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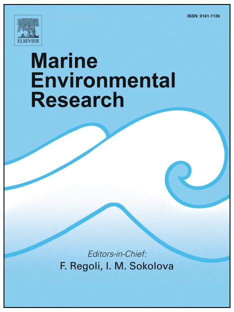

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Multiple effects of a *Gracilaria vermiculophylla* invasion on estuarine mudflat functioning and diversity

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

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

Introduction

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

This species, originating from East Asia, can be found in Europe from Norway to Portugal (Rueness 2005; Hammann et al. 2013 and references therein), as well as along the Atlantic coast of Morocco (Guillemin et al. 2008; Krueger-Hadfield et al. 2017). Its introduction in France likely occurred in the vicinity of oyster farms (Mollet et al. 1998): a recent study on this species using population genetics (Krueger-Hadfield et al. 2017) indicates that the probable main source of the invasion is northeastern Japan, the area from which the majority of *Crassostrea gigas* oysters were imported during the 20th century. In its native range, the species is characterized by a haplo-diplontic life cycle and is attached by holdfasts to hard substrata. Along west and east coasts of the North Atlantic, its populations are dominated by diploid thalli without holdfasts that colonize estuarine mudflats through vegetative fragmentation (Krueger-Hadfield et al. 2016). Similarly, in French mudflats, *G. vermiculophylla* occurs without attaching to a (however small) hard substratum. The presence of *G. vermiculophylla* on mudflats on the northeastern coasts of the United States seems to be linked to, or at least facilitated by, the presence of the tube-dwelling worm *Diopatra cuprea* (Thomsen and McGlathery2005; Wright et al. 2014). Nevertheless, it has been reported in various types of substrata in Europe, including immersed soft sediments and stones, e.g. in the Baltic Sea (Weinberger et al. 2008), where it may constitute a competitor of the native brown alga *Fucus vesiculosus*.

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

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

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

Materials and Methods

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

Ecosystem metabolism

Ecosystem metabolism was measured during low tide with three 0.071 m^2 benthic chambers to estimate CO_2 fluxes at the air-sediment interface using the method described in Migné et al. (2002). Sediment (including *G. vermiculophylla* when present) was enclosed down to 10 cm depth. Changes in air CO_2 concentration (ppm) in the benthic chamber (10 L) were measured with an infrared gas analyzer (LiCor Li-820) for 10-15 min. CO_2 concentrations were recorded in a data logger (LiCor Li-1400) at a 5 s frequency. CO_2 flux was calculated as the slope of the linear regression of CO_2 concentration (μmol mol^{-1}) against time (min) and expressed in mg C m^{-2} h^{-1} assuming a molar volume of 22.4 L at standard temperature and pressure. Transparent chambers were used to estimate the net benthic community production (NCP), the difference between community gross primary production (GPP) and community respiration (CR). Opaque chambers were used to estimate CR. During light incubations, incident photosynthetically available radiation (PAR, 400-700 nm) was monitored with a LiCor SA-190 quantum sensor. On each sampling date, stratified sampling was performed, with three replicates on the *Gracilaria*-colonized area (% cover *G.* vermiculophylla > 50%) and three replicates on the bare-mud area, considered as the control. Benthic chambers were deployed within a few meters of each other to limit any spatial variation.

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

Chlorophyll a

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

$$\text{Chl a } (mg.m^{-2}) = 50 \times [11.85 \times (OD_{664} - OD_{750}) - 1.54 \times (OD_{647} - OD_{750}) - 0.08 \times (OD_{630} - OD_{750})] / 1.96$$

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

Macro- and meiobenthos

On each occasion, three 0.1 m^2 quadrats were sampled on the *Gracilaria*-colonized area and three other quadrats on the bare-mud area for macrofauna identification. Samples were isolated in the field on sieves of 1 mm mesh size, and stored in 4% salted and buffered formalin in the lab. Individuals were identified at the species level and counted.

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

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

Food web

<u>Sample collection and preparation</u>. Invertebrates and the main potential organic matter sources from the sampling area were collected on the four sampling dates. Suspended particulate organic matter (POM) from the site was sampled by collecting 2 L of seawater. POM was obtained by filtration on pre-combusted Whatman GF/F glass fiber membranes within 2 h after collection. Membranes were then acidified (10% HCl) to remove carbonates, briefly rinsed with Milli-Q water, dried (60°C), and kept at -32°C until analysis. Sediment samples were taken by scraping the upper 1 cm of the sediment. For the measurements of carbon isotope ratios in sedimented organic matter (SOM), SOM was acidified (10% HCl) rinsed several times with distilled water, dried (60°C), and ground to a powder (Riera 2010). δ^{13}C measurements were conducted on acidified samples, and measurements of nitrogen isotope ratios were conducted on non-acidified samples. Samples of terrestrial organic matter, dead leaves of terrestrial plants abundant on the muddy sediment, were collected by hand and, back at the laboratory, were rinsed with filtered seawater (pre-combusted GF/F) to clean off epibionts, quickly acidified (10% HCl) to remove any possible residual carbonates, and rinsed with distilled water. The macroalga *G. vermiculophylla* was also collected by hand and washed with distilled water to remove any attached material. In the sampling area, benthic diatoms occurred only as algal mats in May and September and were extracted for stable isotope analyses on these two dates. The absence of dense mats of benthic microalgae at the sediment surface in January 2014 and February 2015 may be due to local strong hydrodynamic conditions and frequent stormy weather during winter. When present, benthic diatom mats were collected at low tide by scraping the surface of the sediment and extracting them using the method in Riera (2007) for intertidal muddy sediment. Diatoms were then collected on pre-combusted glass fiber filters, quickly acidified (10% HCl), and rinsed with distilled water. These samples were then dried (60°C) and kept at -32°C until analysis.

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

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

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

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

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

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

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

Results

Ecosystem metabolism (Figure 2)

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 bare-mud areas (H = 3.853, df = 1, $p < 0.05$), with GPP values for *Gracilaria*-colonized areas being more than twice those of bare-mud areas in spring (Figure 2A), but without interaction.

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

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

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

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

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

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

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

Density of macrofauna (Figure 3B) did not exhibit any seasonal trends (H = 5.343, df = 3, $p > 0.05$) but remained higher in colonized areas (H = 14.313, df = 1, $p < 0.001$). Among the abundant taxa, *N. diversicolor* (Figure 3C) showed higher densities in colonized areas regardless of the season ($H = 14.976$, $df = 1$, $p < 0.001$), as did *A. gracilis* (Figure 3D) ($H = 17.333$, $df = 1$, $p < 0.001$) and oligochaetes ($H = 8.316$, $df = 1$, $p < 0.01$) (Figure 3E).

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

Food web (Figures 5 & 6 and supplementary material)

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

For SIAR calculations, the main potential food sources were considered at each sampling date, namely *G. vermiculophylla*, POM, SOM, and terrestrial organic matter carried by the river to the estuary, and benthic diatoms. However, when the number of sources is greater than five, the solution of Bayesian mixing-models becomes less robust (Parnell et al. 2010). In this study, macroalgae, and, to a lesser extent, benthic diatoms contributed to most of the diet for the three main consumer species considered. During the total sampling period, results of the SIAR models indicated that *G. vermiculophylla* was the main assimilated food source for *S. plana* (mean relative contributions from 67.5 to 83.2%), *H. ulvae* (34.8 to 61.4%), and *N. diversicolor* (55.0 to 92.7%). During spring, and to a lesser extent in summer, benthic diatoms also contributed significantly to consumers' diet with the highest median relative contributions for *N. diversicolor* (35% in May) followed by *S. plana* (23.9% in May) and *H. ulvae* (25.3% in May). Local POM and SOM and terrestrial inputs were much less important in consumers' diet across the entire sampling year (Figure 6).

Discussion

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

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

Typical macrofaunal species were collected both on bare mud and *Gracilaria*-colonized areas. Compared with bare mud, macrobenthic abundance and diversity were positively impacted in the *Gracilaria*-colonized area, corroborating previous studies (e.g. Thomsen et al. 2013). Total density significantly increased in *Gracilaria*-colonized areas due to the rise in density of some common species such as *Nereis diversicolor* and the high abundances of species that were absent or rare in bare mud, such as *Streblospio shrubsolii*. Moreover, species newly recorded in the studied area (e.g. the amphipods *Jassa marmorata*, *Melita palmata* and *Allomelita pellucida*, and the decapods *Pilumnus hirtellus* and *Liocarcinus pusillus*) are generally absent from bare sediments and usually found among seaweeds and hydrozoans on hard substrata or on eelgrass (Hayward and Ryland 1995). These species were found at low densities in *Gracilaria*-colonized areas, likely favored by the complexity of the habitat driven by the settlement of this engineer seaweed (Thomsen et al. 2013). These new epifaunal species led to a significant increase in the number of sampled taxa and therefore in the diversity of the community, due to new available habitat (Wallentinus and Nyberg 2007). Non-native ecosystem engineers, such as the reef-building tubeworm *Ficopomatus enigmaticus*, may also favor the occurrence of opportunistic species (Heiman and Micheli 2010).

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

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

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

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

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

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

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

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

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

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Figures

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

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

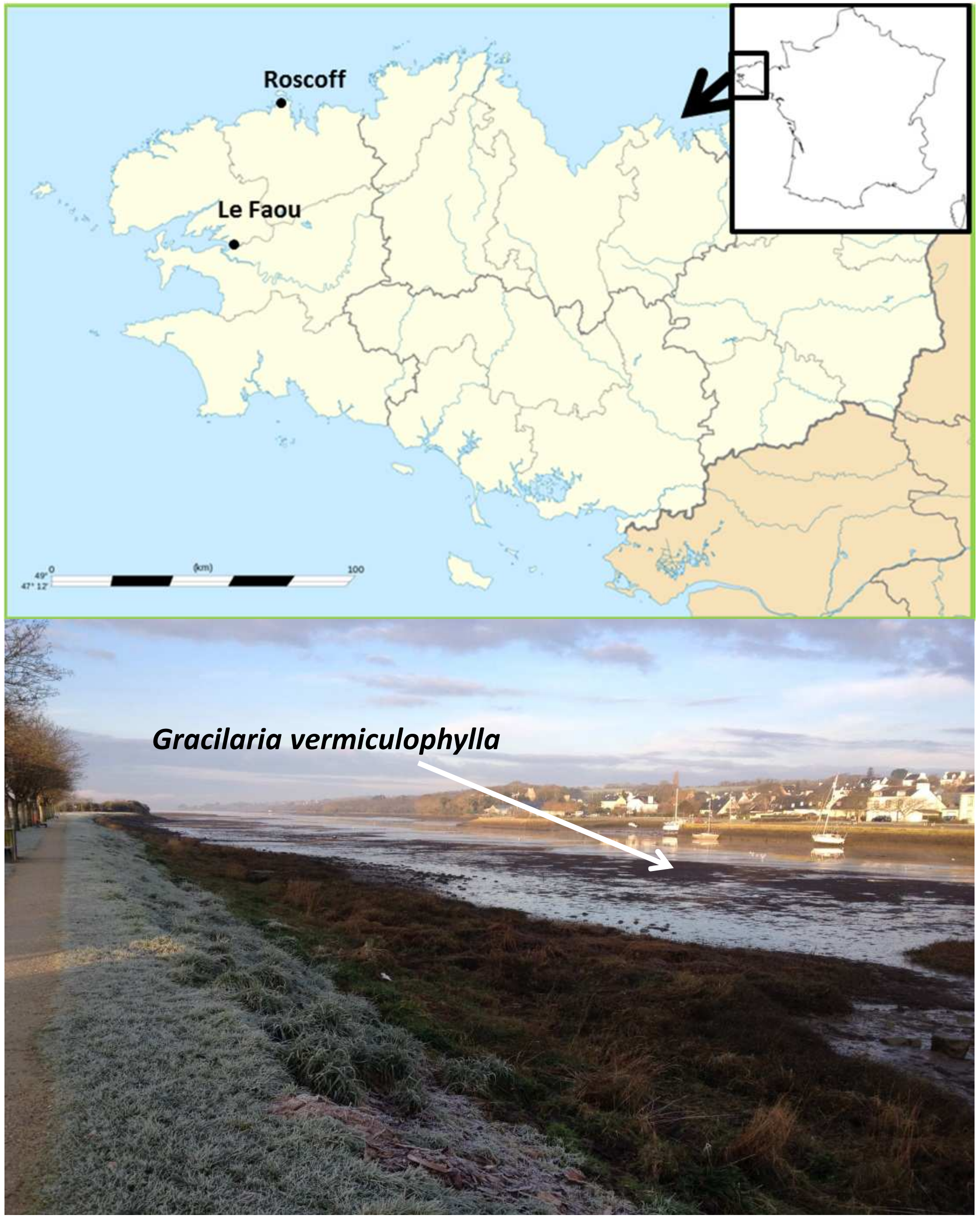
Figure 3. Macrofauna. Number of taxa, total density, density of *Nereis diversicolor*, *Anthura gracilis,* and oligochaetes in the bare-mud area (Control) and the area colonized by *Gracilaria vermiculophylla* (Gracilaria).

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

Figure 5. δ^{13}C vs δ^{15}N (mean ± 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 diets of *Scrobicularia plana, Hydrobia ulvae* and *Nereis diversicolor*, obtained with Bayesian stable isotope mixing models at the four sampling dates. Credibility intervals are 95% (in light gray), 75% (in medium gray) and 50% (in dark gray). POM = particulate organic matter, SOM = sedimented organic matter , Ter-plant = terrestrial plants.

Supplementary material. Carbon and nitrogen isotope ratios (δ^{13}C and δ^{15}N, respectively) (mean values ± SD) of organic matter sources and consumers. Four-date comparisons were performed using the Kruskall-Wallis test and two-date comparisons (benthic diatoms) the Mann-Whitney U test.



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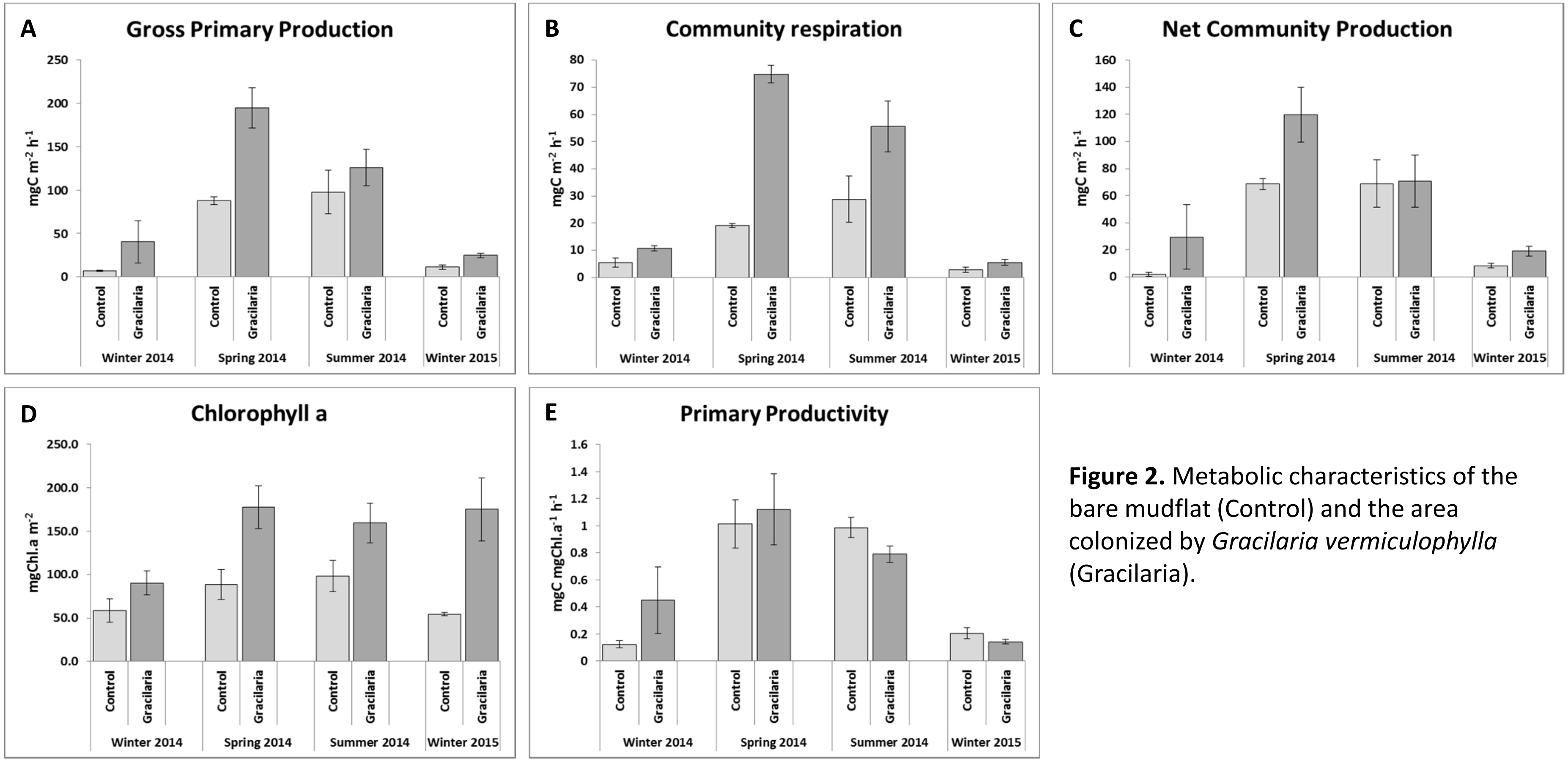

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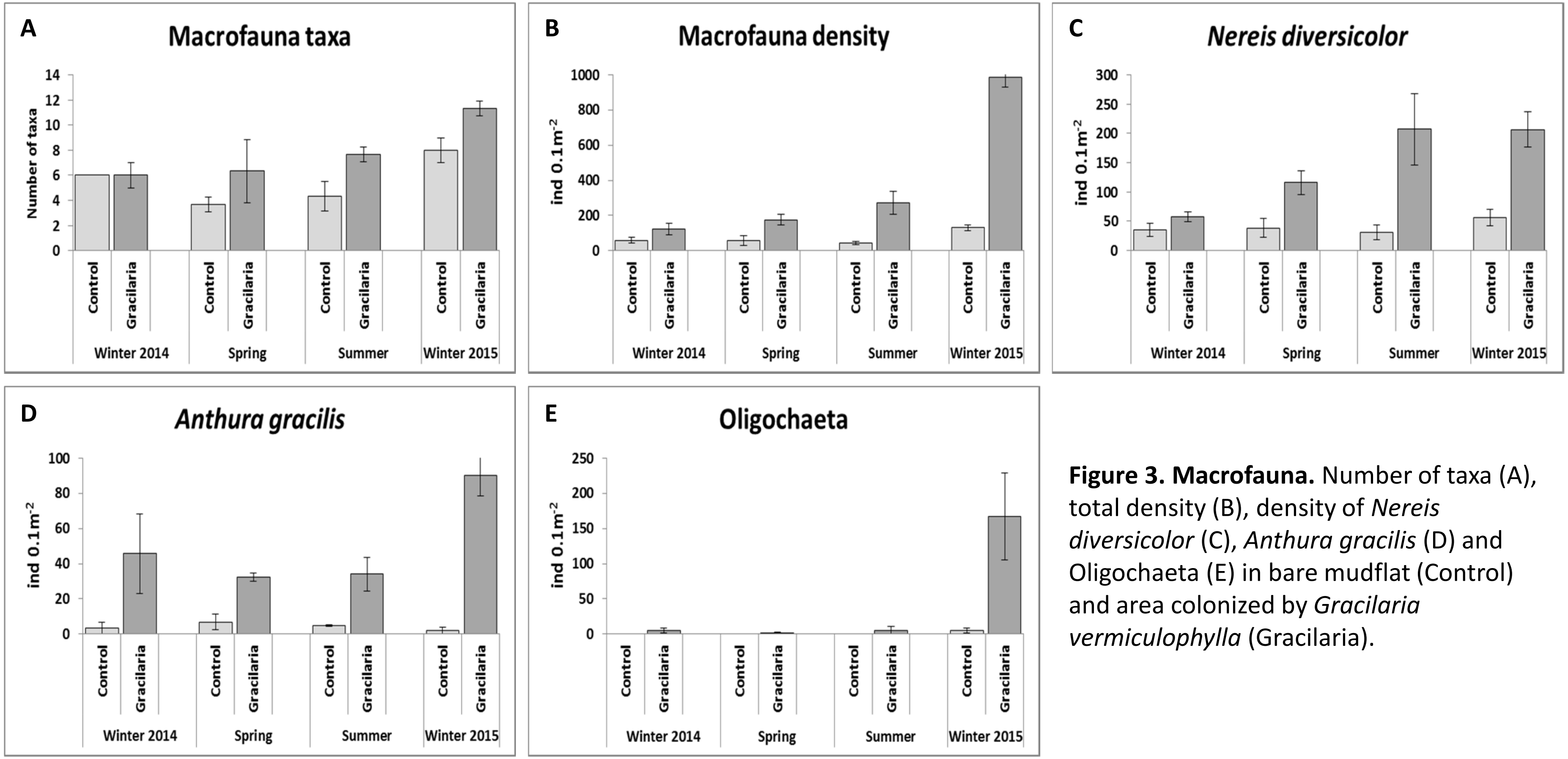

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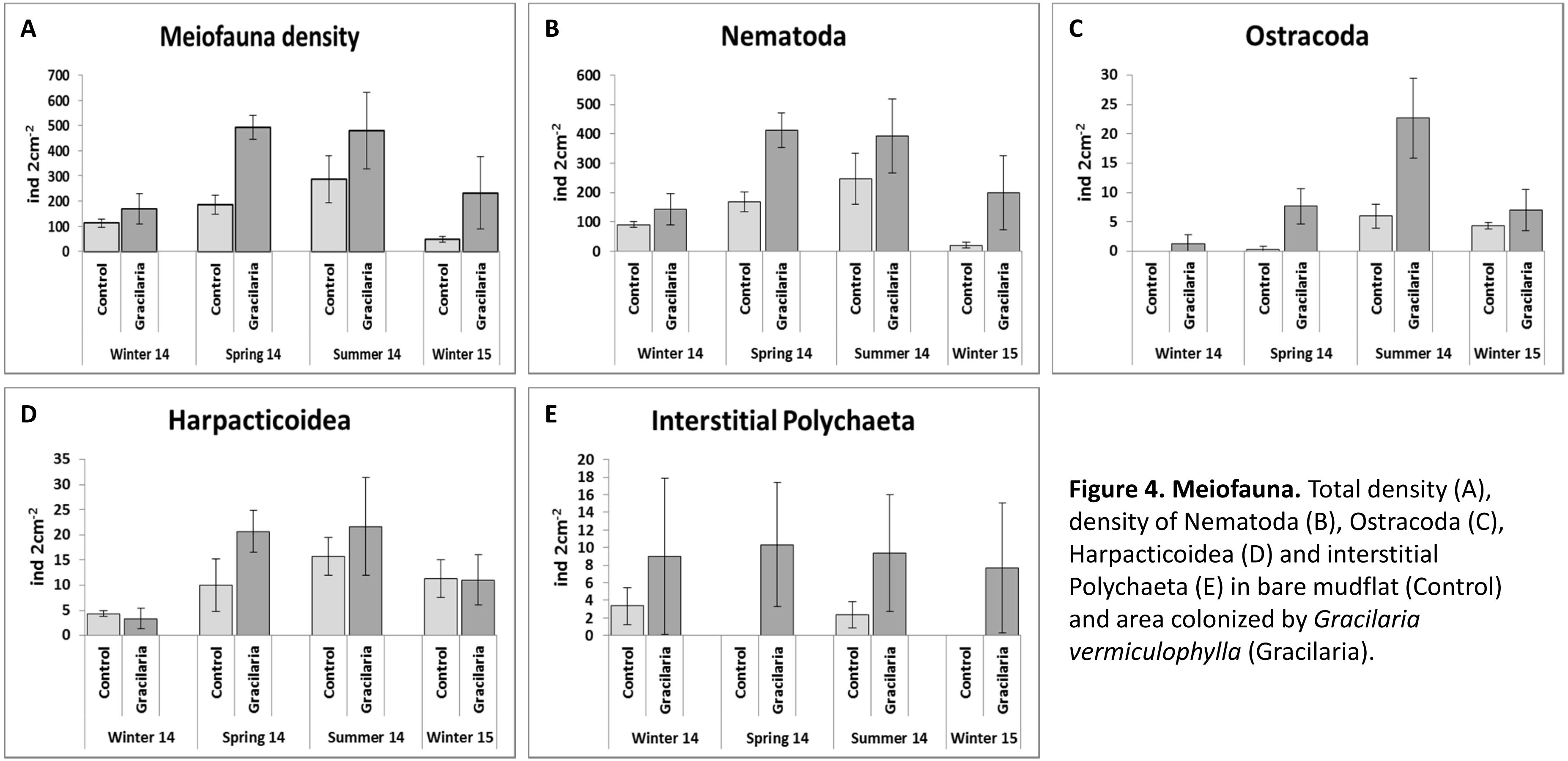

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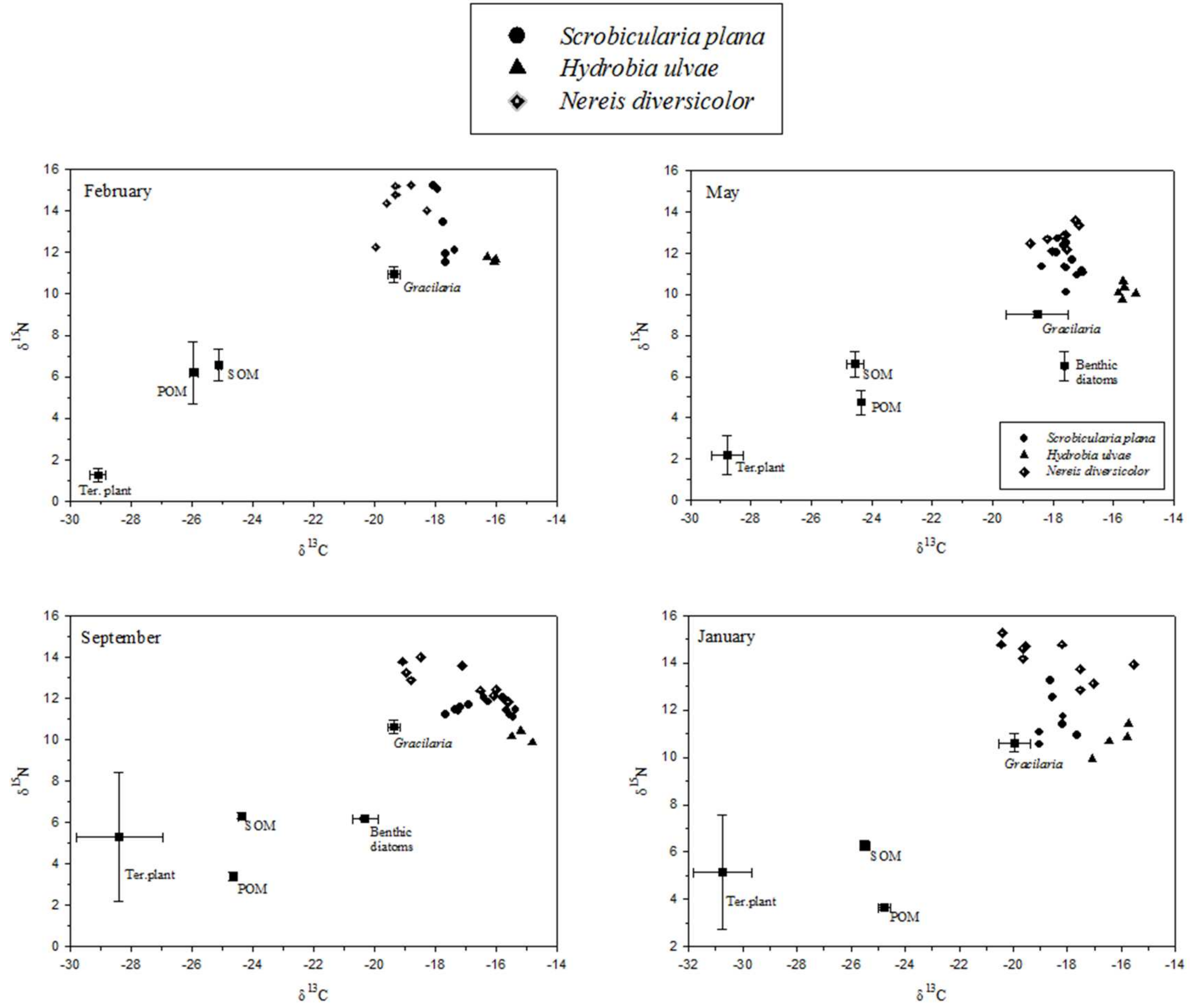

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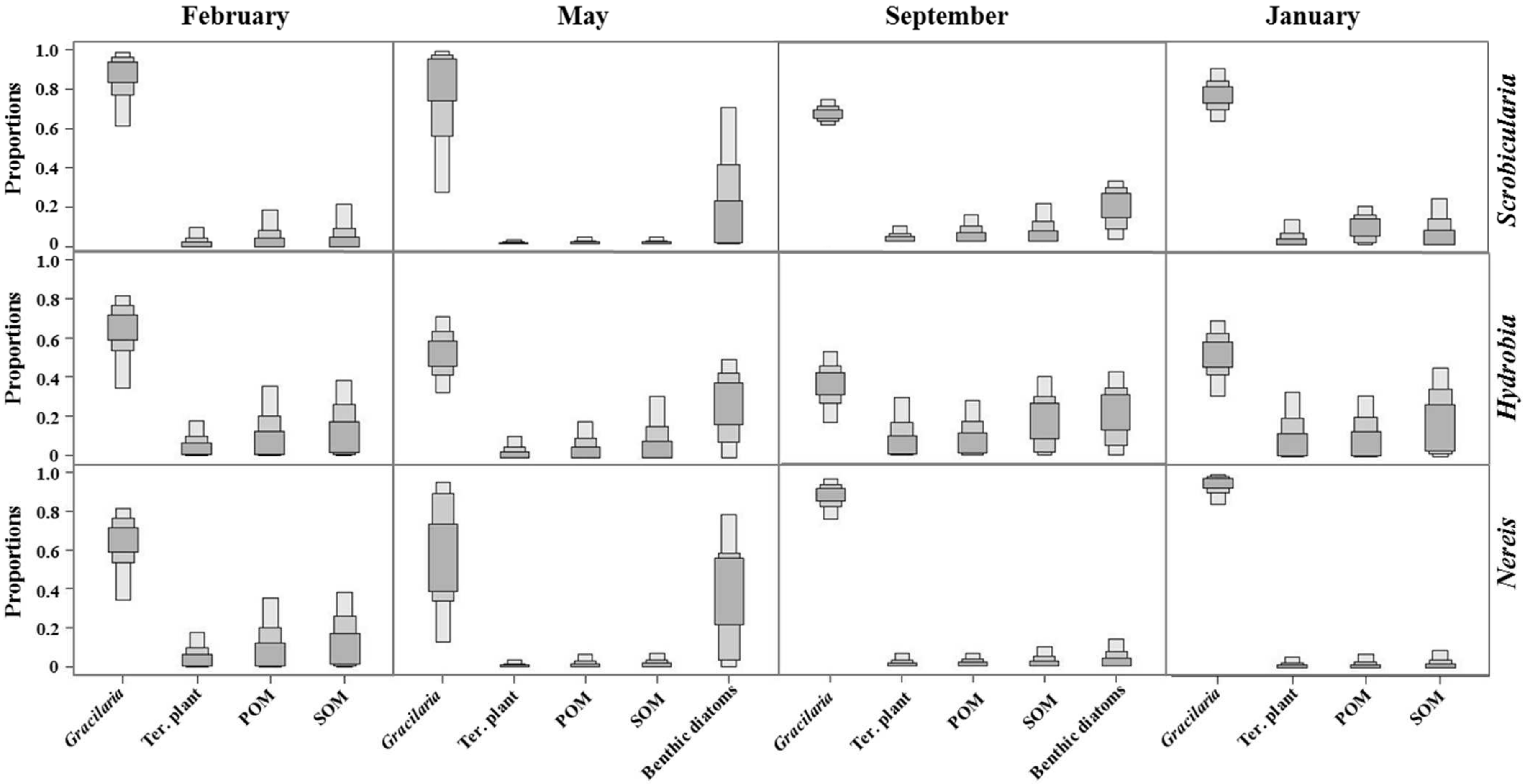

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Highlights – Davoult et al.

A holistic approch 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