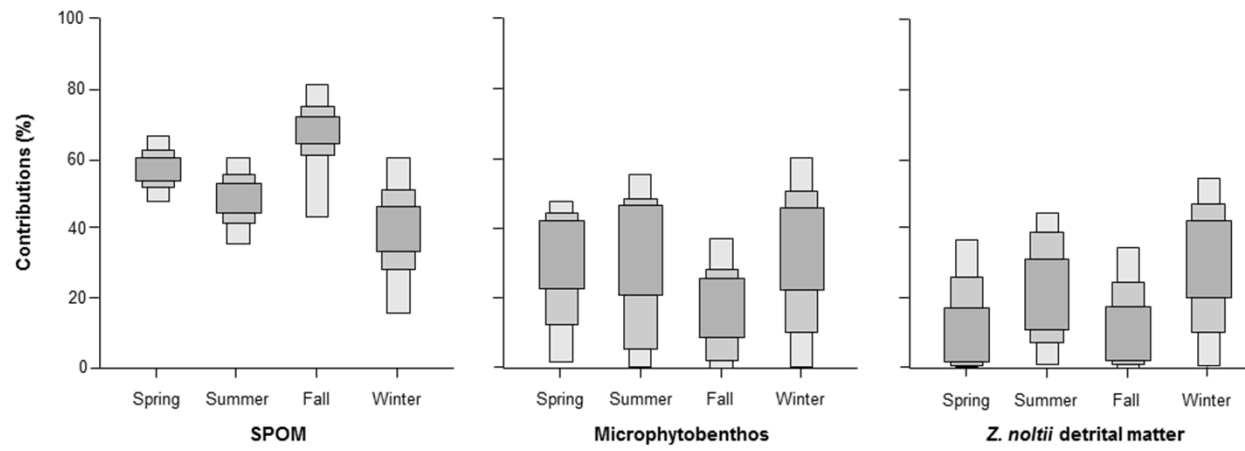
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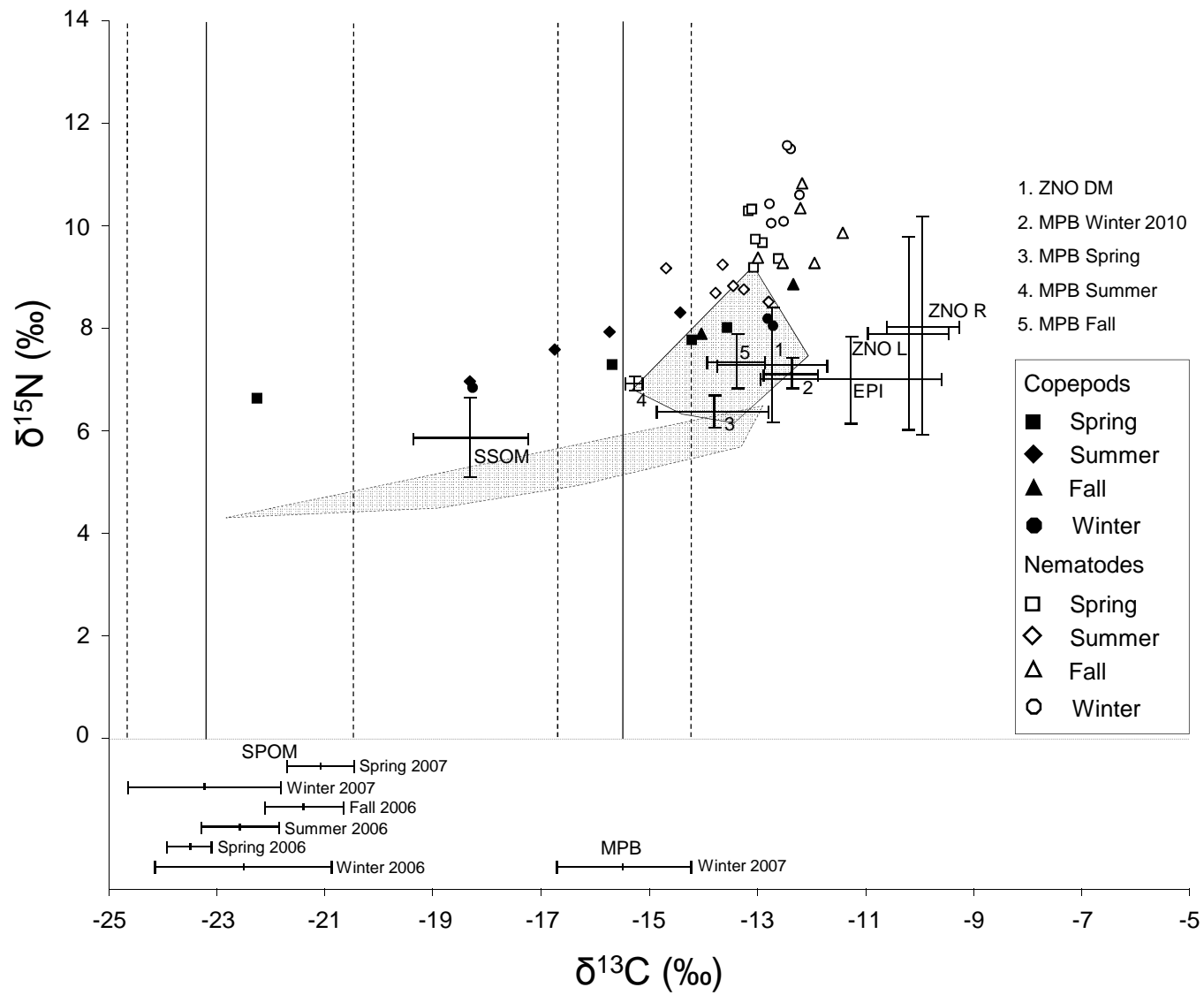
770 **Fig. 2** Plots of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of copepods (‰), nematodes (‰) and of potential food
771 sources (‰, mean \pm SD) in the Marennes-Oléron seagrass bed. For copepods and nematodes,
772 each point corresponds to one sample, *i.e.* about 300 individuals. Grey polygons symbolize
773 ranges of nematodes (full line) and copepods (dotted line) theoretical food source signatures
774 taking into account the trophic enrichment (see material and methods). To make comparisons
775 between food source and consumer signatures easier, seasonal data of food sources which
776 show no seasonal variations (*i.e.* SSOM, *Zostera noltii* detrital matter, see table 1) or which
777 are outside of the grey polygons (*i.e.* epiphytes, *Z. noltii* roots and leaves) are aggregated. No
778 $\delta^{15}\text{N}$ values were available for SPOM and MPB. For SPOM, full line represents annual mean
779 of $\delta^{13}\text{C}$ values and dotted lines represent lowest and highest limits of SD. For MPB sampled
780 in winter 2007, full line represents mean and dotted lines represent SD. Food sources: ZNO R:
781 *Z. noltii* roots; ZNO DM: *Z. noltii* detrital matter; MPB: Microphytobenthos; SSOM: Surface
782 sediment organic matter; SPOM: Suspended particulate organic matter

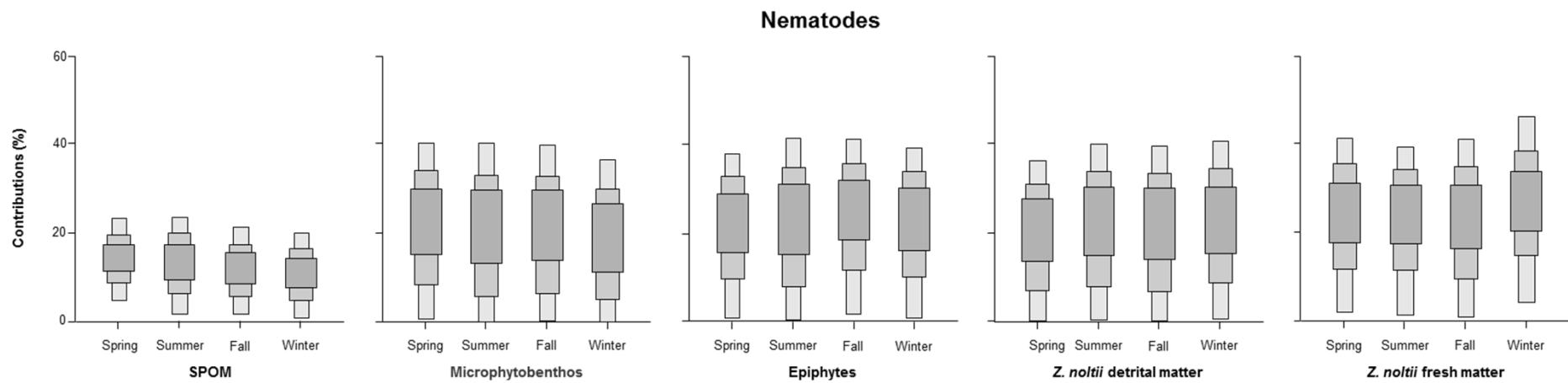
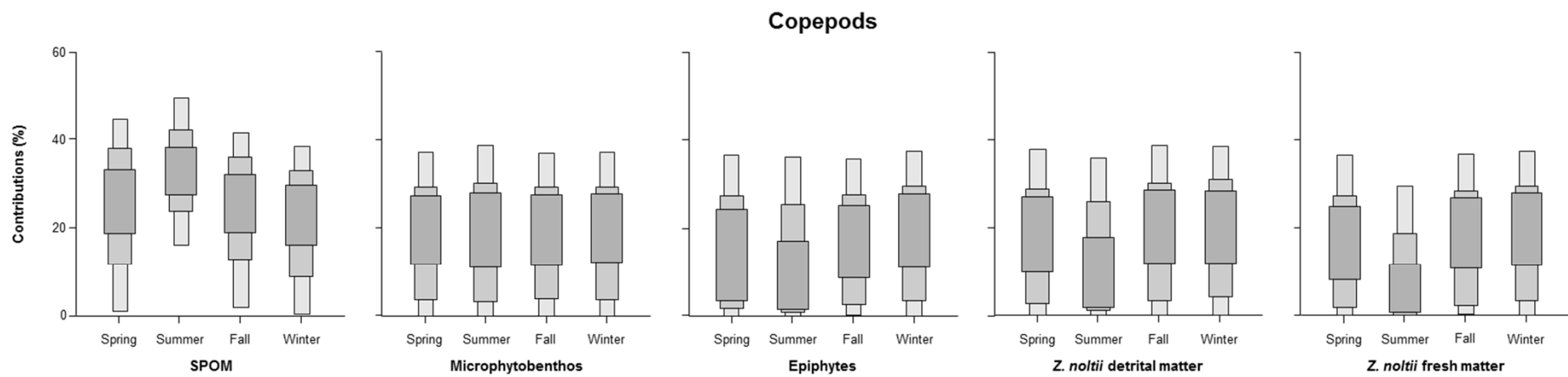
783

784 **Fig. 3** Seasonal comparison of contributions (%) of the potential food sources (SPOM:
785 suspended particulate organic matter, microphytobenthos, epiphytes, *Z. noltii* detrital matter
786 and fresh matter) as carbon sources for nematodes and copepods resulting from the mixing
787 model SIAR. 0.95, 0.75, 0.25 credibility intervals are in dark grey, light gray and white,
788 respectively

789







	Winter	Spring	Summer	Fall	P values	Comparisons of means
$\delta^{13}\text{C}$						
<i>Z. noltii</i> leaves	2006: -9.9 ± 0.1 , N = 3 2007: -9.7 ± 0.5 , N = 3	2006: -10.7 ± 0.2 , N = 3 2007: -11.2 ± 0.2 , N = 3	2006: -9.4 ± 0.2 , N = 6 2007: -11.1 ± 0.2 , N = 3	2006: -10.4 ± 0.6 , N = 3	0.003	Summer 2006 > Spring 2007 Summer 2006 > Summer 2007
<i>Z. noltii</i> roots	2006: -10.2 ± 0.1 , N = 3 2007: -9.8 ± 0.1 , N = 3	2006: -9.3 ± 0.2 , N = 3 2007: -11.1 ± 0.4 , N = 3	2006: -9.3 ± 0.4 , N = 6 2007: -10.1 ± 0.1 , N = 3	2006: -10.4 ± 0.5 , N = 3	0.005	Summer 2006 > Spring 2007
<i>Z. noltii</i> detrital matter	2006: -12.8 ± 1.3 , N = 3	2006: -12.3 ± 1.7 , N = 3	2006: -13.2 ± 0.8 , N = 3 2007: -12.5 ± 0.7 , N = 3	2006: -12.8 ± 0.8 , N = 3	0.894	=
Epiphytes		2007: -10.4 ± 1.5 , N = 5	2007: -12.8 ± 0.1 , N = 3		0.025	Spring 07 > Summer 07
Microphytobenthos	2007: -15.5 ± 1.2 , N = 6 2010: -12.4 ± 0.5 , N = 4	2007: -13.8 ± 1.0 , N = 5	2007: -15.3 ± 0.2 , N = 4	2007: -13.4 ± 0.5 , N = 4	0.002	Winter 2007 < Winter 2010 Summer 2007 < Winter 2010
SSOM	2007: -17.4 ± 1.9 , N = 4	2006: -18.2 ± 0.3 , N = 3 2007: -19.1 ± 0.1 , N = 3	2006: -18.1 ± 1.0 , N = 3 2007: -18.6 ± 0.1 , N = 2	2006: -18.9 ± 0.1 , N = 3	0.067	=
SPOM	2006: -22.5 ± 1.6 , N = 14 2007: -23.2 ± 1.5 , N = 5	2006: -23.5 ± 0.4 , N = 12 2007: -21.1 ± 0.6 , N = 7	2006: -22.6 ± 0.7 , N = 18	2006: -21.4 ± 0.7 , N = 11	> 0.001	Spring 2006 < Fall 2006 Spring 2006 < Spring 2007
$\delta^{15}\text{N}$						
<i>Z. noltii</i> leaves	2006: 4.9 ± 0.3 , N = 3 2007: 6.5 ± 0.5 , N = 3	2006: 8.2 ± 0.5 , N = 3 2007: 7.0 ± 0.4 , N = 3	2006: 8.7 ± 2.0 , N = 3 2007: 9.4 ± 0.4 , N = 3	2006: 9.9 ± 0.8 , N = 3	0.011	Winter 06 < Fall 06
<i>Z. noltii</i> roots	2006: 6.4 ± 0.2 , N = 3 2007: 7.6 ± 0.3 , N = 3	2006: 7.4 ± 1.5 , N = 3 2007: 6.5 ± 0.7 , N = 3	2006: 6.9 ± 0.4 , N = 3 2007: 10.5 ± 1.8 , N = 3	2006: 11.0 ± 2.4 , N = 3	0.017	=
<i>Z. noltii</i> detrital matter	2006: 6.9 ± 0.1 , N = 3	2006: 6.9 ± 1.1 , N = 3	2006: 7.1 ± 0.3 , N = 3 2007: 8.5 ± 0.7 , N = 3	2006: 7.0 ± 2.1 , N = 3	0.213	=
Epiphytes		2007: 7.8, N = 1	2007: 6.2 ± 0.2 , N = 3			
Microphytobenthos	2010: 7.1 ± 0.3 , N = 4	2007: 6.4 ± 0.3 , N = 5	2007: 6.9 ± 0.1 , N = 4	2007: 7.4 ± 0.5 , N = 4	0.016	Spring 2007 < Fall 2007
SSOM	2007: 5.6 ± 0.4 , N = 3	2006: 5.0 ± 0.1 , N = 3 2007: 6.0 ± 0.1 , N = 3	2006: 6.8 ± 1.3 , N = 3 2007: 5.9 ± 0.2 , N = 2	2006: 6.0 ± 0.2 , N = 3	0.038	=

Table 1 Stable isotope ratios (‰, mean \pm SD, number of samples) of primary producers and composite food sources per season and summary of Kruskal-Wallis tests between sampling seasons. Values from winter and summer 2006 and 2007 are issued from Lebreton et al. (2011)

	Spring 2007	Summer 2007	Fall 2007	Winter 2008	P values	Comparisons of means
$\delta^{13}\text{C}$						
Nematodes	$-13.0 \pm 0.2, N = 6$	$-13.6 \pm 0.6, N = 6$	$-12.2 \pm 0.5, N = 6$	$-12.5 \pm 0.2, N = 6$	0.001	Summer < Fall Summer < Winter
Copepods	$-16.4 \pm 4.0, N = 4$	$-16.4 \pm 1.4, N = 5$	$-14.4 \pm 2.3, N = 3$	$-14.6 \pm 3.2, N = 3$	0.490	=
$\delta^{15}\text{N}$						
Nematodes	$9.8 \pm 0.5, N = 6$	$8.9 \pm 0.3, N = 6$	$9.8 \pm 0.7, N = 6$	$10.7 \pm 0.7, N = 6$	0.001	Summer < Winter
Copepods	$7.4 \pm 0.6, N = 4$	$7.7 \pm 0.6, N = 4$	$8.4 \pm 0.7, N = 2$	$7.7 \pm 0.7, N = 3$	0.507	=

Table 2 Stable isotope ratios (‰, mean \pm SD, number of samples) of nematodes and copepods and summary of Kruskal-Wallis tests at the different seasons from spring 2007 to winter 2008

	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Spring 2007	0.400	0.200
Summer 2007	1.000	0.533
Fall 2007	0.400	0.100
Winter 2008	0.400	0.700

Table 3 Summary of P values of Mann-Whitney-Wilcoxon tests between stable isotope signatures of nematodes ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) from top and bottom communities