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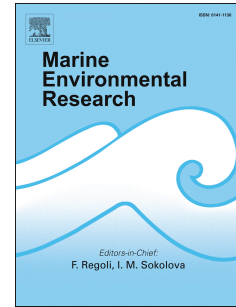
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Multiple effects of a *Gracilaria vermiculophylla* invasion on estuarine mudflat functioning and diversity

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Short title: Effects of *Gracilaria vermiculophylla* on estuaries

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Abstract

The invasive Japanese seaweed *Gracilaria vermiculophylla* has become established over the past several years in numerous European estuaries, from Portugal to Norway. In the Faou estuary (48.295°N-4.179°W, Brittany, France), it forms a dense population at the mud's surface. The effects of *G. vermiculophylla* on metabolism, diversity, and the food web were studied. Community gross primary production (GPP) and respiration (CR) during emersion, chlorophyll-*a* content, macrofaunal and meiofaunal diversity and abundance, and stable

35 isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of representative macrofaunal species and main food sources were
36 measured at low tide in winter, spring, summer 2014, and winter 2015. Results show
37 significant seasonal variation in GPP and CR. Moreover, GPP was significantly higher in
38 areas where *G. vermiculophylla* was present than in the control area (bare mud). However,
39 this high GPP appeared to be linked to the increase in biomass in primary producers, with
40 their efficiency (primary productivity, i.e. assimilation number) remaining relatively stable
41 compared with the control area. Significant variation in abundance of meiofauna and
42 macrofauna was also detected and new epifaunal species were collected, mainly in
43 *Gracilaria*-colonized areas. Isotopic food-web Bayesian mixing models strongly suggested
44 that *G. vermiculophylla* plays a major role in the diet of some dominant species. Mechanisms
45 interacting with the functioning and diversity of the mudflat are discussed. Finally, the
46 invasive seaweed *G. vermiculophylla* affected the mudflat ecosystem in three ways: as a new
47 primary producer (increase in metabolism), as a habitat-forming species (changes in diversity
48 and abundance of macrofauna and meiofauna), and as a new abundant food source, likely
49 through the detrital pathway.

50 Key-words: Invasive – Non-indigenous species – Red alga – Metabolism – Food web –
51 Macrofauna diversity – Meiofauna diversity – Engineer species

52

53

Introduction

54 Among reported marine non-indigenous species (NIS) in Europe, between 20 and 40% are
55 macroalgae (Schaffelke et al. 2006; Stiger-Pouvreau and Thouzeau 2015). Owing that some
56 of them can act as foundation species (Dayton 1972; Ellison et al. 2005) or ecosystem
57 engineers (Jones et al. 1994, 1997), they may deeply alter the structure and functioning of
58 local communities by changing abiotic conditions (Jones et al. 1997), local diversity
59 (Wallentinus and Nyberg 2007 and references therein), and food webs (Hastings et al. 2007).
60 Reports on these changes generally indicate negative effects on indigenous species (Levine et
61 al. 2003), although there may also be some positive effects (Crooks 2002). Studies on
62 macroalgal introductions generally focus on rocky shores and explore potential competition
63 with native seaweeds for space (Schaffelke and Hewitt 2007 and references therein). Less
64 frequently, non-indigenous seaweeds can also colonize biotopes that originally have no
65 significant macroalgal populations. For example, the perennial red seaweed *Gracilaria*
66 *vermiculophylla* (Ohmi) Papenfuss is now reported on the Pacific coast of USA, on the west
67 and east coasts of the North Atlantic (Freshwater et al. 2006; Thomsen et al. 2007, 2009;
68 Krueger-Hadfield et al. 2017) and especially along the French Atlantic coasts (Stiger-
69 Pouvreau and Thouzeau 2015).

70 This species, originating from East Asia, can be found in Europe from Norway to Portugal
71 (Rueness 2005; Hammann et al. 2013 and references therein), as well as along the Atlantic
72 coast of Morocco (Guillemin et al. 2008; Krueger-Hadfield et al. 2017). Its introduction in
73 France likely occurred in the vicinity of oyster farms (Mollet et al. 1998): a recent study on
74 this species using population genetics (Krueger-Hadfield et al. 2017) indicates that the

75 probable main source of the invasion is northeastern Japan, the area from which the majority
76 of *Crassostrea gigas* oysters were imported during the 20th century. In its native range, the
77 species is characterized by a haplo-diplontic life cycle and is attached by holdfasts to hard
78 substrata. Along west and east coasts of the North Atlantic, its populations are dominated by
79 diploid thalli without holdfasts that colonize estuarine mudflats through vegetative
80 fragmentation (Krueger-Hadfield et al. 2016). Similarly, in French mudflats, *G.*
81 *vermiculophylla* occurs without attaching to a (however small) hard substratum. The presence
82 of *G. vermiculophylla* on mudflats on the northeastern coasts of the United States seems to be
83 linked to, or at least facilitated by, the presence of the tube-dwelling worm *Diopatra cuprea*
84 (Thomsen and McGlathery 2005; Wright et al. 2014). Nevertheless, it has been reported in
85 various types of substrata in Europe, including immersed soft sediments and stones, e.g. in the
86 Baltic Sea (Weinberger et al. 2008), where it may constitute a competitor of the native brown
87 alga *Fucus vesiculosus*.

88 Invasive *G. vermiculophylla* may be less palatable than *G. vermiculophylla* in its native range
89 both for grazers from its native range and from its invaded range (Hamman et al. (2013)).
90 Invasive populations of *G. vermiculophylla* defend themselves better against bacterial
91 epibionts isolated from their respective introduced ranges than from the ones from their native
92 range, suggesting a rapid adaptation of its chemical defense mechanism to new bacterial
93 epibionts in the invaded range (Saha et al. 2016).

94 For more than 10 years, *G. vermiculophylla* has been considered as an occasional species on
95 French coasts, without any effect on ecosystems (Martinez-Lüscher and Holmer 2010).
96 Currently, it is now broadly distributed in estuarine ecosystems where it visibly constitutes a
97 habitat-forming species at the surface of the mud. *G. vermiculophylla* can be now considered
98 as invasive because it tends to occupy a large part of the mudflats it has colonized. It also
99 represents a new benthic primary producer on the mudflat, the microphytobenthos being the
100 only primary producers until this invasion.

101 The expansion of the species has been monitored in three estuaries in the Bay of Brest since
102 2013 (Surget et al. 2017). Among them, the Faou estuary appears to be the most heavily
103 colonized field site (Surget et al. 2017). Therefore, this estuary was chosen for a study on the
104 impact of *G. vermiculophylla* that was carried out from February 2014 to January 2015. The
105 aim was to determine whether this alga present in mudflats previously colonized only by
106 microphytobenthos (1) is significantly modifying mudflat metabolism (primary production
107 and respiration) as a new primary producer, (2) is significantly modifying the diversity and
108 abundance of the benthic community (macrofauna and meiofauna) as a habitat-forming
109 species, and (3) is significantly modifying the macrobenthic food web by providing a new
110 food source.

111

112

Materials and Methods

113 All sampling and measurements were performed simultaneously during low tide, about 4.10
 114 m above chart datum, in February, May, September 2014, and January 2015 in the Faou
 115 estuary (48.295°N-4.179°W, Brittany, France) (Figure 1).

116 **Ecosystem metabolism**

117 Ecosystem metabolism was measured during low tide with three 0.071 m² benthic chambers
 118 to estimate CO₂ fluxes at the air-sediment interface using the method described in Migné et al.
 119 (2002). Sediment (including *G. vermiculophylla* when present) was enclosed down to 10 cm
 120 depth. Changes in air CO₂ concentration (ppm) in the benthic chamber (10 L) were measured
 121 with an infrared gas analyzer (LiCor Li-820) for 10-15 min. CO₂ concentrations were
 122 recorded in a data logger (LiCor Li-1400) at a 5 s frequency. CO₂ flux was calculated as the
 123 slope of the linear regression of CO₂ concentration (μmol mol⁻¹) against time (min) and
 124 expressed in mg C m⁻² h⁻¹ assuming a molar volume of 22.4 L at standard temperature and
 125 pressure. Transparent chambers were used to estimate the net benthic community production
 126 (NCP), the difference between community gross primary production (GPP) and community
 127 respiration (CR). Opaque chambers were used to estimate CR. During light incubations,
 128 incident photosynthetically available radiation (PAR, 400-700 nm) was monitored with a
 129 LiCor SA-190 quantum sensor. On each sampling date, stratified sampling was performed,
 130 with three replicates on the *Gracilaria*-colonized area (% cover *G. vermiculophylla* > 50%)
 131 and three replicates on the bare-mud area, considered as the control. Benthic chambers were
 132 deployed within a few meters of each other to limit any spatial variation.

133 Considering the low number of replicates and the absence of homoscedasticity even after
 134 metric transformation, we used the non-parametric Scheirer-Ray-Hare test (Sokal and Rohlf
 135 1995), the non-parametric equivalent of a two-way ANOVA, on sampling date (n = 4) and
 136 colonization status (area) (bare-mud and *G. vermiculophylla*-colonized areas, n = 2).

137 **Chlorophyll a**

138 Four replicates of 1.96 cm² and 1 cm depth (including *G. vermiculophylla* when present) were
 139 sampled within each benthic chamber at each sampling date during low tide. Samples were
 140 kept cool, in the dark, and brought back to the laboratory where they were stored at -24°C
 141 until analysis. Fresh samples were ground 30 sec in pure acetone (5 mL), placed in the dark at
 142 4°C for at least 4 h, and centrifuged (4°C, 3500 rpm, Eppendorf Centrifuge 5810R) to extract
 143 chlorophyll a. Chlorophyll a contents (Chl a) were determined on homogenized supernatants
 144 using spectrophotometry according to the trichromatic method described in Jeffrey and
 145 Humphrey (1975). In microplates (UVStar F-Bottom, Greiner Bio-one), optical density (OD)
 146 of 200 μL samples was read at 630, 647, 664, and 750 nm with a POLARstar Omega
 147 spectrophotometer (BMG Labtech). Chlorophyll a contents were calculated using the
 148 following equation and expressed in mg.m⁻²:

$$149 \text{ Chl a (mg.m}^{-2}\text{)} = 50 \times [11.85 \times (\text{OD}_{664} - \text{OD}_{750}) - 1.54 \times (\text{OD}_{647} - \text{OD}_{750}) - 0.08 \times (\text{OD}_{630} -$$

$$150 \text{ OD}_{750})] / 1.96$$

151 Data were also analyzed using the Scheirer-Ray-Hare test.

152 **Macro- and meiobenthos**

153 On each occasion, three 0.1 m² quadrats were sampled on the *Gracilaria*-colonized area and
154 three other quadrats on the bare-mud area for macrofauna identification. Samples were
155 isolated in the field on sieves of 1 mm mesh size, and stored in 4% salted and buffered
156 formalin in the lab. Individuals were identified at the species level and counted.

157 Three replicates (1.77 cm², 2 cm deep) were also collected on the *Gracilaria*-colonized area
158 along with three other replicates from the bare-mud area, and sieved with a mesh size of 40
159 µm. Individuals of the meiofauna were identified, belonging to the 10 following taxonomic
160 categories: nematodes, platyhelminths, interstitial polychaetes, oligochaetes, harpacticoid
161 copepods, ostracods, halacarid mites, foraminifers, gastropods, and bivalves.

162 Given the low number of replicates and the absence of homoscedasticity even after metric
163 transformation, we used the non-parametric Scheirer-Ray-Hare test.

164

165 **Food web**

166 Sample collection and preparation. Invertebrates and the main potential organic matter
167 sources from the sampling area were collected on the four sampling dates. Suspended
168 particulate organic matter (POM) from the site was sampled by collecting 2 L of seawater.
169 POM was obtained by filtration on pre-combusted Whatman GF/F glass fiber membranes
170 within 2 h after collection. Membranes were then acidified (10% HCl) to remove carbonates,
171 briefly rinsed with Milli-Q water, dried (60°C), and kept at -32°C until analysis. Sediment
172 samples were taken by scraping the upper 1 cm of the sediment. For the measurements of
173 carbon isotope ratios in sedimented organic matter (SOM), SOM was acidified (10% HCl)
174 rinsed several times with distilled water, dried (60°C), and ground to a powder (Riera 2010).
175 δ¹³C measurements were conducted on acidified samples, and measurements of nitrogen
176 isotope ratios were conducted on non-acidified samples. Samples of terrestrial organic matter,
177 dead leaves of terrestrial plants abundant on the muddy sediment, were collected by hand and,
178 back at the laboratory, were rinsed with filtered seawater (pre-combusted GF/F) to clean off
179 epibionts, quickly acidified (10% HCl) to remove any possible residual carbonates, and rinsed
180 with distilled water. The macroalga *G. vermiculophylla* was also collected by hand and
181 washed with distilled water to remove any attached material. In the sampling area, benthic
182 diatoms occurred only as algal mats in May and September and were extracted for stable
183 isotope analyses on these two dates. The absence of dense mats of benthic microalgae at the
184 sediment surface in January 2014 and February 2015 may be due to local strong
185 hydrodynamic conditions and frequent stormy weather during winter. When present, benthic
186 diatom mats were collected at low tide by scraping the surface of the sediment and extracting
187 them using the method in Riera (2007) for intertidal muddy sediment. Diatoms were then
188 collected on pre-combusted glass fiber filters, quickly acidified (10% HCl), and rinsed with
189 distilled water. These samples were then dried (60°C) and kept at -32°C until analysis.

190 Individuals of the surface deposit-/suspension-feeder bivalve *Scrobicularia plana*, the grazing
191 gastropod *Hydrobia ulvae*, and the dominant generalist/suspension-feeder polychaete *Nereis*
192 *diversicolor*, which were among the most representative macrobenthic species of the study
193 area, were collected within the macrobenthic replicates (see above). After collection,
194 specimens were kept alive overnight at the laboratory in filtered water from the sampling site
195 to allow evacuation of gut contents and then frozen. For mollusks, the flesh was dissected
196 from the shell and quickly treated with 10% HCl to remove any carbonate debris, and then
197 rinsed with distilled water. All individuals were then freeze-dried and ground to a powder
198 using mortar and pestle. *N. diversicolor* specimens were treated in the same way with 10%
199 HCl. Collected *H. ulvae* individuals being very small, composite samples (4-5 individuals)
200 were pooled to obtain sufficient tissue for accurate stable isotope analyses. Finally, all
201 samples were dried (60°C) and kept frozen (-32°C) until analysis. Samples, crushed with a
202 mortar and a pestle, were then put in tin capsules before mass-spectrometry analyses.

203 Stable isotope measurements. Carbon and nitrogen isotope ratios were determined using a
204 Flash EA CN analyzer coupled with a Finnigan Delta Plus mass spectrometer, via a Finnigan
205 Con-Flo III interface. Data are expressed in the standard δ unit:

$$206 \quad \delta X (\text{‰}) = [(R_{\text{sample}} / R_{\text{reference}}) - 1] \times 10^3$$

207 with $R = {}^{13}\text{C}/{}^{12}\text{C}$ for carbon and ${}^{15}\text{N}/{}^{14}\text{N}$ for nitrogen. These abundances were calculated
208 relative to the certified reference materials Vienna Pee Dee Belemnite-limestone (V-PDB)
209 and atmospheric dinitrogen (at-air). The VPDB and at-air scaling was carried out using in-
210 house protein standards that have been calibrated against NBS-19 and IAEA N3 reference
211 materials. The standard deviation of repeated measurements of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the
212 laboratory standard was 0.10‰ versus V-PDB and 0.05‰ versus at-air, respectively.

213 Data analysis. The number of individuals analyzed for each species was not sufficient to
214 satisfy the conditions of normality; therefore, non-parametric Kruskal-Wallis tests were
215 carried out.

216 For the food-web study, the isotopic values of consumers were pooled because (1) the
217 consumers considered showed similar isotopic values in sediments with or without apparent
218 macroalgae (see Figure 5) and (2) macroalgae were considered as detritus equally available to
219 consumers within the total sampling area through the important physical reworking and the
220 bioturbation of sediments. The trophic links were then considered for the whole area.

221 The Bayesian mixing model SIAR (Stable Isotope Analysis in R; Parnell et al. 2010) was run
222 to infer the feasible contribution of the potential sources to the consumers' diets at each
223 sampling date. Bayesian statistics have proven to be a powerful tool because they allow
224 models to incorporate the variability in the stable isotope ratios of both sources and
225 consumers, as well as in the isotopic fractionation (Parnell et al. 2010). Trophic enrichment
226 factors ranged from $0.30 \pm 0.21\text{‰}$ for $\delta^{13}\text{C}$ and $2.5 \pm 0.25\text{‰}$ for $\delta^{15}\text{N}$, corresponding to values
227 for invertebrate species (whole body) as reviewed by Caut et al. (2009).

228

229

Results

230 Ecosystem metabolism (Figure 2)

231 Gross primary production (GPP) showed strong seasonal variation (Scheirer-Ray-Hare test: $H = 17.447$, $df = 3$, $p < 0.001$) and significant differences between *Gracilaria*-colonized and
232 bare-mud areas ($H = 3.853$, $df = 1$, $p < 0.05$), with GPP values for *Gracilaria*-colonized areas
233 being more than twice those of bare-mud areas in spring (Figure 2A), but without interaction.
234

235 The same seasonal trend occurred for community respiration (CR) ($H = 18.407$, $df = 3$, $p <$
236 0.001) but no significant differences appeared between *Gracilaria*-colonized and bare-mud
237 areas over the study year ($H = 3.203$, $df = 1$, $p > 0.05$) despite the higher CR values recorded
238 on *Gracilaria*-colonized areas than on bare mud in spring and summer (Figure 2B).

239 Consequently, NPP also exhibited strong seasonal variation ($H = 17.767$, $df = 3$, $p < 0.001$)
240 but there was no difference between the two areas ($H = 2.430$, $df = 1$, $p > 0.05$) across the
241 year (Figure 2C).

242 Chlorophyll *a* content was much higher on areas colonized by *Gracilaria* than in bare-mud
243 areas ($H = 12.813$, $df = 1$, $p < 0.001$), but no seasonal trend occurred ($H = 1.873$, $df = 3$, $p >$
244 0.05) and chlorophyll *a* content remained high in winter 2015 on *Gracilaria*-colonized areas
245 (Figure 2D).

246 Considering primary productivity (i.e. photosynthetic efficiency, also called assimilation
247 number) as the ratio between GPP and chlorophyll *a* and expressed in $\text{mg C mg Chl.a}^{-1} \text{h}^{-1}$, a
248 strong seasonal trend was again highlighted ($H = 17.820$, $df = 3$, $p < 0.001$), with no
249 significant differences ($H = 0.003$, $df = 1$, $p > 0.05$) between the two areas, which showed
250 very similar values each season (Figure 2E).

251 Macro- and meiobenthic diversity and abundances (Figures 3 & 4)

252 A total of 5533 macrofaunal individuals belonging to 21 taxa were collected and identified
253 during the study. The most abundant taxa were the polychaete *N. diversicolor* (40.6% of
254 sampled individuals), the gastropod *H. ulvae* (19.7%), the isopod crustacean *Anthurus gracilis*
255 (11.9%), oligochaetes (9.9%), the polychaete *Streblospio shrubsolii* (8.2%), and the bivalve
256 *Scrobicularia plana* (2.8%), with *S. Shrubsolii* only appearing on the last sampling date and
257 mainly in the area colonized by *Gracilaria*.

258 The number of sampled taxa varied significantly among sampling dates ($H = 11.222$, $df = 3$, p
259 < 0.05) but also between the two areas ($H = 5.026$, $df = 1$, $p < 0.05$), with a strong increase in
260 colonized areas (Figure 3A). This increase can be explained by the appearance of species
261 uncommon on mudflats such as epifaunal amphipods (*Jassa marmorata*, *Melita palmata*,
262 *Allomelita pellucida*), decapods (*Pilumnus hirtellus*, *Liocarcinus pusillus*) and polychaetes
263 (tube-dwelling Sabellidae and *S. shrubsolii*), mainly found in colonized areas.

264 Density of macrofauna (Figure 3B) did not exhibit any seasonal trends ($H = 5.343$, $df = 3$, $p >$
265 0.05) but remained higher in colonized areas ($H = 14.313$, $df = 1$, $p < 0.001$). Among the

266 abundant taxa, *N. diversicolor* (Figure 3C) showed higher densities in colonized areas
 267 regardless of the season ($H = 14.976$, $df = 1$, $p < 0.001$), as did *A. gracilis* (Figure 3D) ($H =$
 268 17.333 , $df = 1$, $p < 0.001$) and oligochaetes ($H = 8.316$, $df = 1$, $p < 0.01$) (Figure 3E).

269 For meiofauna, a total of 12,020 individuals belonging to 10 taxa were collected during this
 270 study. Main taxa were nematodes (83.6% of sampled individuals), harpacticoid copepods
 271 (4.9%), platyhelminths (3.5%), ostracods (2.5%), and interstitial polychaetes (2.1%).
 272 Densities differed significantly (Figure 4A) among dates ($H = 10.800$, $df = 3$, $p < 0.05$) and
 273 between area types ($H = 7.397$, $df = 1$, $p < 0.01$). Among the dominant taxa, the abundance of
 274 nematodes (Figure 4B) varied significantly across seasons ($H = 11.687$, $df = 3$, $p < 0.01$) and
 275 areas ($H = 6.163$, $df = 1$, $p < 0.05$), as did one of the ostracod species (Figure 4C; season: $H =$
 276 12.718 , $df = 3$, $p < 0.01$; area: $H = 5.572$, $df = 1$, $p < 0.05$). The abundance of harpacticoid
 277 copepods (Figure 4D) only varied significantly across seasons ($H = 13.058$, $df = 3$, $p < 0.01$),
 278 and interstitial polychaetes (Figure 4E) were significantly more abundant in *Gracilaria*-
 279 colonized areas ($H = 11.519$, $df = 1$, $p < 0.001$).

280 Food web (Figures 5 & 6 and supplementary material)

281 The $\delta^{13}\text{C}$ of the potential food sources varied widely (from -30.7‰ for terrestrial organic
 282 matter to -17.6‰ for benthic diatoms) due to the presence of ^{13}C -enriched sources (*G.*
 283 *vermiculophylla* and benthic diatoms) compared with more ^{13}C -depleted SOM, POM and
 284 terrestrial inputs. *G. vermiculophylla* was slightly less ^{13}C -depleted and showed $\delta^{15}\text{N}$ values
 285 similar to mean values previously reported in brackish lagoons of Japan (-21.7 and 9.6‰ for
 286 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively) (Kanaya et al. 2008). The range of $\delta^{15}\text{N}$ displayed by the food
 287 sources decreased slightly during the study year ranging from 9.6‰ in February 2014 to 6‰
 288 in January 2015. Except for $\delta^{13}\text{C}$ of benthic diatoms and $\delta^{15}\text{N}$ of terrestrial organic matter,
 289 there were no isotopic differences among sampling dates for the different food sources,
 290 whereas consumer isotopic signatures displayed significant temporal variation for $\delta^{13}\text{C}$ and
 291 $\delta^{15}\text{N}$ (Table 1). At each sampling date, significant differences were observed among the
 292 consumer species for $\delta^{13}\text{C}$ (Kruskal-Wallis, $p = 0.0023$, 0.0025 , 0.017 , and 0.027 in February,
 293 May, September, and January, respectively) and for $\delta^{15}\text{N}$ (Kruskal-Wallis, $p = 0.043$, < 0.001 ,
 294 < 0.001 and < 0.001 in February, May, September, and January, respectively). The $\delta^{15}\text{N}$ for
 295 *G. vermiculophylla* (between 9.0 and 10.9‰) and the primary consumers considered in this
 296 study (between 10.1 and 14.3‰) were relatively high, which may be due to a highly nitrogen-
 297 polluted environment.

298 For SIAR calculations, the main potential food sources were considered at each sampling
 299 date, namely *G. vermiculophylla*, POM, SOM, and terrestrial organic matter carried by the
 300 river to the estuary, and benthic diatoms. However, when the number of sources is greater
 301 than five, the solution of Bayesian mixing-models becomes less robust (Parnell et al. 2010). In
 302 this study, macroalgae, and, to a lesser extent, benthic diatoms contributed to most of the diet
 303 for the three main consumer species considered. During the total sampling period, results of
 304 the SIAR models indicated that *G. vermiculophylla* was the main assimilated food source for
 305 *S. plana* (mean relative contributions from 67.5 to 83.2%), *H. ulvae* (34.8 to 61.4%), and *N.*
 306 *diversicolor* (55.0 to 92.7%). During spring, and to a lesser extent in summer, benthic diatoms

307 also contributed significantly to consumers' diet with the highest median relative
308 contributions for *N. diversicolor* (35% in May) followed by *S. plana* (23.9% in May) and *H.*
309 *ulvae* (25.3% in May). Local POM and SOM and terrestrial inputs were much less important
310 in consumers' diet across the entire sampling year (Figure 6).

311

312

Discussion

313 The seasonal variation in GPP and CR measured in the bare mudflat was characteristic of
314 these systems (Spilmont et al. 2006; Davoult et al. 2009; Migné et al. 2016), but the rates in
315 the area colonized by *G. vermiculophylla* were intermediate to values typical of mudflats and
316 those typical of macroalgae-dominated rocky shores (Golléty et al. 2008; Bordeyne et al.
317 2015). GPP appeared greatly increased in the presence of the red alga *G. vermiculophylla*,
318 with important consequences on ecosystem functioning. However, this increase tended to vary
319 with chlorophyll *a* content, resulting in the same productivity for both areas. Therefore,
320 although *G. vermiculophylla* added biomass to the benthic system, primary productivity
321 hovered around 0.8-1 mg C mg Chl.a⁻¹ h⁻¹ in spring and summer, and dropped in winter, with
322 values typical of mudflats and macroalgae (Davoult et al. 2009). Similarly, a study of a *G.*
323 *vermiculophylla* invasion of a *Zostera noltei* bed showed a decrease in photosynthetic
324 efficiency at low light intensity but higher metabolic performance due to increasing *G.*
325 *vermiculophylla* biomass (Cacabelos et al. 2012). Finally, despite the invasion of a new
326 primary producer, the productivity of the mudflat remained stable, at least under saturating
327 light and during emersion.

328 Nevertheless, our results confirm that this seaweed is well adapted to muddy conditions and
329 remains in good physiological condition even in this extreme environment (Nyberg 2007;
330 Thomsen et al. 2013). Measurements of primary production were not performed during
331 immersion, but water turbidity is likely too high during high tide to allow photosynthesis, as
332 already suggested for bays and estuarine mudflats along the English Channel (Spilmont et al.
333 2006; Migné et al. 2009) and the French Atlantic coast (Migné et al. 2016).

334 Typical macrofaunal species were collected both on bare mud and *Gracilaria*-colonized areas.
335 Compared with bare mud, macrobenthic abundance and diversity were positively impacted in
336 the *Gracilaria*-colonized area, corroborating previous studies (e.g. Thomsen et al. 2013).
337 Total density significantly increased in *Gracilaria*-colonized areas due to the rise in density of
338 some common species such as *Nereis diversicolor* and the high abundances of species that
339 were absent or rare in bare mud, such as *Streblospio shrubsolii*. Moreover, species newly
340 recorded in the studied area (e.g. the amphipods *Jassa marmorata*, *Melita palmata* and
341 *Allomelita pellucida*, and the decapods *Pilumnus hirtellus* and *Liocarcinus pusillus*) are
342 generally absent from bare sediments and usually found among seaweeds and hydrozoans on
343 hard substrata or on eelgrass (Hayward and Ryland 1995). These species were found at low
344 densities in *Gracilaria*-colonized areas, likely favored by the complexity of the habitat driven
345 by the settlement of this engineer seaweed (Thomsen et al. 2013). These new epifaunal
346 species led to a significant increase in the number of sampled taxa and therefore in the

347 diversity of the community, due to new available habitat (Wallentinus and Nyberg 2007).
348 Non-native ecosystem engineers, such as the reef-building tubeworm *Ficopomatus*
349 *enigmaticus*, may also favor the occurrence of opportunistic species (Heiman and Micheli
350 2010).

351 The density of some meiofauna taxa was also higher in the *Gracilaria*-colonized area,
352 particularly the dominant group of nematodes, but also ostracods and interstitial polychaetes.
353 Similar to the macrofaunal polychaete *N. diversicolor*, these taxa belong to endofauna and
354 their density is not expected to be positively influenced by the occurrence of a new habitat-
355 forming seaweed, even some *G. vermiculophylla* are partially buried in the mud.

356 This increase in endofaunal density may be related to a change in food availability, possibly
357 due to the effective use of *G. vermiculophylla* in the diet of these species. Accordingly, for
358 three common species of the community — the bivalve *Scrobicularia plana*, considered as a
359 surface deposit-feeder and a suspension-feeder (Hughes 1969), the gastropod *Hydrobia ulvae*,
360 considered as a grazer of benthic diatoms (Hautbois et al. 2005), and the polychaete *Nereis*
361 *diversicolor*, considered as opportunistic, exhibiting behavior ranging from suspension-
362 feeding to predation and generalist scavenging (Fidalgo e Costa et al. 2006) —, our estimates
363 based on stable isotopes showed that *G. vermiculophylla* could represent their main food
364 source, even if other algae such as *Ulva* sp. could also be sparingly consumed and have not be
365 sampled in the vicinity of our studied site. Nevertheless, benthic diatoms also contributed
366 substantially to their diets in spring and summer, conversely to local POM, SOM, and
367 terrestrial inputs.

368 To our knowledge, there is no previous evidence for the predominance of this invasive
369 macroalga in the diet of suspension- and/or deposit-feeder species. In its native brackish
370 lagoons of Japan, *G. vermiculophylla* does not significantly contribute to the local food web
371 (Kanaya et al. 2008). Moreover, previous feeding experiments and field isotope data show
372 that although the invasive *G. vermiculophylla* can be consumed by the amphipod *Gammarus*
373 *locusta*, this alga is clearly not targeted in its diet compared with other local food sources
374 (Wright et al. 2014). The predominance of *G. vermiculophylla* as a food source appears
375 paradoxical in light of its chemical defense activity that protects it from strong grazing
376 activity (Nylund et al. 2011). However, previous studies have pointed out that macroalgae
377 enter the coastal food web mostly through detrital pathways (Pomeroy 1980; Raffaelli and
378 Hawkins 1996), losing their repellent properties upon degradation. In particular, a fraction of
379 the detrital macroalgal pool degrades *in situ*, becoming available to nearshore pelagic and
380 benthic filter-feeders through resuspension processes (Bustamante and Branch 1996). In
381 addition, when abundantly present, macroalgae also become readily available for the
382 organisms inhabiting sediments due to the lack of lignin content in their tissues and their rapid
383 physical fractionation due to the effect of wave action and sediment motion (Rossi 2007).

384 Interestingly, our results indicate that benthic diatoms were not preferentially used by the
385 benthic consumers, even when they were present at the sediment surface as brown mats. In
386 contrast, several previous studies have highlighted the role of benthic diatoms as a major food
387 source for marine invertebrates inhabiting intertidal sediments (De Jonge and Van Beusekom

388 1992; Decottignies et al. 2007). The significant, but non-dominant, contribution of
389 microphytobenthos (diatoms) to benthic food sources observed in our study may be caused by
390 the irregular presence and abundance of this resource in these estuarine sediments, mainly due
391 to hydrodynamic variability and/or low production, at least in winter. Previous stable isotope
392 analyses have shown that in other types of ecosystems, decaying macroalgae are major
393 contributors to *H. ulvae* and meiofaunal diets in intertidal muddy/sandy sediments compared
394 with benthic diatoms (Riera and Hubas 2003; Riera 2010).

395 Finally, these three macrofaunal species were likely representative of the macrofauna in this
396 estuarine site and, considering their intermediate trophic position of primary consumer
397 species, the food web of this area appears to be largely based on the use of this invasive
398 seaweed which probably generates the main source of detritus in this estuarine environment.

399 Three major points can be highlighted from these results. First of all, there were no
400 differences in consumers' isotopic signatures between bare-mud areas and seaweed-colonized
401 areas. When sampling bare muds, we actually collected some macro-detritus of *G.*
402 *vermiculophylla* which is easily fragmented in the environment, and abundant micro-debris
403 certainly also present, which may thus explain the homogeneous signatures found in the two
404 close sub-systems. The species' diet was therefore similar in the bare-mud and seaweed-
405 colonized areas due to this mixing. The second interesting point was that densities were
406 higher in colonized areas, likely due to higher food availability. Access to more food may
407 directly favor deposit-feeders and grazers that can ingest both microphytobenthos and
408 seaweed micro-debris. In addition, this can indirectly benefit suspension-feeders through
409 resuspension of microphybenthos, when present, and seaweed micro-debris. Finally, this
410 study highlighted that a NIS species can profoundly influence and even potentially change the
411 trophic structure of the macrobenthic community, although NIS are generally not readily
412 consumed by native species (Hammann et al. 2013). This lack of palatability may partly
413 explain NIS success in colonizing new ecosystems (Wright et al. 2014 and references herein).
414 As Pintor and Byers (2015) underlined: "although non-native prey may have a lower per
415 capita value than native prey, they seem to benefit native predators by serving a supplemental
416 prey resource", that could apply here.

417 In a nutshell, the settlement of the NIS *G. vermiculophylla* affected the mudflat ecosystem of
418 the Faou estuary (1) as a new primary producer increasing the local benthic primary
419 production, (2) as a habitat-forming species changing the size and shape of the habitat
420 vertically, thereby favoring the occurrence of epifaunal species, and (3) as a new and
421 abundant food source, confirming its high potential to transform estuaries (Byers et al. 2012).

422 Finally, observations suggest that our study took place during the expansion of the invasive
423 seaweed in this estuary (G. Surget, pers. obs.). Monitoring should be planned with the goal to
424 follow this expansion and measure the consequences on the associated community and
425 potential increase in sedimentation.

426

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572

573 **Figures**

574 **Figure 1.** Location of the study site in the Faou estuary where *Gracilaria vermiculophylla*
575 occupies more than 50% of the mudflat surface.

576

577 **Figure 2.** Metabolic characteristics of the bare-mud area (Control) and the area colonized by
578 *Gracilaria vermiculophylla* (Gracilaria).

579

580 **Figure 3.** Macrofauna. Number of taxa, total density, density of *Nereis diversicolor*, *Anthura*
581 *gracilis*, and oligochaetes in the bare-mud area (Control) and the area colonized by *Gracilaria*
582 *vermiculophylla* (Gracilaria).

583

584 **Figure 4.** Meiofauna. Total density, density of nematodes, ostracods, harpacticoids and
585 interstitial polychaetes in the bare-mud area (Control) and the area colonized by *Gracilaria*
586 *vermiculophylla* (Gracilaria).

587

588 **Figure 5.** $\delta^{13}\text{C}$ vs $\delta^{15}\text{N}$ (mean \pm standard deviation) of food sources (*Gracilaria*
589 *vermiculophylla*, SOM = sedimented organic matter, POM = particulate organic matter, Ter-
590 plant = terrestrial plants, and benthic diatoms) and consumers during February, May and
591 September 2014 and January 2015.

592

593 **Figure 6.** Boxplots of the contributions of potential food sources to the diets of *Scrobicularia*
594 *plana*, *Hydrobia ulvae* and *Nereis diversicolor*, obtained with Bayesian stable isotope mixing
595 models at the four sampling dates. Credibility intervals are 95% (in light gray), 75% (in
596 medium gray) and 50% (in dark gray). POM = particulate organic matter, SOM = sedimented
597 organic matter, Ter-plant = terrestrial plants.

598

599 **Supplementary material.** Carbon and nitrogen isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively)
600 (mean values \pm SD) of organic matter sources and consumers. Four-date comparisons were
601 performed using the Kruskal-Wallis test and two-date comparisons (benthic diatoms) the
602 Mann-Whitney U test.



Figure 1. Location of the studied site in Le Faou estuary. *Gracilaria vermiculophylla* occupying more than 50% of the mudflat.

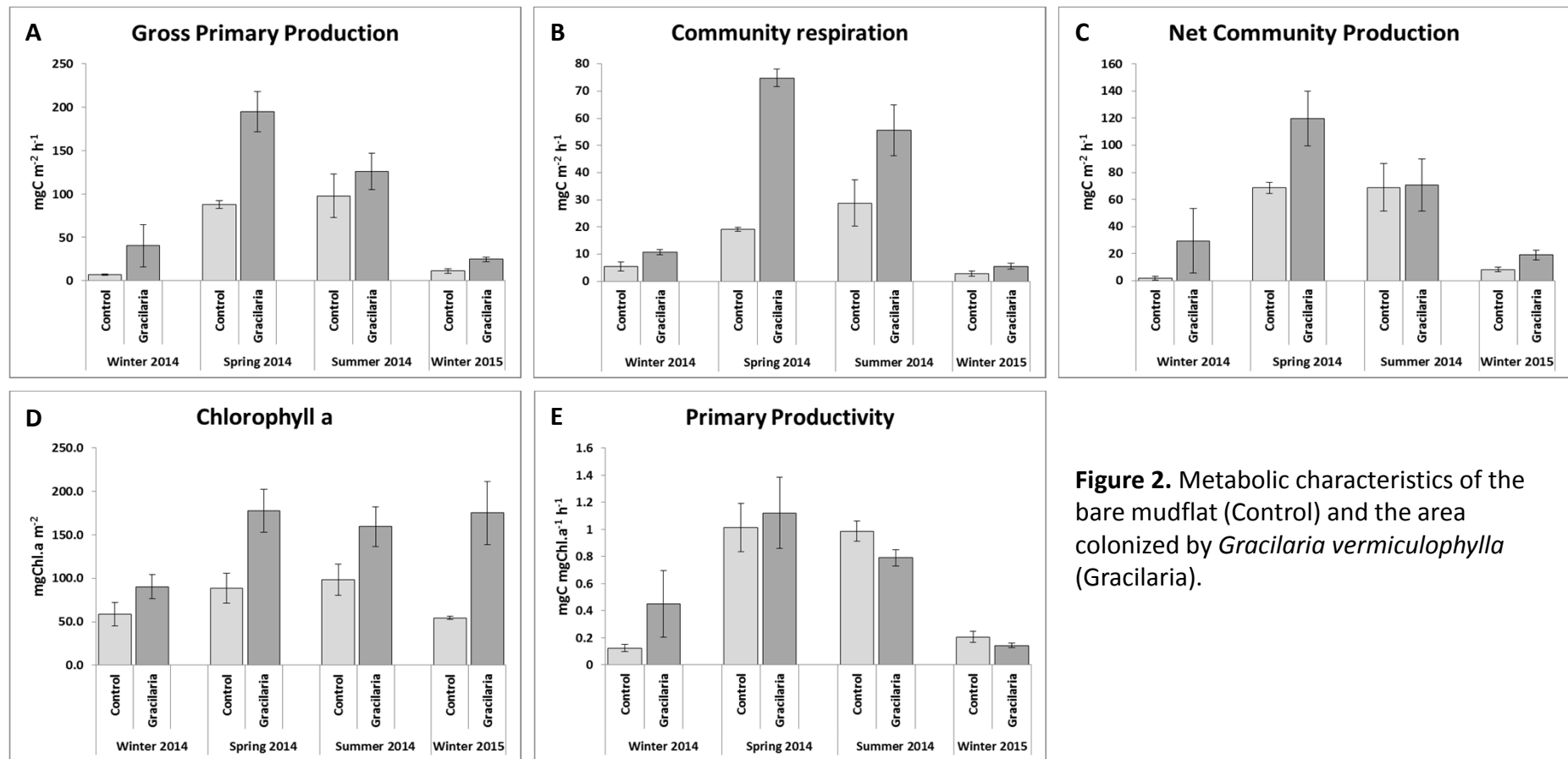


Figure 2. Metabolic characteristics of the bare mudflat (Control) and the area colonized by *Gracilaria vermiculophylla* (Gracilaria).

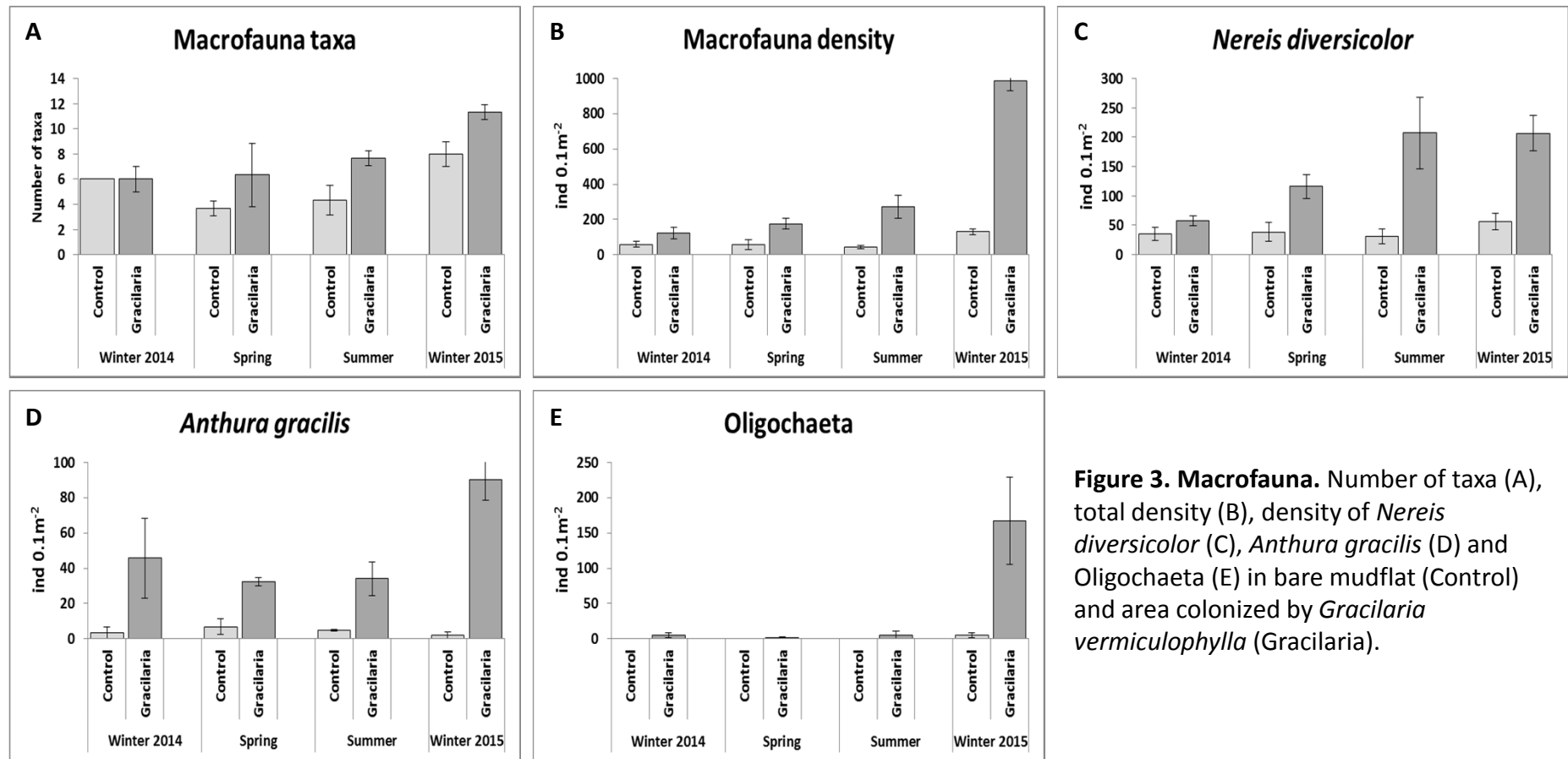


Figure 3. Macrofauna. Number of taxa (A), total density (B), density of *Nereis diversicolor* (C), *Anthura gracilis* (D) and Oligochaeta (E) in bare mudflat (Control) and area colonized by *Gracilaria vermiculophylla* (Gracilaria).

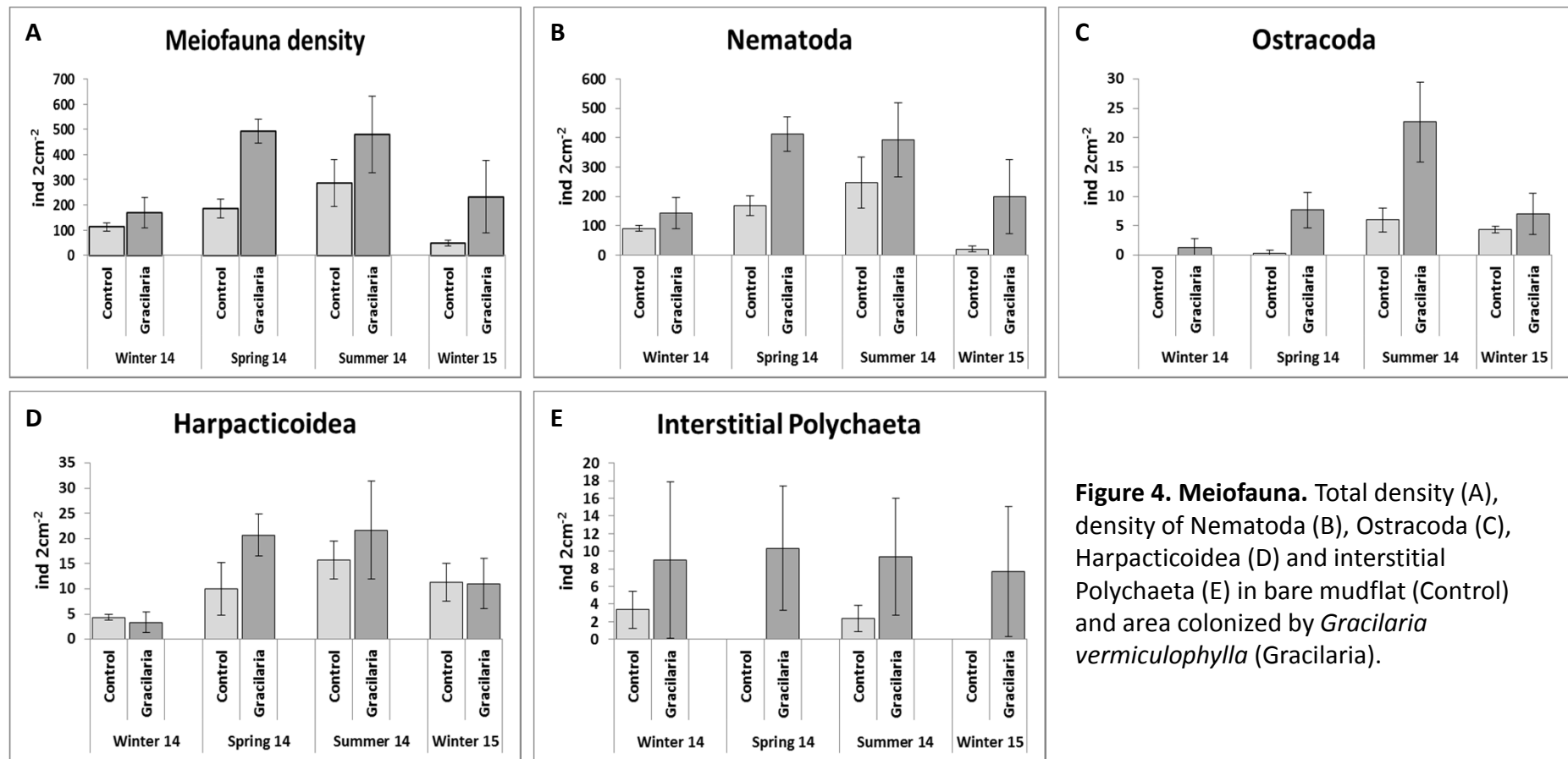


Figure 4. Meiofauna. Total density (A), density of Nematoda (B), Ostracoda (C), Harpacticoida (D) and interstitial Polychaeta (E) in bare mudflat (Control) and area colonized by *Gracilaria vermiculophylla* (*Gracilaria*).

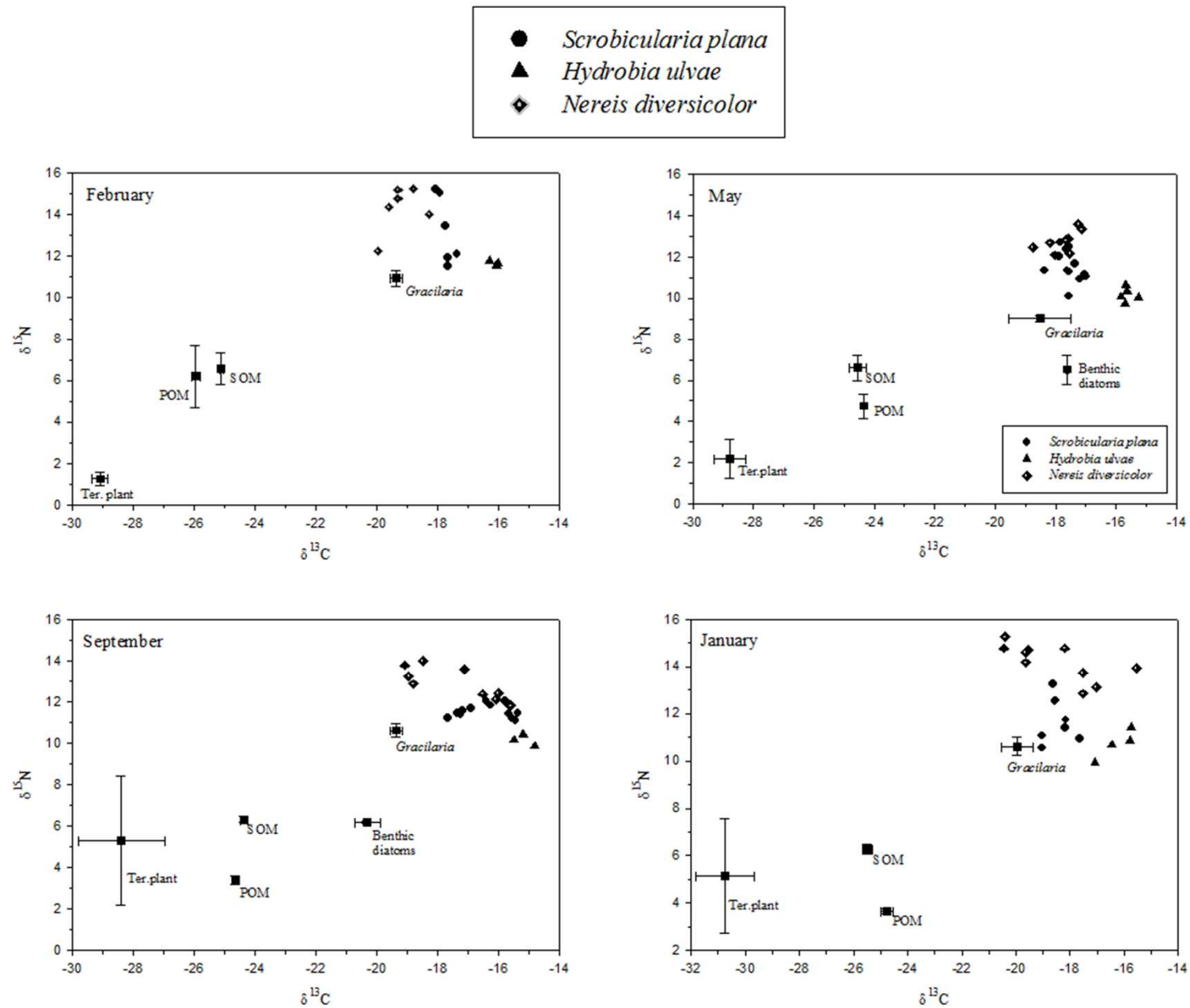


Figure 5. $\delta^{13}\text{C}$ vs $\delta^{15}\text{N}$ (mean \pm standard deviation) of food sources (*Gracilaria vermiculophylla*, SOM = Sedimented Organic Matter, POM = Particulate Organic Matter, Ter-plant = Terrestrial Plants, and benthic diatoms) and consumers during February, May and September 2014, and January 2015.

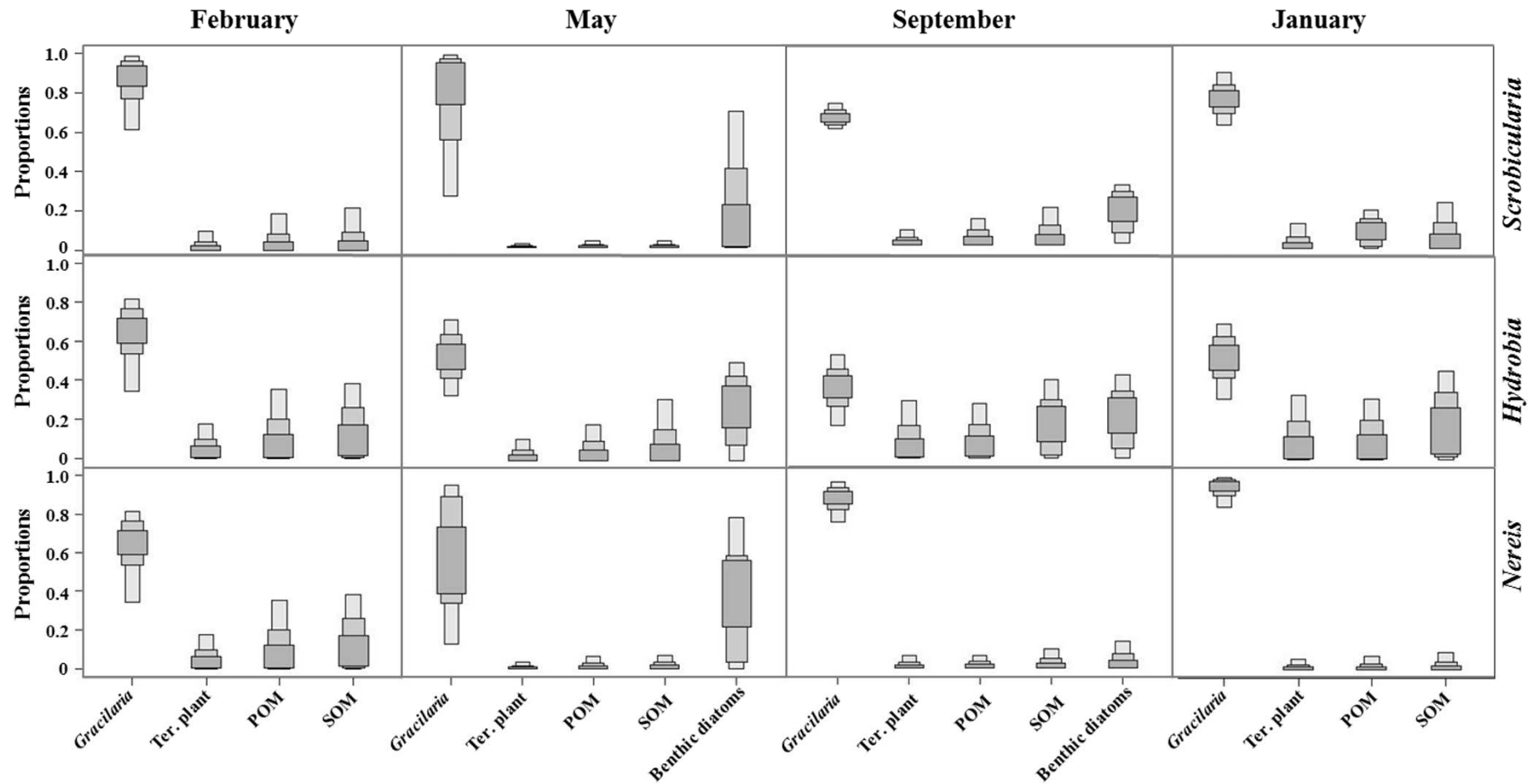


Figure 6. Boxplots of the contributions of potential food sources to the diet of the three species *Scrobicularia plana*, *Hydrobia ulvae* and *Nereis diversicolor*, obtained with Bayesian stable isotope mixing models at four sampling periods. Credibility intervals are 95% (in light grey), 75% (in medium grey) and 50% (in dark grey). POM = Particular Organic Matter, SOM = Sedimented Particular Organic Matter, Ter-plant = Terrestrial Plants.

Highlights – Davoult et al.

A holistic approach is proposed to understand the effects of a *Gracilaria vermiculophylla* invasion on estuarine mudflat

The species affected the ecosystem as a primary producer, as a habitat-forming species and as a new abundant food source

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