Journal of Experimental Marine Biology and Ecology 396 (2010) 18–26

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

The effect of wrack composition and diversity on macrofaunal assemblages in intertidal marine sediments

Celia Olabarria [a,*], Mónica Incera [b], Josefina Garrido [a], Francesca Rossi [c]

[a] Departamento de Ecoloxía e Bioloxía Animal, Universidad de Vigo, Campus As Lagoas-Marcosende, 36310 Vigo, Pontevedra, Spain
[b] Centro Tecnológico del Mar-Fundación CETMAR, C/Eduardo Cabello s/n, 36208 Bouzas-Vigo, Pontevedra, Spain
[c] Laboratoire Ecosystemes Lagunaires, Universite´ Montpellier II, Case 093, UMR CNRS-UMII 5119, 34 095 Montpellier Cedex 5, France

ARTICLE INFO

Article history:
Received 2 June 2010
Received in revised form 27 September 2010
Accepted 5 October 2010

Keywords:
Buried wrack
Intertidal sediments
Macrofauna
Sargassum muticum

ABSTRACT

Wrack (dead, washed-up seaweed and seagrass) buried in soft substrata may increase the organic content and alter the physical structure of sediments. These effects may influence the composition and structure of macrofaunal assemblages in the sediment. Such influences can be expected to vary according to the type and amount of wrack as well as the presence of invasive seaweeds in the wrack. In this study, we deliberately buried different amounts of the invasive species *Sargassum muticum* in isolation or mixed to the native species *Ulva* sp. and *Fucus vesiculosus*, in two intertidal sandflats to test some hypotheses about the response of macrofaunal assemblages. We tested whether (1) diversity of detritus (i.e. different mixtures), and (2) the amount of detritus of *S. muticum* influenced the composition and the relative abundance of macrofaunal assemblages. We also assessed whether the sediment organic carbon and the biomass of benthic microalgae varied depending on the diversity of detritus and the amount of detritus of *S. muticum*. Finally, we tested if these effects of wrack were consistent across sites. Results indicated that buried wrack affected the composition and structure of macrofaunal assemblages in short-term (i.e. 4 weeks), but there were no differences depending on detritus diversity or the amount of *S. muticum*. In addition, sediment organic matter and microalgal biomass were not affected by the addition of wrack. They instead varied greatly among small spatial scales (i.e. plots). Wrack composition or abundance of the invasive species *S. muticum* played thus a small role in shaping the structure of macrofaunal assemblages or the biomass of benthic microalgae in these intertidal sediments, probably because these sediments are frequently affected by various inputs of organic matter and benthic assemblages are already adapted to organically enriched sediments.

1. Introduction

The distribution of the macrofauna in marine systems has long been recognized to vary over a range of spatial and temporal scales (e.g. Thrush, 1991; Morrisey et al., 1992; Lagos et al., 2005; Rossi, 2006). Such variability may depend on the interactions at different scales of physical processes, which mainly affect macrofauna distribution at meso-scales, e.g. among sites 100 to 1000 m apart (Archambault and Bourget, 1999), biological processes and environmental variables such as the availability of food, which affect the small-scale variability of macrofauna, e.g. within sites (Hughes, 1993; Menge, 1995; Pennings and Richards, 1998; Underwood, 1999).

In intertidal soft-sediment habitats, an important small-scale natural disturbance is the stranding of mats of dead plant material, called wrack, that may alter the chemical characteristics of the sediments and provide food to the macrofauna (Raffaelli et al., 1998). Wrack can be locally redistributed through wind and wave action, washing up in some areas of intertidal shores and remaining absent from others. This pattern of deposition creates a mosaic of patches supplemented with different amounts and types of organic matter at different phases of decomposition (Mann, 1988). Very often, the macroalgal detritus is buried and it can represent a relevant supply of organic matter for the benthos (Rossi, 2007).

Buried wrack will decay, releasing nutrients, particularly nitrogen and phosphorous, which may enhance reproduction of benthic microalgae (Posey et al., 1999) and stimulate the growth of aerobic and anaerobic bacteria (Raffaelli et al., 1998). In turn, the availability of detritus, the growth of primary producers and bacteria may facilitate the growth and recruitment of macrofauna grazers and deposit-feeders (Ford et al., 1999; Kelaher and Levinton, 2003). The effects of detritus enrichment can be further complicated by short-term anoxic events that occur at the sediment–water interface when enrichment is excessive. Anoxic conditions in surface sediments can substantially reduce the diversity and cause negative effects on the consumers (Rossi and Underwood, 2002; Kelaher and Levinton, 2003; Rossi, 2006). All these processes are complex and will depend on the

* Corresponding author. Tel.: +34 986812589; fax: +34 986812556.
E-mail address: colabarria@uvigo.es (C. Olabarria).

0022-0981/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jembe.2010.10.003

amount and type of wrack composition, the temporal frequency and spatial distribution of its deposition (Valiela et al., 1997; Raffaelli et al., 1998), and both site- and time-specific environmental conditions (Colombini and Chelazzi, 2003).

The composition of wrack is variable spatially and temporally depending on the shore dynamics, presence of macrophyte species on adjacent rocky shores, decomposition rate and life cycle or nutritional value (Colombini and Chelazzi, 2003). Although several studies have dealt with the effects of buried wrack on macrofaunal assemblages of intertidal flats (Ford et al., 1999; Rossi and Underwood, 2002; Kelaher and Levinton, 2003; Rossi, 2006, 2007) no attention has been paid to the effects of different types of buried wrack on macrofaunal assemblages.

Since the arrival of invasive macroalgae and plants to new areas is an increasing phenomenon during the last decades (Pauchard and Shea, 2006), it is necessary to understand how non-indigenous macroalgae may alter ecosystem properties and subsequently, cause changes in native assemblages. For example, wrack composed of autochthonous or allochthonous species may experience different rates of decomposition and turnover of organic matter (Pedersen et al., 2005) and subsequently, their effects on the composition and structure of macrofaunal assemblages might differ (Kelaher and Levinton, 2003; Rossi, 2006). For instance, detritus derived from the invasive species *Sargassum muticum* decomposed faster and more completely than detritus from other brown native algae such as *Fucus vesiculosus* (Pedersen et al., 2005). In contrast, ephemeral species such as *Ulva* sp., showed the fastest rate of decomposition due to the low content of refractory and phenolic compounds in comparison to other brown and perennial algae (Buchsbaum et al., 1991).

In this study, we deliberately buried different types of macroalgal detritus of the native species *Ulva* sp. and *F. vesiculosus*, and the invasive species *S. muticum* at small scale in two intertidal sandflats to test some hypotheses about the response of macrofaunal assemblages in intertidal estuarine sandflats. The experimental design encompassed spatial replication over sites and plots, to take into account the natural variability of benthic assemblages and the patterns of natural deposition of the algal mats in the area of study. In particular, we tested whether (1) diversity of detritus (i.e. different mixtures), and (2) the amount of detritus of *S. muticum* influenced composition and relative abundance of taxa in macrofaunal assemblages, the content of total organic carbon of the sediment and the biomass of benthic microalgae. Finally, we tested if responses of macrofauna were consistent between sites located on relatively close intertidal sandflats because of their different environmental conditions.

2. Material and methods

2.1. Study area and experimental design

This work was done on the south region of the Galician coast (NW Spain) in two sheltered intertidal sandflats, Bouzas (42°13′N; 8°41′W) and Etea (42°15′N; 8°41′W), located on the southern side of Ria de Vigo. Both sites are organically enriched, although they differed slightly in organic content and sediment size. Bouzas (hereafter Site 1) had a mean (±SE) content of OC (%) of 0.53 (0.03), whereas Etea (hereafter Site 2) had a mean of 0.21 (0.02).

The manipulative experiment lasted 6 weeks, from 17 September 2008, when the different types of macroalgal detritus were buried, to 31 October 2008. This time was considered to be sufficient to detect any possible effects of increase of detritus on benthic macrofaunal assemblages since previous studies have suggested that effects of algal mats on macrofauna occur within 2 to 10 weeks from deposition (Thrush, 1986; Rossi, 2006) and decomposition rate of *Ulva* sp., *F. vesiculosus* and *S. muticum* occur within 1 to 1.5 months (e.g. Rossi, 2006; Olabarria et al., 2007; Urban-Malinga et al., 2008).

The different species of algae were collected from nearby rocky shores situated on the southern side of the ria the Vigo characterised by the leathery algae *Bifurcaria bifurcata*, *Fucus* spp., and *S. muticum*, the articulated calcareus alga *Corallina officinalis*, the corticated alga *Stypocaulon scoparium*, and the green algae *Ulva rigida* and *U. clathrata*. In the laboratory, algae were thoroughly washed and their epiphytes were removed. The cleaned algae were refrigerated (−20 °C), then dried (at 60 °C to constant weight) and shredded (<2 cm diameter) prior to experimental addition. This is a common method used to detect effects of burial of macroalgal detritus on macrobenthos and to follow decomposition rate of plant detritus (Buchsbaum et al., 1991; Ford et al., 1999; Rossi, 2006). Although thawing macroalgae can affect decomposition, causing a more rapid initial release of dissolved intracellular organic pool, after few hours the loss of biomass, POC and PON is similar between frozen and untreated macroalgae (Buchsbaum et al., 1991; Castaldelli et al., 2003).

At each site, we chose an area of 10×10 m and we randomly allocated detrital treatments in 24 plots (0.25 m^{-2}) within this area. The experiment was set up at a tidal height of MLW springs + 0.4 m, because macroalgae mainly deposit and bury at this height on the shore, forming small patches enriched with algal detritus interspersed with bare sediment (Olabarria et al., 2007). We added 40 to 120 g detritus because it is within the biomass naturally deposited in the study areas (Olabarria, C., personal observation). 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 of each plot. Three plots were randomly assigned to each of the following treatments: (1) addition of 120 g of *S. muticum* [S120], (2) addition of 60 g of *S. muticum* and 60 g of *Ulva* sp. [SU], (3) addition of 60 g of *S. muticum* and 60 g of *F. vesiculosus* [SF], (4) addition of 40 g of *S. muticum*, 40 g of *F. vesiculosus* and 40 g of *Ulva* sp. [SFU], (5) addition of 60 g of *S. muticum* [S60], and (6) addition of 40 of *S. muticum* [S40]. Treatments 1 to 4 examined changes in macrofaunal assemblages due to diversity of detritus as biomass compensation during the process of replacement of *S. muticum* by *Ulva* spp. and *F. vesiculosus*, and treatments 1, 5 and 6 assessed whether *S. muticum* has density-dependent effects on macrofaunal assemblages. In addition, we established procedural control plots [PC] were the sediment was physically disturbed but detritus was not added and natural control plots [C].

We sampled both sites 4 and 6 weeks after detritus addition. Macrofauna was sampled from each plot using a core (10 cm in diameter) to the depth of 20 cm, sieved at 0.5 mm and stored in 4% buffered formalin until sorting. In the laboratory samples were sorted, animals were counted and identified to the lowest taxonomical level when possible and the biomass of algal detritus present was weighted.

Sediment for total organic carbon and chlorophyll *a* was collected using a 30 ml cut-off syringe (2 cm inner diameter). In each plot, 2 replicates were taken for chlorophyll *a* (chl *a*). Three small cores were taken for organic carbon to the depth of 5 cm. The cores were then pooled together to have 1 measure per plot. Samples for chlorophyll *a* were kept in dark. In the laboratory, samples were freeze-dried and algal detritus was removed from the sediment under microscope. The sediment was homogenized and sub-samples of about 1 g dry weight were taken. Chlorophyll *a* was extracted according to Lorenzen (1967), in darkness for 24 h at 0–4 °C using a 90% acetone solution. Pigments were measured through a spectrophotometer at the wave length of 665 A, before and after treatment with 0.1 N HCl. Correction for turbidity was done at 750 A. The content of phaeopigments was measured to check for any pigments introduced with the dead macroalgae; no significant differences were found between controls and the plots where different types of algal detritus were added.

The sediment for analyses of total OC was desiccated at 60 °C overnight. Inorganic carbon was removed by aqueous acidification of 0.1 N HCl, following the method of Hedges and Stern (1984). Measurements were made using a LECO CN-2000 element analyser.

2.2. Analyses of data

Non-parametric multivariate analyses of variance (PERMANOVA; Anderson, 2001) were used to test for overall multivariate changes in macrofaunal assemblages structure, which may include differences in composition, richness, and/or individual species abundances. The PERMANOVAs were run on matrices of Bray Curtis dissimilarity, calculated among samples using $\sqrt{(x+1)}$ transformed data. The PERMANOVAs partitioned sources of variation in a similar way to ANOVA and used unrestricted permutation of raw data to assess statistical significance. The *P*-values for each term in the model were generated using 5000 permutations. In order to test for the physical disturbance of detrital manipulations, the effect of diversity of detritus and the amount of *S. muticum*, we utilised different PERMANOVA analyses. First, we ran one-way analyses with two levels (PC, C) testing for the impact of detrital manipulations. Because PC and C did not differ in any analyses, we used indistinctly one of each in the analyses testing for effects of diversity of detritus and amount of *S. muticum*. Second, we ran one-way analyses with the fixed factor "Treatment" (5 levels; S120, SFU, SF, SU, and C) to test for effects of diversity of detritus and one-way analyses with the fixed factor "Treatment" (4 levels; S120, S60, S40, and PC) to test for effects of amount of *S. muticum*. Analyses were run separately for the two sites because differences in the structure of macrofaunal assemblages could overwhelm any effects due to the addition of wrack. In addition, since temporal data (4 and 6 weeks after burial) were not independent because we re-sampled the same replicate plots, we analysed separately the two sampling times. A one-way PERMANOVA with the random factor "Site" (2 levels) was also used to measure the natural variability among sites, as compared to the variability among natural control plots. In addition, a two-way PERMANOVA with the fixed factor "Treatment" (8 levels) and the random factor "Site" (2 levels) was used to test for differences in algal detritus remaining in the sediment after 4 and 6 weeks.

To graphically visualize multivariate patterns of variation among treatments at each site after 4 and 6 weeks, non-metric multidimensional scaling (nMDS) was used to produce two-dimensional ordination plots from the Bray–Curtis similarity matrix. This required plots of the centroids of the 3 cells corresponding to the treatments at each site. Centroids were calculated from principal coordinates obtained from the full Bray–Curtis dissimilarity matrix among the 24 observations at each site. Euclidean distances were then obtained between each pair of centroids and used as the input matrix for the nMDS. Taxa that mostly contributed to the dissimilarity/similarity among/within treatments and sites were identified using SIMPER analysis (Clarke, 1993), and abundances of these taxa were further plotted to determine differences among treatments.

Hypotheses about differences in total number of individuals and species, total biomass, abundances of dominant taxa, and total content of organic carbon and chlorophyll *a* were tested with different analyses of variance (ANOVA), following the procedure illustrated for the PERMANOVA analyses. In addition, since 2 replicate cores were taken for clorophyll *a* concentration in each plot, a two-way nested ANOVA with "Plot" nested in "Treatment" and 2 replicate cores were used to test for differences in microalgal biomass. Most of the ANOVAs used untransformed data, except in the few instances in which Cochran's test indicated heterogeneous abundance data needing $\sqrt{(x+1)}$ or $\ln(x+1)$ transformation. Analyses were followed by post-hoc tests to identify those treatment means that differed significantly. In the case of ANOVAs, these were Student–Newman–Keuls (SNK) tests.

Because data were used multiple times in all multi and univariate analyses, significance levels were adjusted using a sequencial Bonferroni correction method (Holm, 1979) whenever multiple comparisons were used.

Product-moment correlations were also calculated between the abundance of the most abundant taxa and the content of organic carbon and biomass of benthic microalgae (i.e. content of chlorophyll *a*) for each of the treatments (C, PC, S120, S60, S40, SF, SFU and SU).

3. Results

3.1. Analyses of OC and chlorophyll a

The algal biomass remaining in the sediment did not vary significantly between sites after 4 or 6 weeks (after 4 weeks, $F_{1,32}=0.01$, $P>0.05$; after 6 weeks, $F_{1,32}=0.34$, $P>0.05$), but it varied depending on the treatments after 4 weeks ($F_{7,7}$: 10.03, $P<0.001$). Algal biomass was greater in the treatments where *S. muticum* and *Ulva* sp. were added (SU treatment) than in the rest of treatments at both sites (SNK tests, $P<0.05$).

In general, after 4 and 6 weeks from the burial there was no effect of algal detritus to the sediment pool of organic carbon. Only the type of detritus had a significant effect at Site 1 after 4 weeks (Table 1; Fig. 1a, b), with greater content of organic carbon in the SFU plots (SNK tests, $P<0.05$). Only abundance of Nematoda, one of the most abundant taxa, was correlated with the content of OC in 4 out of 8 experimental treatments ($r^2=0.67$, 0.60, 0.86 and 0.61 for C, S40, SF and SU, respectively; $n=12$, $P<0.05$).

Differences in clorophyll *a* between experimental burial treatments and controls (PC and C) were sporadic and variable between sites. Biomass of microalgae varied significantly depending on the diversity of detritus at Site 1 after 4 weeks, with a greater biomass in the SF plots (SNK tests, $P<0.05$). The differences among treatments were generally over a background of large and significant variation among plots and did not result in any significant effects. For instance, chlorophyll *a* increased at S60, but significant differences were not found due to the high variability among plots (Table 1). Relationships between the most abundant taxa and biomass of benthic microalgae were sporadic. In fact, only abundances of three taxa, Nematoda, *Pygospio elegans* and *Hydrobia ulvae* were significantly correlated with biomass of benthic microalgae in 4 out of 8 treatments (Nematoda: $r^2=0.75$, 0.60, for C and SF, respectively; *P. elegans*: $r^2=0.51$ for S60; *H. ulvae*: $r^2=0.76$ for PC; $n=12$, $P<0.05$).

3.2. Natural variability of assemblages

A total of 29 species and 884 individuals were found in the control treatments at Site 1 and Site 2 during the experimental period. The assemblages were mainly dominated by 5 taxa, namely *Capitella capitata*, *P. elegans*, *Scolelepis squamata*, *Polydora cornuta* and Nematoda. The composition of assemblages did vary between sites, but only at the first sampling date (PERMANOVA, after 4 weeks, Site: $F_{1,4}=7.33$, $P<0.05$; after 6 weeks, Site, $F_{1,4}=6.36$, $P>0.05$). The abundances of *S. squamata*, *C. capitata*, *P. elegans*, *P. cornuta*, *Cyathura carinata*, Nematoda and Oligochaeta were important to affect this pattern of variability (SIMPER analysis, 53% of cumulative average dissimilarity between sites).

3.3. Response of macrofauna to burial of algal detritus

There were some changes in the composition of assemblages after 4 weeks of burial of algal detritus (Fig. 2, Table 2). In general, assemblages in controls and procedural controls varied significantly from the other treatments at both sites. Although assemblages varied significantly depending on the diversity of detritus at Site 1, such variation was due to differences between control and the rest of treatments, i.e. S120, SF, SFU, and SU, (PERMANOVA, $F_{4,10}=2.66$, $P<0.01$). At Site 2, the diversity of detritus had an effect on assemblages (PERMANOVA, $F_{4,10}=2.87$, $P<0.01$). Assemblages in controls and SFU treatments differed from the rest of treatments (i.e. S120, SU, and SF). Although marginally non-significant, assemblages

Table 1
ANOVAs of the effects of treatments on the content of organic carbon at 5 cm and chlorophyll *a* (μg g^{-1}) after 4 and 6 weeks. Three different ANOVAs were performed to test different hypotheses (see Analyses of data). Treatment was a fixed factor and plots (in the case of chlorophyll *a*) was a random factor nested in treatments. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

		Site 1				Site 2			
		4 weeks		6 weeks		4 weeks		6 weeks	
	df	MS	F	MS	F	MS	F	MS	F
Organic carbon									
Detrital manipulation									
Treatment	1	0.001	0.08	0.001	0.01	0.063	1.00	0.001	0.74
Residual	4	0.013		0.017		0.063		0.001	
Diversity of detritus									
Treatment	4	0.049	**3.57***	0.009	0.01	1.673	0.49	0.007	0.98
Residual	10	0.013		0.019		3.410		0.007	
Amount of *Sargassum*									
Treatment	3	0.071	2.97	0.002	0.12	0.020	0.13		
Residual	8	0.024		0.024		0.164			
Chlorophyll a									
Detrital manipulation									
Treatment	1	7.415	0.50	0.685	0.02	0.205	0.39	0.045	0.01
Plot (treatment)	4	14.82	**5.06***	41.05	17.01	0.522	**328.94*****	40.177	**237.58*****
Residual		2.93		2.41		0.001		0.169	
Diversity of detritus									
Treatment	4	1.771	**3.99***	0.388	1.28	0.120	0.87	0.497	0.77
Plot (treatment)	10	0.444	1.49	0.303	**3.76***	0.138	**3.07***	0.648	**8.64*****
Residual		0.297		0.080		0.045		0.075	
Amount of *Sargassum*									
Treatment	3	0.227	0.29	26.69	0.72	0.219	0.83	0.024	0.14
Plot (treatment)	8	0.787	**5.27****	37.314	**41.18*****	0.264	**5.20****	0.174	**3.34***
Residual		0.150		0.906		0.05		0.052	

in the SF plots also tended to differ from the rest of treatments (Fig. 2). However, the amount of *S. muticum* did not affect the composition of assemblages at any site (Site 1, PERMANOVA, $F_{3,8}=0.89$, $P>0.05$; Site 2, PERMANOVA, $F_{3,8}=1.76$, $P>0.05$).

After 6 weeks of wrack burial, neither the diversity of detritus (Site 1, PERMANOVA, $F_{4,10}=1.41$, $P>0.05$; Site 2, PERMANOVA, $F_{4,10}=1.41$, $P>0.05$) nor the amount of *S. muticum* had an effect on macrofaunal assemblages (Site 1, PERMANOVA, $F_{3,8}=1.25$, $P>0.05$;

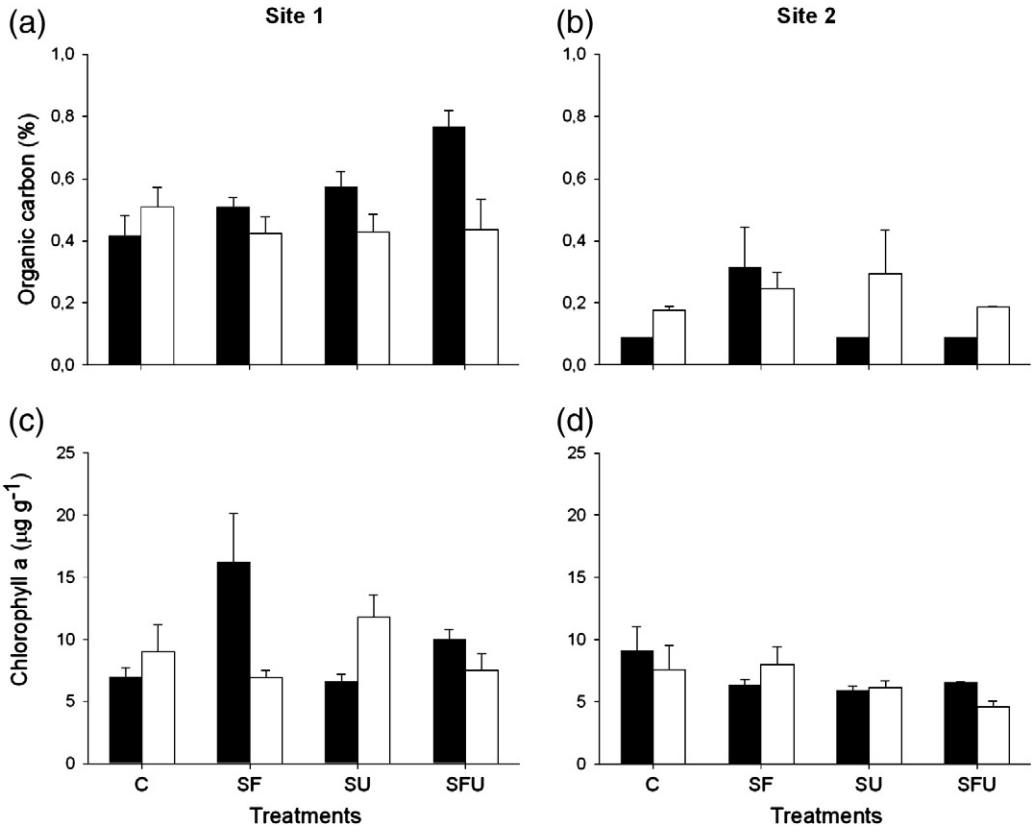

Fig. 1. Mean (+SE) amounts of organic carbon ($n=3$) at Site 1 (a) and Site 2 (b), and chlorophyll *a* ($n=6$) at Site 1 (c) and Site 2 (d) averaged from 2 replicates in each of the 3 plots. Black and white bars indicate after 4 and 6 weeks, respectively. Only treatments to test for effects of diversity of detritus are shown.

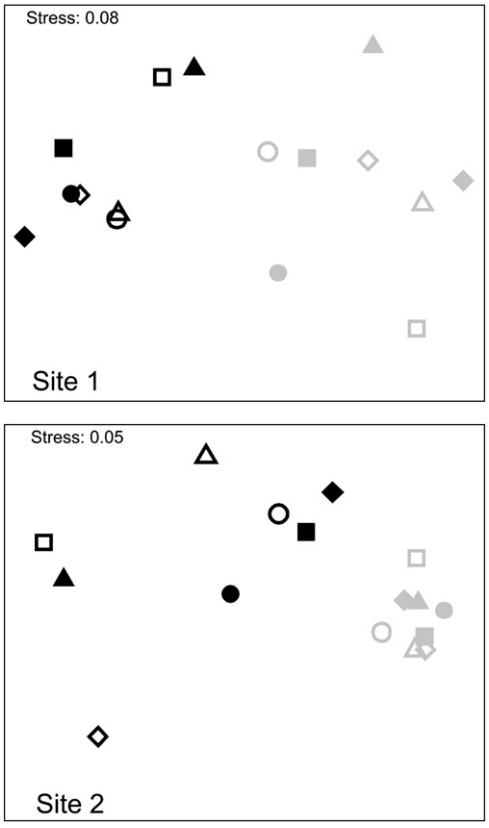

Fig. 2. Non-metric multi-dimensional scaling (nMDS) for differences of assemblages among the treatments at each site after 4 and 6 weeks of wrack burial. Data are centroid for each of 3 replicates. Black and grey lines indicate after 4 and 6 weeks, respectively. ▲ C, □ PC, ● S120, ○ S40, ■ S60, △ SF, ◇ SFU, ◆ SU.

Site 2, PERMANOVA, $F_{3,8} = 2.01$, $P > 0.05$). These results evidenced that any disturbance caused by wrack burial on macrofaunal assemblages disappeared after 6 weeks.

Although there were no major patterns of variation among treatments after 4 weeks of burial of algal detritus, the total number of species varied depending on the diversity of detritus, but this variation was not consistent between sites and it did not respond to any pattern of addition of algal detritus (Table 2; Fig. 3a, b). There was a trend to a larger number of species in the controls than in the other treatments after 4 weeks of burial at Site 2 (Fig. 3b). The total number of individuals varied significantly depending on the diversity of detritus after 4 weeks at Site 1 (Table 2; Fig. 3c, d), but no clear alternative hypothesis could be defined because only controls differed significantly from SU treatments (Fig. 3c, d). Although total biomass did not vary significantly depending on the diversity of detritus or amount of *S. muticum* at any time (Table 2), there was a trend to a greater biomass in C, PC, S120 and SU treatments at Site 1 and in C treatments at Site 2 after 4 weeks. After 6 weeks biomass was, however, greater in the control plots than in the rest of treatments at both sites. Biomass was generally much smaller after 6 weeks of burial.

In general, the most abundant and widespread species did not present any significant trend related to diversity of detritus or amount of *S. muticum*. Only one polychaete species, *C. capitata*, presented smaller number of individuals in the C and PC treatments (SNK tests, $P < 0.05$, Fig. 4), but only after 4 weeks of burial. Abundances of three species, *S. squamata*, *P. cornuta* and *Spio filicornis*, tended to be larger in the C and PC treatments after 4 weeks of burial, but these trends were not significant (illustrated by *S. squamata* in Fig. 4). In turn, abundances of *P. elegans* did not follow any significant pattern or trend after 4 weeks (Fig. 4).

4. Discussion

4.1. Total organic content and chlorophyll a

The composition of nutrients (N, P) and the amount of organic carbon can vary considerably among plants. Therefore, we expected that there would be differences in the amount of organic carbon released in the sediment during the decomposition of different types of wrack (Banta et al., 2004; Castaldelli et al., 2003). However, we found that the amount of organic carbon changed between treatments, but it did not depend on the type of buried wrack. Increases of organic carbon, although significant for many invertebrates, might not be easily detectable (Rossi, 2006), because excess organic carbon may be rapidly mineralised by bacteria or enter in the food web, through bacterivore and detritivore consumers (Fontaine et al., 2004; Rossi, 2007). Decomposition of detritus might also stimulate microalgal growth, since microalgae may be easily N-limited in marine sediments (Heip et al., 1995). Nevertheless, the amount of chlorophyll *a* was not consistently greater in those treatments with different buried wrack than in controls. The effect of marine plant detritus on benthic microalgae may vary. In fact, some studies have detected a clear increase of microalgal biomass, when wrack was added (Levinton, 1985; Sundback et al., 1996; Rossi and Underwood, 2002), whereas others have found neutral patterns (Rossi, 2006). It is possible that in some habitats, macroalgae are not nutrient-limited or they are limited by both availability of nutrients and grazing, and that an addition of nutrients that stimulate their growth and also increases grazing pressure, might result in neutral or even negative effects on microalgal growth. For instance, the addition of *Ulva rotundata* increased standing stocks of benthic diatoms only in the absence of grazers (Levinton, 1985). Grazers such as *Hydrobia ulvae* may indeed play an important role in determining structure and diversity of benthic microalgae (see Rossi, 2006). In this study, however, we did not find any evidence of increased grazing pressure along with macroalgal addition. *H. ulvae*, the most abundant grazer, did not change in numbers and there was no correlation with microalgal biomass, apart from the PC plots. Nevertheless, their effect cannot be excluded because these grazers are highly mobile and they can rapidly move from one patch to another to feed on the microalgae (Orvain et al., 2007).

4.2. Macrofaunal assemblages

Inputs of macroalgal detritus can clearly influence patterns of spatial and temporal variation of macrofaunal assemblages in soft-sediment habitats (e.g. Rossi and Underwood, 2002; Cardoso et al., 2004; Bishop et al., 2010). Responses of macrofaunal assemblages to input of detritus are variable and quite complex. Generalities in invertebrates' response to algal wrack are, however, difficult to make because of differences related to the temporal scales of sampling and the extent of disturbance, as well as to the quality of seaweeds composing the wrack and their position on the substratum (e.g. drifting, deposited on the surface or buried) (Raffaelli et al., 1998).

Here, the number of individuals and the composition of macrofauna assemblages responded, sometimes, to the addition of wrack but there were no differences among the type of wrack or the abundance of *S. muticum*. These results are in contrast to previous studies that have pointed out differential composition (i.e. phenolics, C:N ratio), decomposition rates or nutritional value of wrack species as important factors influencing colonisation by macrofaunal assemblages (e.g. Pennings et al., 2000; Mews et al., 2006). It is possible that pre-treatment of macroalgae (thawing and drying) could alter their composition and, therefore, affect processes such as decomposition rate and release of nutrients, minimising differences among wrack species. In fact, the initial release of dissolved intracellular organic pool (leaching phase) can be more rapid in thawed than in untreated macroalgae (Castaldelli et al., 2003).

Table 2
ANOVAs of the effect of treatments on the number of species, number of individuals and biomass after 4 and 6 weeks. Three different ANOVAs were performed to test different hypotheses (see Analyses of data). Treatment was a fixed factor. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

	Site 1					Site 2			
	4 weeks			6 weeks		4 weeks		6 weeks	
	df	MS	F	MS	F	MS	F	MS	F
Number of species									
Detrital manipulation									
Treatment	1	4.16	2.27	4.16	0.74	1.50	0.15	2.66	1.60
Residual	4	1.83		5.66		10.33		1.66	
Diversity of detritus									
Treatment	4	6.56	2.59	0.93	0.38	32.43	**6.01****	1.07	0.33
Residual	10	2.53		2.46		5.40		3.26	
Amount of *Sargassum*									
Treatment	3	3.19	0.72	8.55	2.5	7.33	1.69	1.41	0.55
Residual	8	4.41		3.41		4.33		2.58	
Number of individuals									
Detrital manipulation									
Treatment	1	15.95	0.60	104.16	0.54	3037.50	1.76	2730.66	2.67
Residual	4	26.50		191.16		1725.33		1022.66	
Diversity of detritus									
Treatment	4	9177.66	**3.94***	0.88	3.22	1392.89	0.66	7327.10	0.70
Residual	10	2327.93		0.27		2100.76		10467.52	
Amount of *Sargassum*									
Treatment	3	21804.00	1.03	1837.44	2.37	7.75	2.52	10908.74	1.73
Residual	8	21259.50		776.66		3.07		6299.22	
Biomass									
Detrital manipulation									
Treatment	1	190.41	0.01	6.73	1.24	5355.69	0.47	11094.00	2.76
Residual	4	37946.43		5.44		11314.56		4012.33	
Diversity of detritus									
Treatment	4	23541.18	0.45	0.60	0.19	1.96	1.09	29461.40	0.71
Residual	10	52364.08		3.18		1.79		41729.93	
Amount of *Sargassum*									
Treatment	3	32.25	0.66	57.63	0.68	0.61	1.15	43812.30	1.72
Residual	8	49.06		84.79		0.53		25414.66	

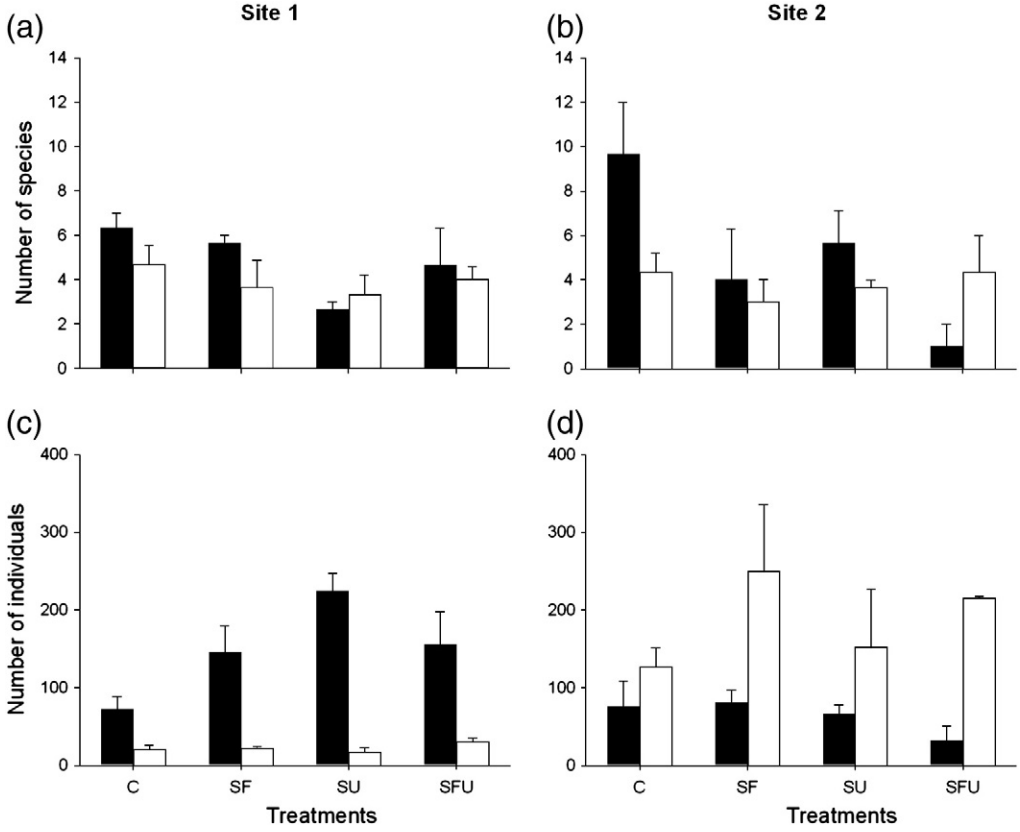

Fig. 3. Mean (+SE) ($n=3$) number of species and number of individuals in experimental treatments at Site 1 and Site 2. Black and white bars indicate after 4 and 6 weeks, respectively. Only treatments to test for effects of diversity of detritus are shown.

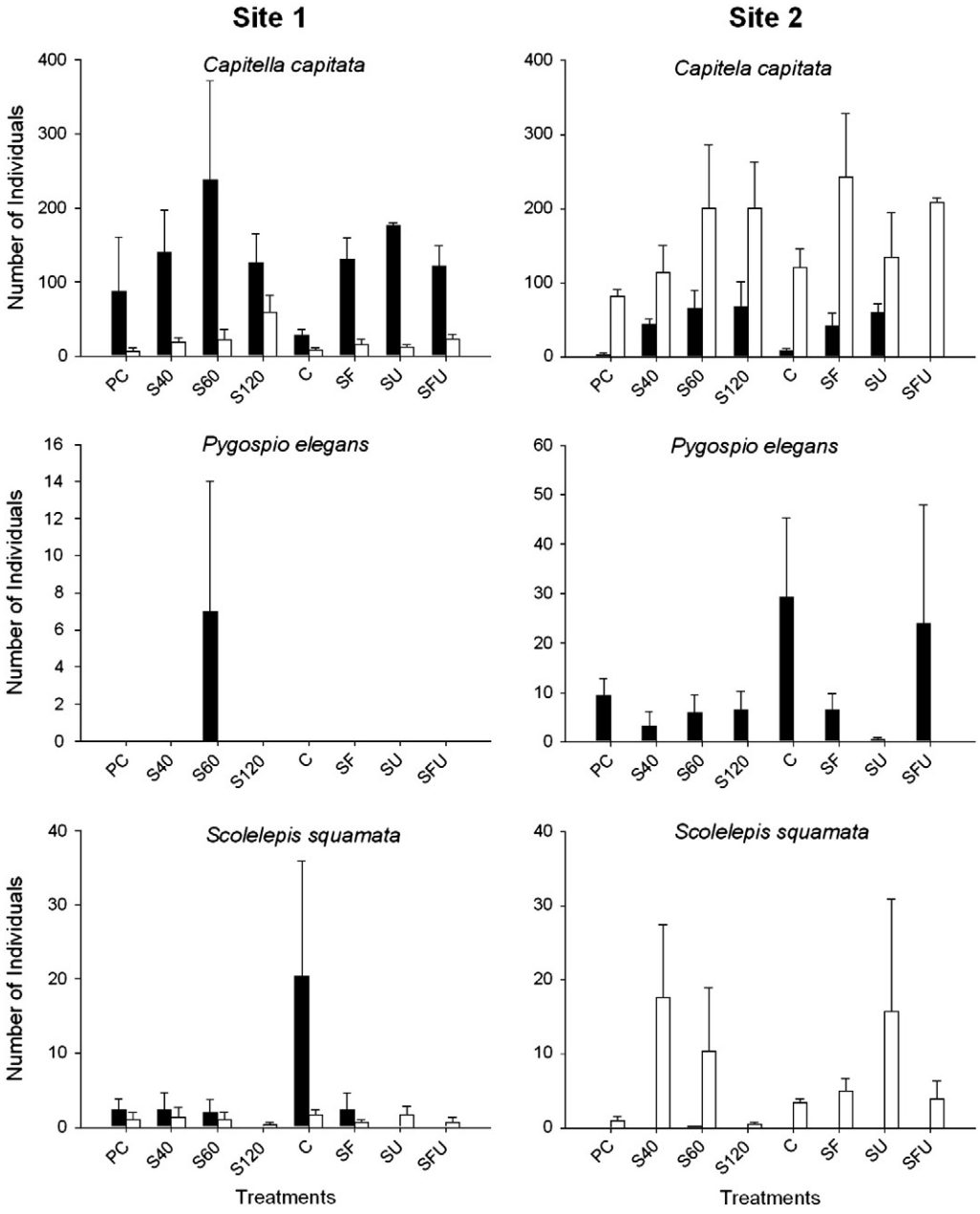

Fig. 4. Mean (+SE) ($n = 3$) number of individuals of *Capitella capitata*, *Scolelepis squamata* and *Pygospio elegans* at Site 1 and Site 2. Black and white bars indicate after 4 and 6 weeks, respectively.

The input of detritus can cause anoxia in the sediment, but also increase the availability of food for organisms (Norkko and Bonsdorff, 1996; Cummins et al., 2004; Rossi, 2006). Wrack can also be a refuge from predators or an obstacle to the settlement and recruitment of larvae (Hull, 1987; Ford et al., 1999). The opportunistic worm *C. capitata,* which is well-known to increase at high levels of organic matter and well tolerate the production of sulphides derived from anaerobic decomposition (Rosenberg et al., 2004) was the only species that increased in abundance when wrack was added. The other species such as the spionid worms *S. squamata*, *P. cornuta* and *S. filiformis* decreased, indicating that addition of wrack could stimulate benthic metabolism and enhance anaerobic decomposition and production of toxic compounds. This explanation of increased hypoxia caused by the addition of wrack may explain why there were no differences in the type of wrack or why we did not detect any differences in microalgal biomass.

The processes behind the response of macrofauna to organic inputs, such as the availability of food or hypoxia may occur at different time scales and vary with the environmental conditions, thereby resulting in variable patterns of response (Thrush, 1986; Ford et al., 1999; Lopes et al., 2000; Kelaher and Levinton, 2003). Macrofauna may, for instance, recover faster at lower temperature, that limits the occurrence of hypoxia (Ford et al., 1999) or have first positive reaction due to the sudden increase of food, then respond negatively, when excess food consume the oxygen reaching the benthos (Lopes et al., 2000). Conversely, there can be first negative effects, during the decomposition of organic matter that leads to hypoxia and sulphide production, followed by positive effects, when there are patches of substratum with low consumer abundance and large availability of resources (Kelaher and Levinton, 2003). Our results, at the time scales analysed, suggested that assemblages of macrofauna followed the latter pattern of response. These results are well in agreement with other experimental works. Short-term positive response (i.e. increase in abundance) of *C. capitata* has often been reported for this species under small patches of dead

seaweeds on the surface of sediment or buried in sediments (e.g. Thrush, 1986; Rossi and Underwood, 2002; Rossi, 2006) as well as the decline of surface-feeding worms such as spionids (Everett, 1994; Norkko and Bonsdorff, 1996).

Here, it is possible that the addition of wrack may have stimulated oxygen consumption and bacterial growth, thus reducing availability of oxygen and stimulating the growth of sulphur-oxidising bacteria and the production of intermediate products derived from the mineralisation of organic matter such as the toxic sulphides (Dauwe et al., 2001). Hypoxia and major differences in macrofaunal assemblages could have occurred within 4 weeks from burial wrack was indeed found at small biomass during sampling, showing that decomposition rate had reached its final stage and the remaining detritus could have been mechanically removed by, for instance, hydrodynamism. After 4 weeks from burial, macrofaunal assemblages could thus, represent already a recovery stage, which was completed within 6 weeks, when no effects were observed. This fast recovery of macrofauna suggested that these assemblages may have developed a high resilience in response to this type of disturbance. The sites where the experiment was done, although characterised by different organic loading, are both affected by repeated input of different wrack species and other organic matter sources during the year. The small severity of disturbance, the presence of a fauna characteristic of organic-enriched sediments and frequently affected by the deposition of wrack and other organic matter inputs may be among the reasons why only minor changes in assemblages and negative effects on some taxa were observed. Alternatively, it is also possible that these systems may quickly recycle excess organic matter in the sediments, as indicated by the lack of any effects in the sediment organic carbon. This fast recycling can mitigate the effects of detritus and increase the stability of the system to organic input. Increasing the severity of disturbance, i.e. amount and extent of wrack, would not reflect the natural processes observed in the area of study.

In summary, results suggest that buried wrack affected the composition and structure of macrofaunal assemblages in short-term (i.e. 4 weeks) and that the assemblage showed high resilience in response to this intensity of the disturbance. However, the diversity of detritus and the presence of the invasive seaweed *S. muticum* did not seem to play an important role in shaping differences among macrofaunal assemblages.

Acknowledgements

This research has been supported by the Spanish Government through the Ministry of Education and Science-FEDER (PROJECT CGL2006-27880-E) and the Ministry of Science and Innovation-FEDER (PROJECT CGL2009-07205). The thoughtful comments of 2 anonymous reviewers improved the quality of this manuscript. **[ST]**

References

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Aust. Ecol. 26, 32–46.
Archambault, P., Bourget, E., 1999. Influence of shoreline configuration on spatial variation of meroplanktonic larvae, recruitment and diversity of benthic subtidal communities. J. Exp. Mar. Biol. Ecol. 238, 161–184.
Banta, G.T., Pedersen, M.F., Nielsen, S.L., 2004. Decomposition of marine primary producers: consequences for nutrient recycling and retention in coastal ecosystems. In: Nielsen, S.L., Banta, G.T., Pedersen, M.F. (Eds.), Estuarine nutrient cycling: the influence of primary producers. Kluwer Academic Publishers, The Netherlands, pp. 187–216.
Bishop, M.J., Coleman, M.A., Kelaher, B.P., 2010. Cross-habitat impacts of species decline: response of estuarine sediment communities to changing detrital resources. Oecologia 163, 517–525.
Buchsbaum, R., Valiela, I., Swain, T., Dzierzeski, M., Allen, S., 1991. Available and refractory nitrogen in detritus of coastal vascular plants and macroalgae. Mar. Ecol. Prog. Ser. 72, 131–143.
Cardoso, P.G., Pardal, M.A., Raffaelli, D., Baeta, A., Marques, J.C., 2004. Macroinvertebrate response to different species of macroalgal mats and the role of the disturbance history. J. Exp. Mar. Biol. Ecol. 308, 207–220.
Castaldelli, G., Welsh, D.T., Flachi, G., Zucchini, G., Colombo, G., Rossi, R., Fano, E.A., 2003. Decomposition dynamics of the bloom forming macroalga *Ulva rigida* C. Agardh determined using a C-14-carbon radio-tracer technique. Aquat. Bot. 75, 111–122.
Clarke, K.R., 1993. Non-parametric multivariate analyses of changes in community structure. Aust. J. Ecol. 18, 117–143.
Colombini, I., Chelazzi, L., 2003. Influence of marine allochthonous input on sandy beach communities. Oceanogr. Mar. Biol. Annu. Rev. 41, 115–159.
Cummins, S.P., Roberts, D.E., Zimmerman, K.D., 2004. Effects of the green macroalga *Enteromorpha intestinalis* on macrobenthic and seagrass assemblages in a shallow coastal estuary. Mar. Ecol. Prog. Ser. 266, 77–87.
Dauwe, B., Middelburg, J.J., Herman, P.M.J., 2001. Effect of oxygen on the degradability of organic matter in subtidal and intertidal sediments of the North Sea area. Mar. Ecol. Prog. Ser. 215, 13–22.
Everett, R.A., 1994. Macroalgae in soft-sediment communities: effects on benthic faunal assemblages. J. Exp. Mar. Biol. Ecol. 175, 253–274.
Fontaine, S., Bardoux, G., Abbadie, V., Mariotti, A., 2004. Carbon input to soil may decrease soil carbon content. Ecol. Lett. 7, 314–320.
Ford, R.B., Thrush, S.F., Probert, P.K., 1999. Macrobenthic colonisation of disturbances on an intertidal sandflat: the influence of season and buried algae. Mar. Ecol. Prog. Ser. 191, 163–174.
Hedges, J.I., Stern, J.H., 1984. Carbon and nitrogen determinations of carbonate-containing solids. Limnol. Oceanogr. 29, 657–663.
Heip, C.H.R., Goosen, N.K., Herman, P.M.J., Krokamp, J., Middelburg, J.J., Soetaert, K., 1995. Production and consumption of biological particles in temperate tidal estuaries. Oceanogr. Mar. Biol. Annu. Rev. 33, 1–149.
Holm, S., 1979. A simple sequentially rejective multiple test procedure. Scand. J. Stat. 6, 65–70.
Hughes, R.N., 1993. Diet selection. An Interdisciplinary Approach to Foraging Behaviour. Blackwell Scientific Publication, London.
Hull, S.C., 1987. Macroalgal mats and species abundance — a field experiment. Estuar. Coast. Shelf Sci. 25, 519–532.
Kelaher, B.P., Levinton, J.S., 2003. Variation in detrital enrichment causes spatio-temporal variation in soft-sediment assemblages. Mar. Ecol. Prog. Ser. 261, 85–97.
Lagos, N.A., Navarrete, S.A., Véliz, F., Masuero, A., Castilla, J.C., 2005. Meso-scale spatial variation in settlement and recruitment of intertidal barnacles along the coast of central Chile. Mar. Ecol. Prog. Ser. 290, 165–178.
Levinton, J.S., 1985. Complex interactions of a deposit feeder with its resources: roles of density, a competitor, and detrital addition in the growth and survival of the mudsnail *Hydrobia totteni*. Mar. Ecol. Prog. Ser. 22, 31–40.
Lopes, R.J., Pardal, M.A., Marques, J.C., 2000. Impact of macroalgal blooms and wader predation on intertidal macroinvertebrates: experimental evidence from the Mondego estuary (Portugal). J. Exp. Mar. Biol. Ecol. 249, 165–179.
Lorenzen, C., 1967. Determination of chlorophyll and pheopigments: spectrophotometric equations. Limnol. Oceanogr. 12, 343–346.
Mann, K.H., 1988. Production and use of detritus in various fresh-water, estuarine, and coastal marine ecosystems. Limnol. Oceanogr. 33, 910–930.
Menge, B.A., 1995. Indirect effects in marine rocky intertidal interaction webs: patterns and importance. Ecol. Monogr. 65, 21–74.
Mews, M., Zimmer, M., Jelinski, D.E., 2006. Species-specific decomposition rates of beach cast wrack in Barkley Sound, British Columbia, Canada. Mar. Ecol. Prog. Ser. 328, 155–160.
Morrisey, D.J., Howitt, L., Underwood, A.J., Stark, J.S., 1992. Spatial variation in soft-sediment benthos. Mar. Ecol. Prog. Ser. 81, 197–204.
Norkko, A., Bonsdorff, E., 1996. Rapid zoobenthic community responses to accumulation of drifting algae. Mar. Ecol. Prog. Ser. 140, 141–151.
Olabarria, C., Lastra, M., Garrido, J., 2007. Succession of macrofauna on macroalgal wrack of an exposed sandy beach: effects of patch size and site. Mar. Environ. Res. 63, 19–40.
Orvain, F., Sauriau, P.G., Le Hir, P., Guillou, G., Cann, P., Paillard, M., 2007. Spatio-temporal variations in intertidal mudflat erodability: Marennes-Oléron Bay, western France. Cont. Shelf Res. 27, 1153–1173.
Pauchard, A., Shea, K., 2006. Integrating the study of non-native plant invasions across spatial scales. Biol. Invasions 8, 399–413.
Pedersen, M.F., Staehr, P.A., Wernberg, T., Thomsen, M.S., 2005. Biomass dynamics of exotic *Sargassum muticum* and native *Halidrys siliquosa* in Limfjorden, Denmark — implications of species replacements on turnover rates. Aquat. Bot. 83, 31–47.
Pennings, S.C., Richards, C.L., 1998. Effects of wrack burial in salt-stressed habitats: *Batis maritima* in a southwest Atlantic salt marsh. Ecography 21, 630–638.
Pennings, S.C., Carefoot, T.H., Zimmer, M., Danko, J.P., Ziegler, A., 2000. Feeding preferences of supralittoral isopods and amphipods. Can. J. Zool. 78, 1918–1929.
Posey, M.H., Alphin, T.D., Cahoon, L., Lindquist, D., Becker, M.E., 1999. Interactive effects of nutrient additions and predation on infaunal communities. Estuaries 22, 785–792.
Raffaelli, D.G., Raven, J.A., Poole, L.J., 1998. Ecological impacts of green macroalgal blooms. Oceanogr. Mar. Biol. Annu. Rev. 36, 97–125.
Rosenberg, R., Blomqvist, M., Nilsson, H.C., Cederwall, H., Dimming, A., 2004. Marine quality assessment by use of benthic species-abundance distributions: a proposed new protocol within the European Union Water Framework Directive. Mar. Pollut. Bull. 49, 728–739.
Rossi, F., 2006. Small-scale burial of macroalgal detritus in marine sediments: effects of *Ulva* spp. on the spatial distribution of macrofauna assemblages. J. Exp. Mar. Biol. Ecol. 332, 84–95.
Rossi, F., 2007. Recycle of buried macroalgal detritus in sediments: use of dual-labelling experiments in the field. Mar. Biol. 150, 1073–1081.
Rossi, F., Underwood, A.J., 2002. Small-scale disturbance and increased nutrients as influences on intertidal macrobenthic assemblages, experimental burial of wrack in different intertidal environments. Mar. Ecol. Prog. Ser. 241, 29–39.

Sundback, K., Nilsson, P., Nilsson, C., Jonsson, B., 1996. Balance between autotrophic and heterotrophic components and processes in microbenthic communities of sandy sediments: a field study. Estuar. Coast. Shelf Sci. 43, 689–706.

Thrush, S.F., 1986. The sublittoral macrobenthic community structure of an Irish sea-lough, effect of decomposing accumulations of seaweed. J. Exp. Mar. Biol. Ecol. 96, 199–212.

Thrush, S.F., 1991. Spatial patterns in soft-bottom communities. Trends Ecol. Evol. 6, 75–79.

Underwood, A.J., 1999. History and recruitment in structure of intertidal assemblages on rocky shores: an introduction to problems for interpretation of natural change. In: Whitfield, M., Matthews, J., Reynolds, C. (Eds.), Aquatic life cycle strategies: survival in a variable environment. Marine Biological Association, UK, pp. 79–96.

Urban-Malinga, B., Gheskiere, T., Degraer, S., Derycke, S., Opalinski, K.W., Moens, T., 2008. Gradients in biodiversity and macroalgal wrack decomposition rate across a macrotidal, ultradissipative sandy beach. Mar. Biol. 155, 79–90.

Valiela, I., McClelland, J., Hauxwell, J., Behr, P.J., Hersh, D., Foreman, K., 1997. Macroalgal blooms in shallow estuaries: controls and ecophysiological and ecosystem consequences. Limnol. Oceanogr. 42, 1105–1118.