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Effects of detrital non-native and native macroalgae on the nitrogen and carbon cycling in intertidal sediments

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Abstract The decay of non-native and native seaweed mixing may modify sediment biogeochemistry and organic matter transfers within benthic food webs according to their composition and biomass. The non-native species *Sargassum muticum* was deliberately added to the sediment of an intertidal sandflat at different biomass and mixed to the native species *Ulva* sp. and *Fucus vesiculosus.* The sediment porewater was then ^{13}C and ^{15}N enriched to test whether both detrital diversity and biomass influenced the transfer of porewater carbon and nitrogen to the sediment and to the macrofauna consumers. More ^{15}N-nitrogen was mobilized to sediments and macrofauna when the 3-species detrital mixing was buried, probably because this mixing provided species-specific compounds such as polyphenols due to the presence of *S. muticum and F. vesiculosus,* as well as large amounts of nitrogen due to the presence of *Ulva*. Our study revealed the importance of detrital diversity and non-native seaweeds for the nitrogen cycling in the benthic food web.

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Introduction

Biological invasions are increasing, especially in marine environments, where they are linked to the intensification of international shipping, aquaculture and aquarium activity (Verlaque 1994; Ribera and Bouderesque 1995). Biological invasions together with climate change are among the most serious global environmental threats (Stachowicz et al. 2002). The replacement of native species or the creation of new habitats by non-native species may influence not only community dynamics and biodiversity but also ecosystem functions, including energy and nutrient flow through food webs (Covich et al. 2004; Hooper et al. 2005; Byrnes et al. 2007). Invasive plants, for instance, may alter communities by providing different amounts of nutrients and food of different qualities, via both living plants and detritus (Wolkovich et al. 2009).

In the marine environment, the detritus food web can be very important since most primary production is not consumed by herbivores but returns to the environment as detritus (Cebrian 2004; Moore et al. 2004). For example, detached seaweeds often deposit as detritus on the substratum of marine intertidal zone where they can be locally buried by sediment reworking and start decomposing (Ford et al. 1999; Kelaher and Levinton 2003; Rossi 2007). The deposition of such marine detritus, often called wrack, may greatly vary in diversity or amounts, and detrital invasive seaweeds may occur in isolation or mixed to native detrital macroalgae replacing partially or completely their biomass (Rossi and Underwood 2002; Olabarria et al. 2007, 2010; Bishop and Kelaher 2008). Despite the importance of

detritus for marine ecosystems, studies that focus on how ecosystem functioning may change following changes in biodiversity have given little attention to the regenerative processes, such as the recycling of the decomposing detritus (Godbold et al. 2009).

The effect of wrack on community dynamics and ecosystem functioning can vary considerably depending on the amounts of wrack deposited on the substratum and their identity (Bishop et al. 2010). At moderate amounts, these detrital seaweeds may represent an important food source for invertebrates and enhance bacterial and microalgal production. However, when seaweed detritus is of great quantities due to eutrophication, anoxic decomposition may prevail and lead to the production of toxic compounds (Rossi 2006). The contribution of wrack as carbon and nutrient providers or as microhabitat builders greatly depends on the chemical composition and the structure of the plant and, as a consequence, on its decomposition rate and edibility. Overall ephemeral green seaweeds such as Ulvaceae have high nitrogen content, decompose rapidly and serve as food for grazers and detritivores. Conversely, brown seaweeds such as Fucales have great lignin content, decompose relatively slowly and produce phenol compounds that are usually associated with a chemical defence against grazers (Buchsbaum et al. 1991; Banta et al. 2004; Pedersen et al. 2005; Rossi et al. 2010). In addition, wrack is often composed of various seaweeds and, sometimes, seagrasses (Rossi and Underwood 2002). Such detrital plant mixing commonly leads to non-additive, identity-dependent effects on decay processes (Moore and Fairweather 2006) and on macroinvertebrates (Bishop and Kelaher 2008). Recently, Olabarria et al. (2010) have also observed variability in the macrofauna response to the burial of non-native and native macroalgal mixing, depending on their species composition and sensitivity to this stress. The diversity of plant detritus has given similar mixed results even in freshwater and terrestrial systems (Hattenschwiler and Gasser 2005), where the mixing of detrital plants sometimes affected mass loss and carbon and nitrogen dynamics according to species identity, through fungi-driven nutrient transfer among litter species of different nitrogen contents or through chemical interactions among specific plant compounds (Hattenschwiler and Gasser 2005; Moore and Fairweather 2006).

The arrival of detrital invasive seaweeds that can appear mixed to native species or in isolation could alter the carbon and nitrogen provision and modify both benthic community structure and carbon or nitrogen cycling. Nonetheless, little attention has been given to the role of invasive detrital seaweeds despite the fact they often fulfil coastal sediments (Rodil et al. 2008; Rossi et al. 2010).

In this study, we have focused on the effects of the non-native brown algae *Sargassum muticum* (Yendo) Fensholt, which is nowadays an important component of intertidal habitats along the Galician coast, NW Spain (Incera et al. 2011). We have specifically studied how changes in *S. muticum* detrital biomass and mixing affected the carbon and nitrogen transport to the benthic food web of intertidal sediments. We have asked whether (1) the burial of detrital mixing of *S. muticum* with the native species *Ulva* sp. and *Fucus vesiculosus* or (2) the biomass of *S. muticum* buried as monospecific detritus changed nitrogen and carbon incorporation by macrobenthic consumers, sediment quality and macrofauna trophic structure.

Materials and methods

Study area and experimental design

This work was done on the south region of the Galician coast (NW Spain) in an organically enriched intertidal sandflat located on the southern side of Ria de Vigo, called "A Punta beach" (42°15′N; 8°42′W). The site is organically enriched with a mean (±SE) organic carbon content of 0.21(±0.02) (Olabarria et al. 2010). In addition to the invasive alga *S. muticum,* the native species *Ulva* sp., and *Fucus vesiculosus* Linnaeus were chosen because they coexist with *S. muticum* and they are the most abundant species on mid- and low-intertidal rocky shores in the area of study. All the seaweeds used in the experiment were collected the same day from nearby rocky shores. In the laboratory, the seaweeds were thoroughly washed and their epiphytes were removed. The cleaned algae were frozen (−20°C), dried (at 60°C to constant weight) and shredded (<2 cm diameter) prior to experimental addition. The treatment was done to avoid algal growth phenomena after experimental addition and to focus our study on the effect of detritus as organic enrichment for biogeochemical cycling, avoiding effects related to changes in sediment microcomplexity. In addition, dried and shredded algae can naturally occur when algae are dried by insolation on the upper intertidal area and then incorporate into the sediment (Ford et al. 1999).

At "A Punta beach", we chose an area of 10 × 10 m at a tidal height of MLW springs +0.4 m. Then, we randomly selected 24 plots of 50 × 50 cm at a distance of at least 3 m from each others. We manipulated detritus at a small spatial scale because natural topographical features of sandflats produce patchiness in the accumulation of detritus at the scale of metres (Kelaher and Levinton 2003). Plots were marked with four bamboo stakes at their corners, and the distance of each bamboo was measured from two fixed reference points so that plots could be relocated. Detritus was added to the experimental plots at low tide by evenly hand-churning dried and shredded algae into the top

0.05 m of sediment. We added 40–120 g seaweed detritus because this amount is within the range of detritus biomass that naturally deposit on the substratum of the study area (Olabarria C., unpub data).

Three plots were randomly assigned to each of the following 6 treatments: (1) 40 g of *S. muticum* (S40), (2) 60 g of *S. muticum* (S60), (3) 120 g of *S. muticum* (S120), (4) 60 g of *S. muticum* and 60 g of *F. vesiculosus* (SF), (5) 60 g of *S. muticum* and 60 g of *Ulva* sp. (SU) and (6) 40 g of *S. muticum*, 40 g of *F. vesiculosus* and 40 g of *Ulva* sp. (SUF). Treatments 1–3 assessed whether *S. muticum* have biomass-dependent effects, whereas treatments 4–6 examined changes due to the diversity of detritus when *S. muticum* mix to the native species *Ulva* spp. and *F. vesiculosus*. In addition, we established 3 procedural control plots (PC), where the sediment was physically disturbed but detritus was not added, and 3 natural control plots (C).

On 18 September 2008, the different types of macroalgal detritus were buried. Then, on 13 October 2008, we sprayed the sediment surface of each plot with 250 mg ^{13}C bicarbonate (99% pure) and 17.3 mg potassium ^{15}N-nitrate (99% pure). The spraying was done at the beginning of the low tide to allow microorganisms assimilate the inorganic carbon and the nutrients. Inorganic carbon was expected to be assimilated primarily by microphytobenthos, while nitrogen could be used by both autotrophic and heterotrophic organisms, including saprophytes.

At the time of the tracing experiment, effects of algal addition were expected on sediment macrofauna since previous studies have suggested that the effects of algal mats occur within 2–10 weeks from deposition (Thrush 1986; Rossi 2006) and decomposition rate of *Ulva sp.*, *F. vesiculosus* and *S. muticum* occur within 1–1.5 months (Pedersen et al. 2005; Olabarria et al. 2007; Urban-Malinga et al. 2008). Indeed, decomposition rate generally follows the exponential equation: $B_t = B_0 \cdot \exp(-k \cdot t)$, where B_0 and B_t are the initial biomass and the biomass at the time t, respectively, and k is the constant of decay. The constant k can vary considerably according to the biochemical composition of the species and environmental conditions (Rossi 2007). From the literature, under laboratory conditions, the constant k is often 0.04 days^{-1} for *Ulva lactuca* and 0.016 and 0.028 days^{-1} for *Sargassum* and *Fucus*, respectively (Banta et al. 2004; Pedersen et al. 2005). Assuming that such values may hold for this field experiment, at the time of the isotope tracing experiment, the detritus still available for *S. muticum* would be of 26, 39 and 78 g dry weight (DW) at the initial biomass of 40, 60 or 120 g, corresponding to about 60% of the initial biomass. For the detrital mixing, we expected a total detritus availability of 67, 59 or 58 g DW, corresponding to percentages of the initial biomass of 56, 49 and 48% for the SF, SU or SUF treatments, respectively (Fig. 1a).

Samples of sediment and macrofauna were taken outside the plots for isotope reference values prior to tracer addition. Bulk sediment was sampled at the end of the low tide, 3 h after the tracer addition and again after 24 h. Macrofauna and sediment were simultaneously sampled at the end of the experiment, after 5 days (120 h). We followed the tracer incorporation by macrofauna after 5 days from tracer addition because in tracing experiments done on

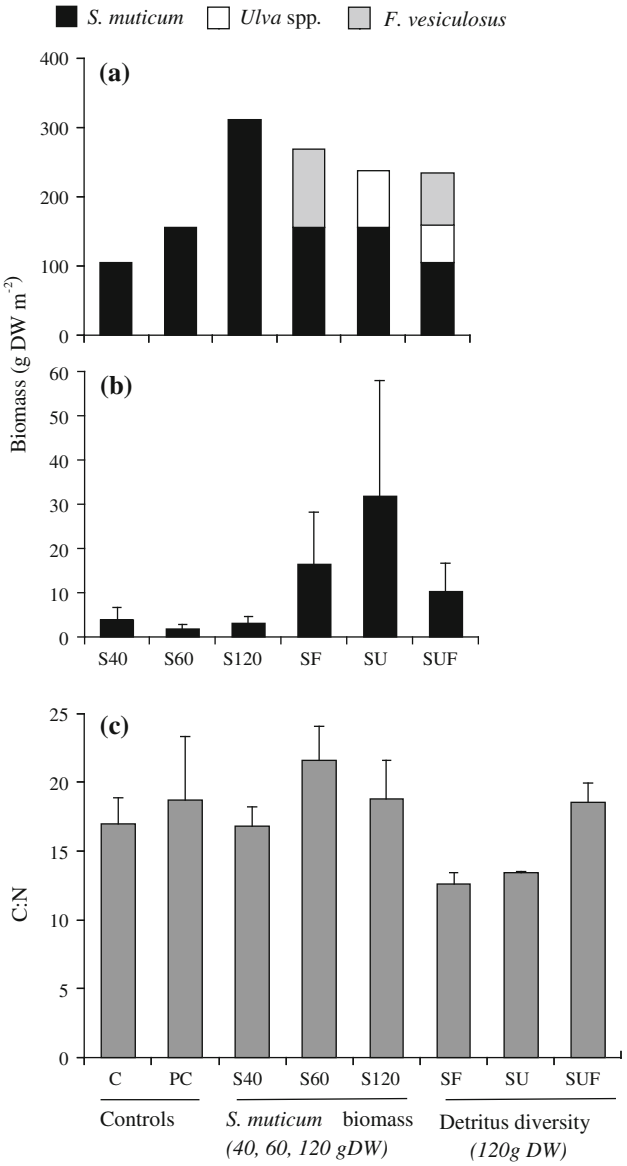

Fig. 1 Mean (±SE) values at each treatment of **a** expected detrital biomass at the time of sampling estimated by the decaying rates of each species; **b** remaining detrital biomass collected after sieving the sediment at 0.5 mm; **c** sediment quality indicated as the sediment organic carbon to nitrogen ratio (C/N). *C* control plots, *PC* procedural control plots, *S40* 40 g of *S. muticum*, *S60* 60 g of *S. muticum*, *S120* 120 g of *S. muticum*, *SF* 60 g of *S. muticum* and 60 g of *F. vesiculosus*, *SU* 60 g of *S. muticum* and 60 g of *Ulva* sp., *SFU* 40 g of *S. muticum*, 40 g of *F. vesiculosus*, 40 g of *Ulva* sp

other intertidal flats, generalist and specialist invertebrates that feed on sediment generally show signal uptake after about 48 h from the addition and reach a steady state with their food sources within 1 week (Herman et al. 2000; Rossi 2007; Rossi et al. 2009). This is a whole system study where tracers are added to the environment under natural conditions. By prolonging the time after the addition, the tracers will start disappearing because of complex biogeochemical and physical processes.

At each plot, one large sediment core (10 cm internal diameter, i. d.) was sampled to the depth of 20 cm for macrofauna analyses. Three additional cores were collected to the depth of 1 cm using a cut-off syringe for sediment organic carbon and nitrogen (2 cm i. d.). The three small cores were then pooled before analyses to maintain a feasible number of sampling. Multiple core sampling allowed more precise estimates of average sediment content of organic carbon and nitrogen since nutrients often show great variability at a very small scale (Rossi and Underwood 2002).

The sediment for macrofauna was immediately sieved at 0.5 mm and stored in 4% buffered formol until sorting, taxonomic identification and isotope analyses. Formol may alter isotopic composition of roughly 1‰ (Kaehler and Pakhomov 2001). Therefore, in this case, the error was irrelevant since tracing experiments increase the isotopic values of one or two orders of magnitude and render the small effect of formol negligible (Rossi et al. 2009). Furthermore, we calculated incorporation as the differences between labelled and background values, both treated with formol, eliminating any other potential bias. In the laboratory, macrofauna samples were sorted and animals were counted and identified to the lowest taxonomical level. Seaweed detritus retained by the sieve mesh was also collected, dried and weighted. The fauna was dried at 60°C for 48 h to determine both the biomass and the label incorporation. The macrofauna species were then grouped in trophic groups, based on their diet and feeding mode (detritivores, grazers and omnivores) or on the origin of their food sources (e.g. sediment surface or subsurface, overlying water) (Table 1; Pearson 2001; Carlier et al. 2007; http://www.marinespecies.org/index.php).

The remaining sediment was freeze-dried, after removing visible seaweed residuals to measure bulk organic carbon, total nitrogen and label incorporation. The organic carbon and nitrogen were determined using a Flash EA 1112 Series elemental analyser, after acidification with HCl to eliminate carbonates. We used the ratio between the sediment nitrogen and organic carbon (C/N) as a bulk indicator of nutrient remineralization and sediment quality.

The label incorporation was evaluated as $^{13}\delta$C and $^{15}\delta$N ‰ increment as compared to natural background $^{13}\delta$C and $^{15}\delta$N values. This increment is expressed as $\Delta\delta^{13}$C and $\Delta\delta^{15}$N. The incorporation was evaluated separately for the different trophic groups of macrofauna to estimate label incorporation independently of changes in biomass, which were estimated separately. No correction for carbon or nitrogen values or biomass was therefore done. The values of $^{13}\delta$C and $^{15}\delta$N were measured using the Flash EA 1112 Series elemental analyser coupled on line via Finningan conflo II interface to a Thermo delta V S mass spectrometer, following the formula:

$$\delta^{13}\mathrm{C} \text{ or } \delta^{15}\mathrm{N} = \left[\left(R_{\text{sample}}/R_{\text{standard}}\right) - 1\right] \times 1{,}000,$$

where $R = {}^{13}\mathrm{C}/{}^{12}\mathrm{C}$ or ${}^{15}\mathrm{N}/{}^{14}\mathrm{N}$.

The standard material was PDB limestone for δ^{13}C and atmospheric nitrogen for δ ^{15}N. Precision in the overall preparation and analysis was better than 0.2‰.

Analyses of data

Hypotheses about differences among treatments were tested with one-way linear models of analyses of variance (ANOVA) for the biomass of algal detritus that remained in the sediment, the biomass of the most common macrobenthic trophic groups, the C/N ratio, the $\Delta\delta^{13}$C and $\Delta\delta^{15}$N for the bulk sediment and for the most common trophic groups. Data were not transformed, because Cochran's test for homogeneity of residual variance was not significant. The analyses that showed significant differences among treatments were followed by the Student–Newman–Keuls (SNK) post hoc tests to identify those treatments that differed significantly ($P < 0.05$). In order to test for the physical disturbance of detrital manipulation, the effect of diversity of detritus (e.g. *S. muticum* mixed to one or two native species) and the biomass of *S. muticum*, we utilized different ANOVA analyses. First, we ran one-way analyses with two levels (PC, C) and 3 replicates testing for the impact of detrital manipulation. Because PC and C did not differ in any analyses ($P > 0.05$), except for the $\Delta\delta^{13}$C of bulk sediment, we used indistinctly one of each treatment in the analyses testing for effects of diversity of detritus and biomass of *S. muticum*. For the $\Delta\delta^{13}$C of bulk sediment, we used PC. We did not pool the controls to maintain the design as balanced as possible, with 3 replicate plots for each treatment. Second, we ran one-way analyses with the fixed factor "Treatment" (4 levels: SFU, SF, SU, C) to test for effects of detrital diversity and one-way analyses with the fixed factor "Treatment" (4 levels: S120, S60, S40, PC) and to test for effects of biomass of *S. muticum*. Unfortunately, 1 out of 3 replicate plots were lost for SFU, SF and S120 during the experiment, and the analyses were done correcting for the unbalanced number of replicates (see Underwood 1997). All analyses were done with GMAV software (Institute of Marine Sciences, University of Sydney).

Table 1 Biomass (mg core^{-1}; average ± SE) of macrofauna species grouped by their major feeding habits

Taxa	C	PC	S40	S60	S120	SF	SU	SUF
Surface deposit feeders	6.33 ± 2.91	2.43 ± 1.09	1.17 ± 0.83	3.88 ± 3.32	1.24 ± 1.14	1.88 ± 0.93	0.46 ± 0.04	8.89 ± 8.29
Corophium sp.								0.08 ± 0.08
Cyatura carinata (Krøyer, 1847)	0.43 ± 0.43	0.26 ± 0.26						
Malacoceros fuliginosus (Claparède, 1869)	0.12 ± 0.08	0.45 ± 0.4	0.05 ± 0.05	0.08 ± 0.08	0.19 ± 0.19		0.05 ± 0.05	0.71 ± 0.71
Melita palmata (Montagu, 1804)	0.10 ± 0.10			0.08 ± 0.08				
Paraonidae	0.11 ± 0.11	0.03 ± 0.03	0.17 ± 0.17	0.16 ± 0.11		0.78 ± 0.09	0.25 ± 0.15	0.43 ± 0.17
Polydora cornuta (Bosc, 1802)	3.07 ± 1.73	0.72 ± 0.34	0.34 ± 0.34	0.03 ± 0.03	0.41 ± 0.41	0.56 ± 0.56		1.40 ± 1.40
Pygospio elegans (Claparède, 1863)	1.90 ± 0.86	0.71 ± 0.29	0.40 ± 0.31	0.51 ± 0.37	0.59 ± 0.59	0.44 ± 0.36	0.03 ± 0.03	3.18 ± 3.18
Scolelepis squamata (Müller, 1806)				2.93 ± 2.93				0.54 ± 0.54
Spio filicornis (Müller, 1776)	0.50 ± 0.39	0.26 ± 0.13	0.22 ± 0.22	0.07 ± 0.07	0.05 ± 0.05	0.11 ± 0.11		2.56 ± 2.56
Spionidae (juveniles)							0.13 ± 0.13	
Subsurface deposit feeders	4.19 ± 2.98	1.1 ± 0.85	3.11 ± 0.31	3.95 ± 0.89	4.11 ± 3.41	3.04 ± 1.62	7.11 ± 2.93	1.94 ± 0.07
Capitella sp.	0.54 ± 0.13	0.21 ± 0.16	2.36 ± 0.47	3.63 ± 0.78	2.93 ± 2.23	2.32 ± 1.35	5.15 ± 1.93	1.50 ± 0.52
Heteromastus filiformis (Claparède, 1864)	0.43 ± 0.43	0.89 ± 0.89	0.12 ± 0.12			0.23 ± 0.23	1.56 ± 1.12	
Oligochaeta	0.10 ± 0.07						0.02 ± 0.02	
Scalibregmidae	3.12 ± 3.12							0.44 ± 0.44
Suspension feeders	1.65 ± 1.25	29.64 ± 25.33	0.73 ± 0.55	0.02 ± 0.02	8.76 ± 8.25	0.39 ± 0.16		27.16 ± 27.16
Ampharetidae						0.27 ± 0.27		
Bivalvia (juveniles)				0.02 ± 0.02				
Cerastoderma edule (Linnaeus, 1758)	1.46 ± 1.33	2.16 ± 1.21	0.73 ± 0.55		8.76 ± 8.25	0.11 ± 0.11		27.16 ± 27.16
Scrobicularia plana (da Costa, 1778)		1.77 ± 1.77						
Sipunculidae	0.17 ± 0.17							
Ruditapes philippinarum (Adams & Reeve, 1850)	0.02 ± 0.02	24.23 ± 24.23						
Tellina tenuis (da Costa, 1778)		1.48 ± 1.48						
Omnivores	0.43 ± 0.43	0.43 ± 0.43	0.01 ± 0.01	0.18 ± 0.18	0.01 ± 0.01	1.03 ± 1		0.01 ± 0.01
Eteone longa (Fabricius, 1780)	0.43 ± 0.43							
Eulalia sp.						0.45 ± 0.45		
Glycera sp.		0.37 ± 0.37				0.57 ± 0.57		
Nereidae Juv		0.05 ± 0.05						
Palaemon sp.				0.18 ± 0.18				
Syllidae		0.01 ± 0.01	0.01 ± 0.01		0.01 ± 0.01	0.02 ± 0.02		0.01 ± 0.01
Grazers	0.23 ± 0.23	0.44 ± 0.22		0.18 ± 0.18	0.52 ± 0.52			0.04 ± 0.04
Littorina littorea (Linnaeus, 1758)	80.96 ± 80.96							
Hydrobia ulvae (Pennant, 1777)	0.23 ± 0.23	0.44 ± 0.22		0.18 ± 0.18	0.52 ± 0.52			0.04 ± 0.04

All animals were adults, except when indicated otherwise

Results

Detrital biomass, sediment quality and biomass of trophic groups

The biomass of the remaining algal detritus greatly varied among plots, and there were no significant differences among treatments for both *S. muticum* biomass ($F_{(2,\ 5)} = 0.58$, $P = 0.59$) and detrital diversity ($F_{(2,4)} = 0.18$, $P = 0.84$). The percentage of macrodetritus found in the samples corresponded to about 1–6% initial detritus, and the only species found was *S. muticum* (Fig. 1b). Such biomass was far smaller than the biomass calculated using values of the constant k from laboratory experiments (Fig. 1a, b), probably because of variable environmental conditions such as the sediment re-working due to the hydrodynamic conditions at that particular time of the year (Rossi F., unpub data).

As for the detrital biomass, there were no differences among treatments for the sediment quality as estimated by the C/N ratio when *S. muticum* biomass was increased (Fig. 1c; $F_{(3,7)} = 0.43$, $P = 0.78$). Detrital diversity also did not increase significantly the sediment quality ($F_{(3,6)} = 4.23$, $P = 0.06$). Conversely, sediment quality tended to be decreased in the SU and SF treatments (Fig. 1c).

The most represented trophic groups of macrofauna were the surface and the subsurface feeders. Their biomass was variably distributed among all treatments. The other trophic groups had less biomass and greatly varied among plots (Table 1). Thus, they were not analysed any further for differences among treatments. There were no significant differences for the biomass of the subsurface and the surface feeders at different levels of *S. muticum* biomass (S40, S60 and S120; surface deposit feeders: $F_{(3,7)} = 0.37$, $P = 0.83$; subsurface deposit feeders: $F_{(3,7)} = 0.86$, $P = 0.53$) or of detrital diversity, when comparing similar biomasses of the detritus composed of *S. muticum* at different combinations with the native species (SF, SU, SUF; surface deposit feeders: $F_{(3,6)} = 1.16$, $P = 0.40$; subsurface deposit feeders: $F_{(3,6)} = 0.69$, $P = 0.59$).

Sediment isotope uptake

The addition of ^{15}N-ammonium and ^{13}C-bicarbonate increased the ^{15}N and ^{13}C pool in the bulk sediment within 3 h (Fig. 2). After 24 h, the amount of heavy isotopes in the bulk sediment decreased considerably for all treatments and the controls (C) and procedural controls (PC). Such decrease was expected, due to the water flushing and porewater exchange during the tide. Values slightly dropped at the end of sampling, 5 days after the tracer addition, except for those plots where the mixing of *S. muticum*, *F. vesiculosus* and *Ulva* spp. was added (SUF in Fig. 2). Indeed, after 5 days, there was a significantly higher ^{15}N incorporation in the bulk sediment of the most diverse detrital mixing than in the sediment of the other treatments (Table 2; SUF > SU = SF, SNK test, $P < 0.05$). No differences were found according to the biomass of *S. muticum* added (Table 2).

There was a strong positive correlation between the $\Delta\delta^{15}$N and the $\Delta\delta^{13}$C of bulk sediment ($r_s = 0.91$, $N = 22$, $P < 0.001$). However, there was no significantly high ^{13}C incorporation for the diversity of detritus (Table 2).

Consumer incorporation

After 5 days from the addition of the tracers, the animals had assimilated a large part of the heavy isotopes

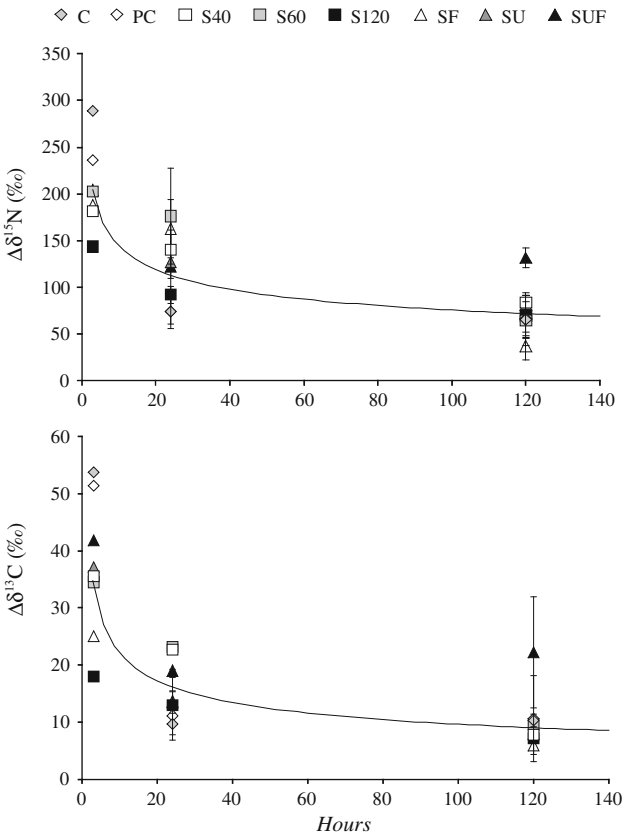

Fig. 2 Mean (±SE) temporal variation of ^{15}N and ^{13}C tracer incorporation ($\Delta\delta^{15}$N and $\Delta\delta^{13}$C) for the bulk sediment at each of the 8 treatments (C, PC, S40, S60, S120, SF, SU, SUF). Treatment abbreviations as in Fig. 1. The *line* indicates the overall decrease in label incorporation during the study period. It is the best-fitted line for all the pooled treatments ($r^2 = 0.69$ and 0.71 for $\Delta\delta^{15}$N and $\Delta\delta^{13}$C, respectively). Statistical analyses for the differences among treatments were done at the end of the experiment (after 5 days from label addition), when tracer incorporation was more stable and the steady state for invertebrate assimilation was likely (see "Materials and methods")

Table 2 One-way analyses of variances on the label uptake ($\Delta\delta^{15}$N and $\Delta\delta^{13}$C) after 5 days from the tracer addition in the bulk sediment and the most abundant macrofaunal trophic groups

	df	$\Delta\delta^{15}$N		$\Delta\delta^{13}$C	
		MS	*F*	MS	*F*
Bulk sediment					
Detrital manipulation					
Treatment	1	0.39	0.00	21.29	0.20
Residual	4	1,699.78		103.87	
Diversity of detritus					
Treatment	3	3,203.62	5.61*	120.74	1.85
Residual	6	571.52		65.41	
Biomass of sargassum					
Treatment	3	179.37	0.15	4.87	0.06
Residual	7	1,165.89		76.6	
Surface deposit feeders					
Detrital manipulation					
Treatment	1	71.85	1.29	30.03	0.97
Residual	4	55.63		37.93	
Diversity of detritus					
Treatment	3	1,418.31	4.66*	99.82	0.95
Residual	6	304.15		104.94	
Biomass of sargassum					
Treatment	3	56.33	0.46	33.01	1.52
Residual	6	123.4		50.26	
Subsurface deposit feeders					
Detrital manipulation					
Treatment	1	120.79	2.40	16.46	0.8
Residual	4	50.25		20.7	
Diversity of detritus					
Treatment	3	469.82	0.64	0.94	0.11
Residual	6	356.66		8.43	
Biomass of sargassum					
Treatment	3	85.47	0.55	8.36	0.53
Residual	7	155.64		15.82	

Three different ANOVAs were performed to assess differences between the procedural and natural controls PC and C, the effect of detrital diversity (C, SF, SU and SUF) and detrital biomass of *S. muticum* (PC, S40, S60 and S120; see also "Materials and methods")

* $P < 0.05$

incorporated in the bulk sediment. Overall, the tracers taken up mainly by the surface and subsurface feeders and by the grazers when present (Figs. 3, 4). The surface deposit feeders showed differences in the ^{15}N uptake for the detrital diversity, with the highest uptake in the most diverse treatment (Table 2), according to the ^{15}N uptake in the bulk sediment. There were instead no differences concerning the biomass of *Sargassum* added. The subsurface deposit feeders did not show any differences among the detrital mixings or the biomass of *S. muticum* (Table 2).

The ^{13}C incorporation by surface deposit feeders did not follow the patterns observed for the ^{15}N uptake ($r_s = 0.12$, $N = 21$, $P > 0.05$), and there were no differences in the uptake according to the detrital mixing or the biomass of *S. muticum* (Table 2). Rather, they showed great variability among plots, particularly in the most diverse treatment (Fig. 4).

Discussion

By spraying a seawater solution of ^{13}C-bicarbonate and ^{15}N-nitrate on the sediment surface, we added ^{13}C and ^{15}N tracers to the sediment porewater and studied the tracer incorporation in the bulk sediment and the macrofauna consumers in relation to the presence of detrital seaweeds varying in diversity and in the biomass of the invasive seaweed *S. muticum*. The tracers were added at the beginning of the low tide allowing benthic microorganisms incorporate them for the entire duration of the low tide. Incoming tide was expected to wash up the excess tracers not incorporated by the microorganisms (Middelburg et al. 2000; Veuger et al. 2005). Indeed, both $\Delta\delta^{15}$N and $\Delta\delta^{13}$C of the bulk sediment followed the expected trend, and values dropped considerably after the incoming tide. We then expected that a part of the tracers would be mobilized

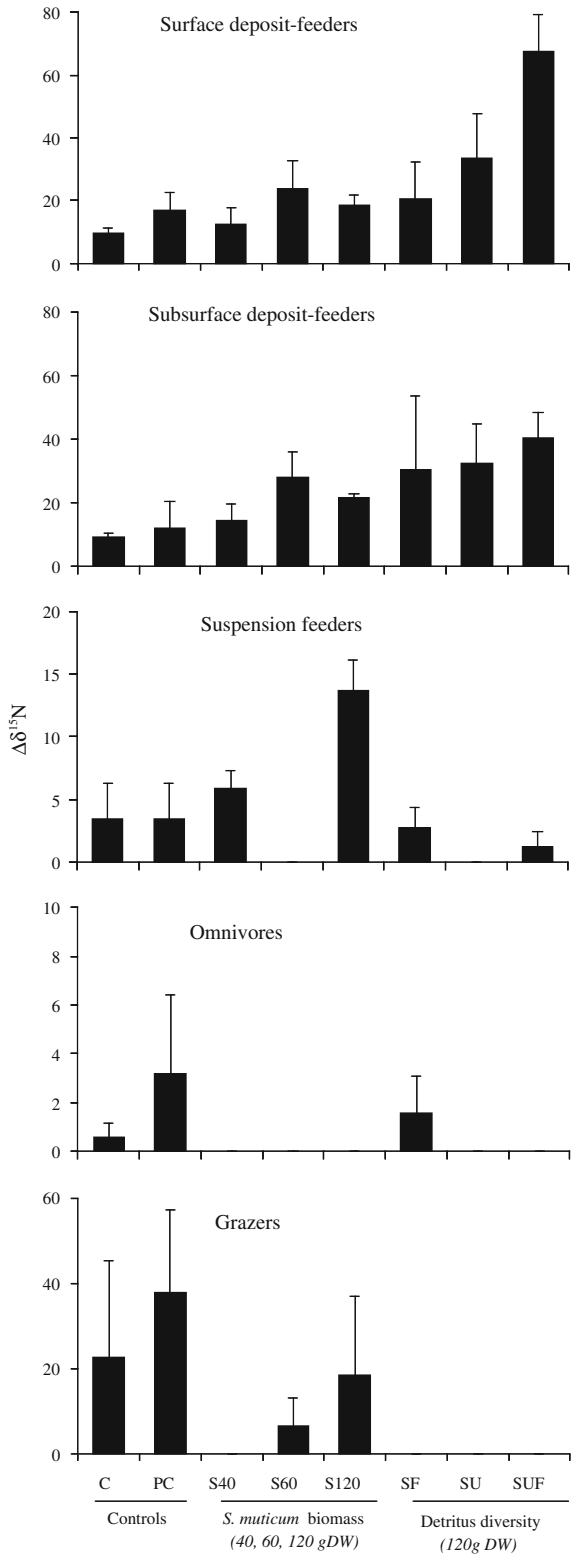

Fig. 3 Mean (±SE) ^{15}N incorporation by the different trophic groups of macrofauna ($\Delta\delta^{15}N$) at each treatment (see Fig. 1 for treatment abbreviations). Incorporation of each trophic group was estimated as average values over all species included in the trophic group at each replicate plot

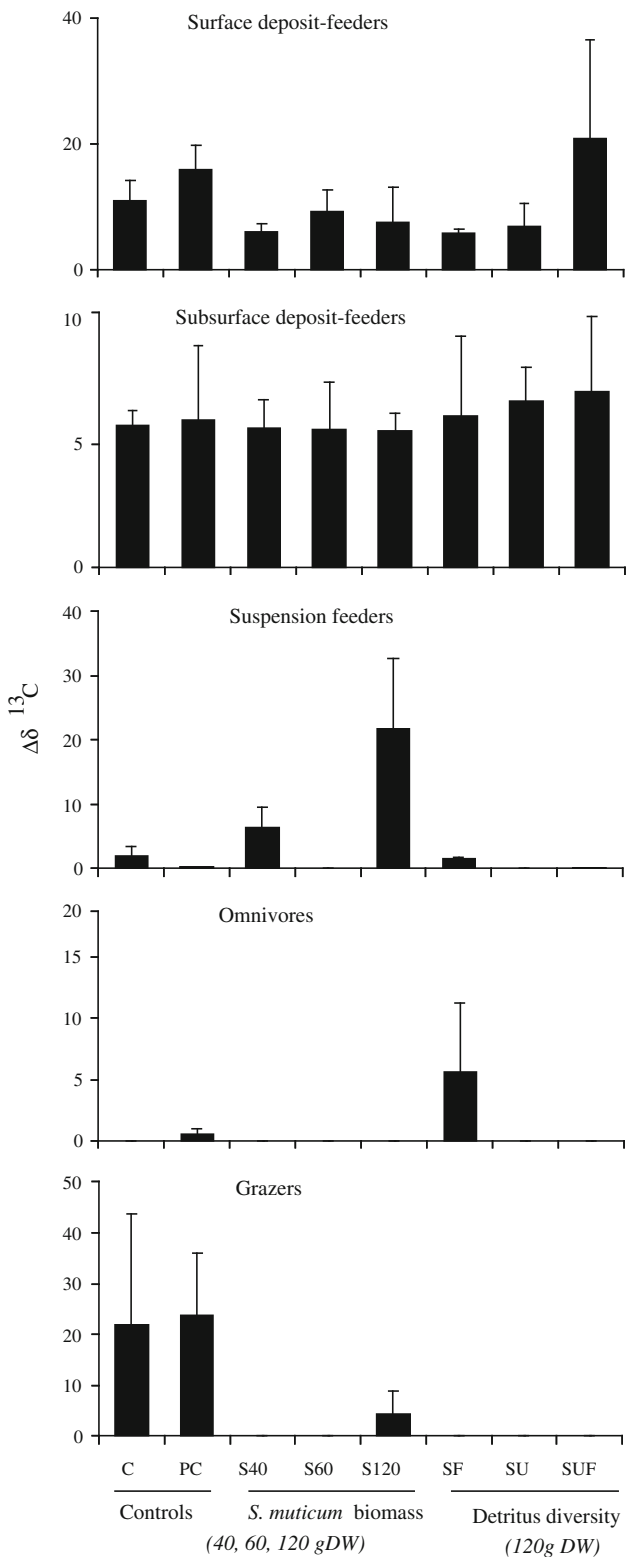

Fig. 4 Mean (±SE) ^{13}C incorporation by the different trophic groups of macrofauna ($\Delta\delta^{13}C$) at each treatment (see Fig. 1 for treatment abbreviations). Incorporation of each trophic group was estimated as average values over all species included in the trophic group at each replicate plot

to the consumers within few days from the tracer addition (Herman et al. 2000; Rossi 2007; Rossi et al. 2009). Accordingly, after 5 days, at the end of the tracer experiment, the isotope signatures of the macrofauna consumers were ^{13}C and ^{15}N enriched.

An increased ^{15}N-nitrogen retention in the bulk sediment was observed for the 3-species macroalgal mixing, composed of equal quantities (40 gDW plot^{-1}) of the invasive *S. muticum* and of the native seaweeds *F. vesiculosus* and *Ulva* sp. The mobilization of the ^{13}C to the sediment followed the pattern observed for ^{15}N, but the effect of detrital diversity on ^{13}C cycling was weaker and more variable than that on ^{15}N cycling and no clear-cut differences were found. Probably, microbial respiration actively eliminated excess carbon over a very short time scale. Increased respiration has been measured in soil added with organic matter (Fontaine et al. 2004) and observed in experimental burial of *Ulvaceae* (Rossi F., unpub data).

The increased retention of ^{15}N-nitrogen in the sediment added with the most diverse detrital mixing did not seem to be in relation to an increased amount of the detrital nitrogen added through the burial of wrack. The nitrogen content for both *S. muticum* and *F. vesiculosus* is roughly 2% of the dry-weight biomass, whereas that of *Ulva* spp. is around 4% dry weight (Rice and Tenore 1981; Rossi 2007). Thus, the total nitrogen added to each plot through the burial of the most diverse detrital mixing (*Sargassum* + *Fucus* + *Ulva*) was 3.2 g, which is lower than that added through the *Sargassum* + *Ulva* mixing, estimated to be 3.6 g. In addition, assuming that the decay constants for seaweed nitrogen follow those of the seaweed biomass, at the time of sampling the amount of nitrogen released by the detrital mixing would be 1.8 g for the 3-species mixing, a value intermediate between the 2-species combination of *S. muticum* and *F. vesiculosus* and that of *S. muticum* and *Ulva* (1.1 and 2.0 g, respectively).These estimates are based on the assumptions that decomposition in mixing is additive, e.g. each species decomposes equally in detrital mixing or in monospecific detritus. Non-additive effects of detrital mixing may, however, occur and alter the decomposition rate, thus affecting the availability of the nitrogen released from the plant detritus (Kominoski et al. 2007). In the marine environment, for instance, the diversity of different macrophytes and their identity can modify their decomposition rate in comparison with monospecific detritus (Moore and Fairweather 2006). Our study does not report any evidence of changes in decomposition rate related to the detrital diversity, since the remaining detrital biomass did not change across treatments. Our experimental conditions were, however, different from the other field studies and could have modified the effects of diversity on decomposition rate. For instance, we did not use litter bags to prevent detritus dispersal. Instead, we buried macroalgal detritus to mimic a natural situation, exposing detritus to tides and waves. Indeed, the biomass of detritus collected was far less abundant than that expected according to the decay constant, which evidenced an additional effect due to natural hydrodynamic conditions. Moreover, decomposition rate might have been affected by the pre-treatment of drying and shredding, which was done to resemble the natural situation when algae are first deposited on sediment surface on the upper intertidal area, dried by insolation and incorporated into the sediment by tides.

In freshwater and soil systems, the diversity of terrestrial litters may affect the nitrogen cycling through a range of complex mechanisms that include interactions between litter nitrogen and specific litter chemical compounds, such as phenols (Hattenschwiler and Gasser 2005; Meier and Bowman 2008). Polyphenols are commonly viewed as a group of secondary plant metabolites that typically interfere with sediment processes, by changing microbial activity and having complex effects on decomposition rate or nitrogen mineralization (Hattenschwiler and Gasser 2005; Meier and Bowman 2008). Similarly to the terrestrial litters, both *Sargassum* and *Fucus* contain phenols and tannins in the form of phlorotannins (Zubia et al. 2008). In the 3-species mixing, their association with *Ulva*, characterized by high nitrogen content (Buchsbaum et al. 1991), could represent an ideal combination of phenols and nitrogen that could increase microbial activity and, in turn, the porewater nitrogen mobilization to these organisms, thereby enhancing nitrogen retention in the sediment. Our study, however, did not allow to identify how much the nitrogen cycling could be affected by *Sargassum* as compared to *Fucus,* and more experimental evidence is needed.

In concomitant to the increased ^{15}N uptake in the bulk sediment, the surface deposit feeders incorporated more ^{15}N label. Macrofauna influence on nitrogen transfer to the food web can be very important, although often underestimated due to their mobility (Rossi 2007) and to the concomitant bottom-up effects of nutrient availability that enhances both microbial growth and activity (Godbold et al. 2009). By feeding on sediment microbes, certain groups of macrofauna consumers can mobilize the microbial nitrogen to the benthic food web and in turn to the highest trophic levels, when they are preys for fish and aquatic birds (Rossi 2007). The effect of detrital diversity on the functional role of macrofauna in the nitrogen cycling may be mediated by changes in the trophic structure and consumer biomass. For example, detrital diversity and identity may change community structure and composition (Bishop et al. 2010). In this study, however, no major changes were observed following the increased detrital

diversity as also confirmed by a more comprehensive study that specifically measured the effects of detrital mixing on macrofauna assemblages (Olabarria et al. 2010). As an alternative, detrital diversity may affect macrofauna functional role indirectly, by modifying microbial activity and growth and rendering available more nitrogen to the macrofauna or changing their feeding behaviour. The positive relationship between sediment and macrofauna ^{15}N incorporation supported this former explanation, but changes in macrofauna behaviour should be further considered. Therefore, the detrital mixing composed of the invasive seaweed *S. muticum* associated with other native species of Fucales and Ulvaceae could have increased nitrogen mobilization to the benthic food web, probably due to changes in the detrital chemical composition that altered microbial activity and, in turn, the use of the microbial nitrogen by macrofauna surface deposit feeders. Such increased mobilization may reflect an improved capacity of the system to recycle sediment nitrogen, which should be further investigated to the light of increasing biological invasions. Indeed, our experiment did not allow to understand how much *S. muticum* could determine these changes and experiments that quantify the role of this invasive species should be done.

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