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Article *in* Marine Ecology Progress Series · April 2012

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Vol. 455: 13–31, 2012
doi: 10.3354/meps09676

MARINE ECOLOGY PROGRESS SERIES
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Importance of phytodetritus and microphytobenthos for heterotrophs in a shallow subtidal sandy sediment

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ABSTRACT: The relative importance of allochthonous phytodetritus deposition and autochthonous microphytobenthos (MPB) production for benthic consumers in an organic carbon (C_{org})-poor sandy sediment was assessed using a ^{13}C-stable isotope natural abundance study combined with a dual ^{13}C-tracer addition approach. In a first experiment (Expt 1), a set of sediment cores received a pulse of ^{13}C-labelled phytodetritus and the fate of that organic matter was followed in the benthic food web (bacteria, meiofauna and macrofauna) over a period of 72 h. In a second experiment (Expt 2), the MPB present in a set of sediment cores was labelled with ^{13}C-bicarbonate and the fate of labelled MPB was followed the same way over a period of 96 h. Natural ^{13}C abundances of sources and consumers revealed that the benthic food web likely relied primarily on MPB. In particular, diatoms contributed at least 40% to the diet of 12 out of the 16 taxonomic groups identified. The dual approach revealed the complexity of the trophic interactions and gave evidence for resource partitioning between 2 species of harpacticoid copepods. Both ^{13}C-tracer addition experiments showed a fast transfer of label to most heterotrophs. Bacteria, which comprised the largest fraction of the heterotroph biomass, incorporated more ^{13}C than other consumers. Meiofauna had similar relative incorporations in both experiments and likely relied equally on benthic and pelagic inputs. Macrofauna relied significantly more on MPB. In both experiments, most of the ^{13}C-label that was incorporated by heterotrophs was respired. While phytodetritus-derived C_{org} consumed by heterotrophs was 41 mg C m^{-2}, MPB-derived was at least one order of magnitude higher. The benthic community growth efficiency in Expt 2 (40%) was higher than that of Expt 1 (25%), confirming the pivotal role of MPB.

KEY WORDS: Stable isotopes · ^{13}C · Food web · Benthic microalgae · Phytoplankton · Meiofauna · Macrofauna · Bacteria

INTRODUCTION

Sandy sediments, due to their low standing stocks of organic carbon (C_{org}), have long been falsely considered as biogeochemically inactive environments (Boudreau et al. 2001). These dynamic environments, where food resources and consumers are heterogeneously distributed, are usually characterized by coarse-grained sediment and high permeability, and the different heterotrophs inhabiting these sedi-

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ments (i.e. heterotrophic bacteria, microfauna, meiofauna and macrofauna) undergo frequent reworking due to physical forces. However, recent studies have provided strong evidence of high primary production (Billerbeck et al. 2007, Cook et al. 2007, Evrard et al. 2008) and enhanced remineralization rates supported by advective porewater transport (Huettel & Gust 1992a, Rusch et al. 2001, Ehrenhauss et al. 2004).

The relative importance of the heterotrophic compartments has been assessed and some generic distribution patterns related to grain size have been illustrated. Firstly, the biomass of macrofauna (which generally comprises the metazoans larger than 1 mm) shows a decreasing trend towards coarser sediments (Heip et al. 1992). This has been attributed to the greater influence of hydrodynamics on coarser grains as well as the patchiness of resources. Secondly, there is a coupled decrease in densities and increase in diversities and morphological variability of the meiofauna with increasing sediment grain size (Vanaverbeke et al. 2000, Rodriguez et al. 2003, Gheskiere et al. 2005, Soetaert et al. 2009). Thirdly, although the preponderance of heterotrophic bacteria with regard to respiration and remineralization is clear for silty sediments (Gerlach 1971, Koop & Griffiths 1982, Sundback et al. 1996, van Oevelen et al. 2006a), some studies available for sandy sediments have shown significant contribution of meiofauna (Evrard et al. 2010), which sometimes outcompete bacteria (Sundback et al. 1996).

Most of the benthic food web studies have been carried out in accreting silty and muddy sediments (e.g. depositional shelf sediments, estuaries or coastal lagoons) and more specifically in intertidal settings (Reise 1979, Heip et al. 1995, Deegan & Garritt 1997, Middelburg et al. 2000, Carman & Fry 2002), leaving sublittoral sandy sediments poorly documented (Sundback et al. 1996, Buhring et al. 2006, Evrard et al. 2010). However, sandy sediments cover ~70% of the continental shelf (Emery 1968). In contrast with fine sediments, where grazing and mineralization occur within the top millimetres of sediment and solute transfer is diffusion-limited, these processes can occur much deeper in permeable sediments and solute transfer is strongly enhanced through pore water advection (Huettel & Rusch 2000, Rusch & Huettel 2000). Considering that depth-integrated microphytobenthos (MPB) production can significantly exceed that of intertidal muddy areas (Evrard et al. 2008) and that permeable sediments can act as particle traps, actively filtering the overlying water through pore water advection (Huettel et al. 2007), food webs in sandy sediments might also contribute significantly to C flows at the scale of the global shelf.

In general, benthic food web studies are limited to one compartment (i.e. microfauna, meiofauna or macrofauna) or to one specific taxon or size class, while neglecting interactions with other size classes or taxa. For example nematodes, which are omnipresent in the meiofauna, have received greater attention than other smaller animals like ostracods and tardigrades. Also, microscopic juveniles of macrofauna (i.e. bivalves, gastropods), which can occur in dense cohorts and can contribute significantly to MPB grazing (Evrard et al. 2010), are often neglected. The smaller and most abundant organisms at the base of the food web (i.e. the microbial domain) are usually either ignored or not resolved and lumped with detritus into the bulk sediment compartment. This is unfortunate since many studies have reported variable dependence of metazoans on algae, bacteria and detritus (Sherr & Sherr 1987, Epstein et al. 1992, Epstein & Shiaris 1992). This lack of resolution in the lower trophic domain also hinders disentangling detritivory, bacterivory and grazing on MPB (van Oevelen et al. 2006a).

While stable isotopes offer a powerful way to unravel trophic interactions in benthic communities (Fry & Sherr 1988, Herman et al. 2000, Ponsard & Arditi 2000), they can fail at identifying the actual food sources, making it difficult to distinguish between pelagic and benthic input. Although it is widely accepted that ^{13}C natural abundance of MPB and phytoplankton are often distinct (France 1995), evidence shows that this assumption can be misleading when the pool of benthic primary producers comprising the MPB is heterogeneous, with microalgae spanning a wide range of $\delta^{13}C$ signatures. For example, animals selectively feeding on cyanobacteria in a heterogeneous MPB community would get more ^{13}C-depleted compared to those feeding exclusively on diatoms (with high $\delta^{13}C$ values) or feeding indiscriminately (Evrard et al. 2010). This is particularly true for the smaller metazoans for which the feeding apparatus limits the size of the food they can ingest. Stable isotope enrichment experiments, combined with the study of stable isotope natural abundances, offer a powerful way to circumvent the problem of food sources with overlapping stable isotope signatures (Herman et al. 2000, Carman & Fry 2002). Some studies have shown that it is possible to selectively label different food sources (phytodetritus: Blair et al. 1996; MPB: Middelburg et al. 2000; bacteria: van Oevelen et al. 2006b) and follow their fate within the benthic food web.

In the present study, we investigate the trophic interactions in a C_{org}-poor subtidal sandy sediment. We combine the results of natural abundance stable isotope measurements of food sources and consumers with the results of a twofold stable isotope addition approach to assess the relative contributions of benthic (autochthonous, living) and pelagic sources (allochtonous, dead organic matter) to the diet of benthic consumers. In a first experiment (Expt 1), we evaluated the importance of pelagic organic matter input to the benthos through an addition of freeze-dried ^{13}C-labelled diatoms to the sediment surface. In the second experiment (Expt 2), we evaluated the significance of autochthonous organic matter in the benthic foodweb by labelling the MPB in a ^{13}C-bicarbonate pulse-chase experiment. During both experiments, the fate of the added label was quantified and followed over time in the different food web compartments.

MATERIALS AND METHODS

Study site

The research was carried out in August 2003 in Hel (Hel peninsula, Poland), situated in the northwestern part of the Gulf of Gdansk in the Baltic Sea. The sampling site (54° 36′ 19″ N, 18° 48′ 01″ E) was ~50 m off the shore, in shallow water (~1.5 m) and the sediment consisted of fine and well-sorted quartz sand, with a median grain size of 210 µm. The tidal amplitude was negligible and the water was brackish with a salinity of 7. The water temperature at the time of the study was 20°C.

Experimental settings

Expt 1: ^{13}C-labelled phytodetritus addition

Ten sediment cores (19 cm diameter × 15 cm high) were sampled with overlying water from the study site and brought back to the laboratory. The core liners used for sampling consisted of transparent acrylic cylinders (19 × 33 cm, inner diameter × height; sediment surface equivalent to 283.5 cm^2) that were capped at both ends and used as flux chambers. The bottom of each core was capped with a PVC lid. Each top end was fitted with a transparent acrylic lid that supported an electric motor rotating a 15 cm diameter transparent disc, stirring the water above the sediment cores. The motor maintained an electronically controlled angular velocity set to 40 rpm which generated a pressure gradient of 1.9 Pa between the circumference and the centre of the sediment surface (~0.2 Pa cm^{-1}). This pressure gradient corresponds to gradients produced by slow bottom currents (~10 cm s^{-1}) interacting with sediment ripple topography (15 mm ripple amplitude) as were typical for the study site during our investigations. Such pressure gradients force bottom water to enter the sediment in the ripple troughs and to emerge at the ripple crests. Similarly, the simulated pressure gradients in the chamber forced water to enter the sediment near the chamber wall and to emerge from the centre of the core. The functioning and deployment of these chambers have been described in detail by Huettel & Gust (1992b), Huettel et al. (1996), and Janssen et al. (2005a,b).

The set of chambers was immersed in a water tank filled with 100 µm filtered decanted seawater from the sampling site and the system was maintained under *in situ* temperature (20°C) and constantly aerated. Initially, chambers were kept open by elevating the upper lids about 1 cm above the chambers edge to allow exchange between the chamber water and the aerated water of the tank. Water recirculation in the tank was enhanced using a submersible pump placed at the bottom of the tank, which ensured that all incubated sediments were exposed to aerated water with the same qualities. The system was left to settle 24 h prior to the start of the experiment.

An axenic clone of the benthic diatom *Amphora coffeaeformis* (UTCC 58) was cultured beforehand at 16°C under 32 W incandescent lights. The artificial seawater (F2 medium) contained 50% ^{13}C-enriched bicarbonate (98% ^{13}C; Isotech) to label the diatoms. After 3 wk, the labelled diatoms were concentrated by centrifugation, washed several times to remove adhering ^{13}C-bicarbonate and subsequently freeze-dried. The final product was 11% ^{13}C-labelled (mg ^{13}C per mg C). The axenic state of the diatom culture was verified microscopically and needed for studying transfer of carbon from phytodetritus to heterotrophic bacteria.

After the acclimation period of 24 h, one core was taken out of the system and sampled (see below) to provide background values for the different parameters investigated. Prior to labelling, the freeze-dried diatoms were resuspended and gently homogenised in 50 ml 0.2 µm filtered seawater from the tank. The 9 remaining cores were closed, and 5 ml of the phytodetritus solution was injected in their overlying water and allowed to settle while the stirrers were stopped. The phytodetritus addition was equivalent to a pulse of 377 mg C_{org} m^{-2} (i.e. 41.5 mg $^{13}C_{org}$ m^{-2}). After 1 h,

another core was taken out of the system and sampled in order to provide a t_0 data point (considered as the beginning of the incubation period), and the stirring of the 8 remaining cores were switched on again. At each sampling time (t = 12, 24, 48 and 72 h), duplicate cores were taken out of the system and processed (see Chamber Sampling). All cores were incubated in the dark so as to minimize any potential incidental uptake of stable isotope label by the MPB (through remineralisation of phytodetritus or cell leakage).

Expt 2: ^{13}C pulse-chase labelling

Five days after Expt 1, an additional 5 sediment cores with overlying water were sampled the same way as in Expt 1 and from the same sampling site. The sediment cores were transferred to the laboratory and immersed in a tank filled with 100 µm filtered decanted seawater. After 24 h of acclimation, 1 core was taken out of the system and sampled in order to provide ^{13}C-background data. The remaining cores received a pulse of 25 mg ^{13}C-bicarbonate in their overlying water, equivalent to a 6.7% ^{13}C-labelling of the dissolved inorganic carbon (DIC) pool (1.2 mmol l^{-1}). All the cores were immediately closed and illuminated for 8 h by incandescent light providing an irradiance of 150 µmol quanta m^{-2} s^{-1} at the sediment surface. The water column of each core was stirred as described above throughout the light incubation. After the light exposure, one transparent core was taken out of the system and sampled in order to provide a data point representing the end of the labelling period (t_1 = 8 h). Then the overlying water was flushed out of the system twice to remove any excess ^{13}C-bicarbonate. This was done by gently siphoning the water above the sediment cores and replacing it with fresh 100 µm filtered decanted seawater from the field. To avoid disturbance at the sediment surface, a 5 mm layer of water was left before refilling the chamber, and water was then gently added on top of a piece of bubble wrap floating at the water surface. The efficiency of label removal was confirmed through ^{13}C-DIC measurements (> 99.9% of label removal).

A 8:16 h light:dark cycle was maintained during the 4 d of the experiment. The cores were always kept open and immersed in the tank to allow aeration and equilibration of the water within the whole system (except during DIC flux measurements, see below). At t = 24, 48 and 96 h, immediately after each illumination period, one core was taken out of the system and processed as described below.

Sampling and analysis

Chamber sampling

All sediment cores were subsampled using 4 smaller core liners (3.56 cm inner diameter, ~10 cm^2). For bulk sediment C_{org} content and stable isotope analysis, and for phospholipid-derived fatty acid (PLFA) analysis, 2 of the small cores were sliced in 3 layers: 0–1, 1–2, and 2–3 cm. The equivalent depth layers were pooled to obtain enough material for PLFA analysis and subsequently freeze-dried. Another small core was sliced the same way and used for meiofauna analysis. The last small core was sliced in 6 thinner layers (0–2, 2–4, 4–6, 6–8, 8–10 and 10–20 mm), freeze-dried and used for the analysis of pigment concentrations. Prior to the experiments, 4 l of water from the field were sampled: 2 l were filtered onto glass-fibre filters no. 6 for pigment analysis (Schleicher & Schuell) and the remaining 2 l were filtered onto GF/F filters for suspended particulate matter (SPM) stable isotope analysis. All samples were stored in a freezer until analysed. Sediment and SPM samples used for pigment analysis were stored in a –80°C freezer.

Total respired ^{13}C was estimated for both experiments from the variation in ^{13}C-DIC concentrations in the dark. Each day, cores were closed for a period of 4 h and a small amount of the overlying water was sampled at the start and at the end of that period (n = 2 for Expt 1 and n = 1 for Expt 2). Water samples were drawn into a 12 ml gas-tight vials (Exetainer, Labco) to which 12 µl saturated HgCl solution (6% w/v) was added to kill the sample. The ^{13}C respiration rate, based on the increase in ^{13}C-DIC over 4 h in the dark, was extrapolated to 24 h periods and integrated over the whole time scale of each experiment to estimate total respired ^{13}C in mg m^{-2}.

Fauna extraction

Remaining sediment from each large core was sieved onto a 1 mm mesh and the macrofauna handpicked and pooled in a large container filled with sea water. Macrofauna found in slices from the small cores were added to the container as well. The macrofauna from the entire chamber sediment was thus extracted (28.35×10^{-3} m^2). The animals were gently cleaned to remove mucus, faeces and particles and then sorted to species level, pooled in different glass vials and stored in the freezer until analysed. Prior to analysis, animals were thawed and dried in

an oven for 48 h at 60°C to assess their dry weight. Finally, they were ground into a fine homogeneous powder.

Meiofauna was neither stained nor fixed to avoid any addition of exogenous C that could have contaminated the samples with a substance of different isotopic signature. Frozen sediment layers were thawed at room temperature and thoroughly rinsed with distilled water onto a 38 µm sieve. Meiofauna was extracted from the sediment with colloidal silica (Ludox HS 40, DuPont) with a density of 1.31 g cm^{-3}, following the protocol proposed by Burgess (2001). Briefly, sediments are washed from the sieve with Ludox into a 50 ml disposable polypropylene centrifuge tube. The tube is capped and thoroughly mixed using a vortex at a gradually decreasing speed and finally centrifuged. The supernatant was rinsed again with distilled water on the sieve and finally poured into a Petri dish for counting and picking. The sediment pellets were set aside for verification of remaining meiofauna content. All samples were treated consecutively to avoid degradation of material. The animals were sorted to higher taxonomic levels or, for some, to the genus or species level and counted under a microscope. They were finally transferred to tin cups for stable isotope analysis. Animals with $CaCO_3$ shells were transferred to silver cups to be acidified to remove inorganic C (see next subsection).

Analyses and data handling

Pigment samples were analysed by reverse-phase high-performance liquid chromatography (Barranguet et al. 1998). The phytoplankton (from SPM samples) and MPB taxonomic compositions were estimated using the CHEMTAX program (Mackey et al. 1996).

Small fractions of the dried sediment samples were ground in an agate mortar to obtain a homogeneous and fine powder. C_{org} content and isotopic composition ($\delta^{13}C$) of sediment, macrofauna and meiofauna were measured using a Carlo Erba/Fisons/Interscience elemental analyser coupled on-line via a conflo interface to a Finnigan Delta S isotope ratio mass spectrometer. Prior to the analysis, the inorganic carbon fraction of all sediment samples and all fauna samples containing $CaCO_3$ shells was removed by adding drops of 10% HCl directly into the silver cup until no effervescence was visible anymore.

PLFA for the top 1 cm were extracted from approximately 6 g of sediment, following the method of Boschker et al. (1999) and Middelburg et al. (2000) and their concentrations were determined by gas chromatograph-flame ionization detection (Carlo Erba HRGC mega 2 GC). PLFA carbon isotopic composition was determined using a gas-chromatograph combustion-interface isotope ratio mass spectrometer (Hewlett Packard 6890 GC coupled via a Thermo combustion interface III to a Thermo Delta Plus isotope ratio mass spectrometer). Bacterial carbon content in the sediment was estimated from the PLFA concentrations. The bacterial carbon biomass (C_{bac}, expressed in mg C m^{-2}) was calculated from bacterial PLFA ($PLFA_{bac}$) as $C_{bac} = PLFA_{bac}/a$, where a is the average PLFA concentration in bacteria (0.073 mg of PLFA carbon per mg of C_{bac} in oxidised sediment; Brinch-Iversen & King 1990). $PLFA_{bac}$ was estimated from the bacteria-specific PLFA ($PLFA_{bacsp}$ = iC14:0, iC15:0, aC15:0 iC16:0) as $PLFA_{bac} = \Sigma\ PLFA_{bacsp}/b$, where b is the average fraction-specific bacterial PLFA (0.14 mg of $PLFA_{bacsp}$ carbon per mg of $PLFA_{bac}$ carbon; Moodley et al. 2000).

Stable isotope data are expressed in the delta notation ($\delta^{13}C$, per mille ‰) relative to Vienna Pee Dee Belemnite standard (VPDB) and calculated from the stable isotope ratio:

$$\delta^{13}C = \left(\frac{R}{R_{VPDB}} - 1\right) \times 1000 \quad (1)$$

where R is the $^{13}C/^{12}C$ ratio measured in the sample and R_{VPDB} in the standard (R_{VPDB} = 0.0111797). Following Maddi et al. (2006) we use the enrichment notation (δ^{E}), as a measure of label enrichment in a sample:

$$^{13}C\delta^{E} = \left(\frac{R_s}{R_b} - 1\right) \times 1000 \quad (2)$$

where R_b is the ratio in the background and R_s in the sample. The incorporation (I), i.e. the total uptake of label, is expressed in mg ^{13}C m^{-2}, as a product of the atomic excess (E) and a quantity (C_{org} or C_{PLFA}). $E = F_s - F_b$, the difference between the fraction (F) of the sample and the one of the background, with $F = R / (R + 1)$.

Direct and indirect contribution of food sources to the diet of the consumers were estimated based on the natural ^{13}C stable isotopic signatures of the primary food sources and those of the consumers, with the assumption that the stable isotopic signature of a consumer is a weighted average of the different food sources. In the case of 2 food sources, the solution follows a simple 2-source mixing model and is unique. With more food sources and only one stable isotopic measurement (^{13}C), the contribution cannot be

uniquely determined and only ranges of the potential food contributions can be determined (Phillips & Gregg 2003, Lubetkin & Simenstad 2004). An extra complicating factor is that the stable isotope compositions of the food sources and consumers are only approximately known (Moore & Semmens 2008, van Oevelen et al. 2010). We therefore applied an isotopic mixing model including all potential food sources, using the δ-value ranges (min.–max.) of food sources and consumers to account for the variability in isotopic ratios. With n sources, the linear model to solve is

$$\delta^{13}C_M = \sum_{i=1}^{n} p_i \cdot \delta^{13}C_i \quad (3)$$

$$1 = \sum_{i=1}^{n} p_i \quad (4)$$

where $\delta^{13}C_M$ and $\delta^{13}C_i$ are the carbon stable isotopic $\delta^{13}C$ values of consumer M and source i respectively, and p_i (≥ 0) is the relative proportion of food item i in the diet of consumer M. In the case where $n > 2$ and with only 2 equations, the model can be solved using linear programming techniques yielding either no solution or a range of solutions. The food web model was solved in the open-source software R (R Development Core Team 2007), using the package limSolve (Soetaert et al. 2008).

RESULTS

Benthos composition

Bulk sediment C_{org} content was low (~0.05% C) and similar for Expt 1 (~9.3 g C m^{-2} and 21.6 g C m^{-2} for the top 1 cm and the top 3 cm, respectively) and Expt 2 (~10 g C m^{-2} and 22.4 g C m^{-2} for the top 1 and top 3 cm, respectively). There were no differences for chl *a* concentrations in the water column or in the sediment between the first and the second sampling (Fig. 1A,B vs. Fig. 1C,D). Despite similarly low chl *a* concentrations between the first and the second sampling, phytoplankton compositions were different, largely dominated by cyanobacteria before Expt 1 and more evenly distributed between diatoms, cyanobacteria and green algae before Expt 2 (Fig. 1A,C). Sediment chl *a* contents before Expt 1 and Expt 2 were 94 and 95.1 mg chl *a* m^{-2} in the top 1 cm, respectively. Using C:chl *a* ratios of 32 and 30 for the first and the second sampling respectively, calculated from the weighted average of C:chl *a* ratios of microalgae making up the ambient MPB communities in this study (Dijkman & Kromkamp 2006, N. A. Dijkman pers. comm.), we derived MPB biomasses for the top 1 cm of sediment of 3006 and 2851 mg C m^{-2} for Expt 1 and Expt 2, respectively. Microphytobenthos (MPB) composition based on CHEMTAX analysis for the top 1 cm of sediment was 69% diatoms, 23% cyanobacteria and 8% green algae for Expt 1 and 84%, 12% and 4% of the same taxa for Expt 2, respectively (Fig. 1B,D).

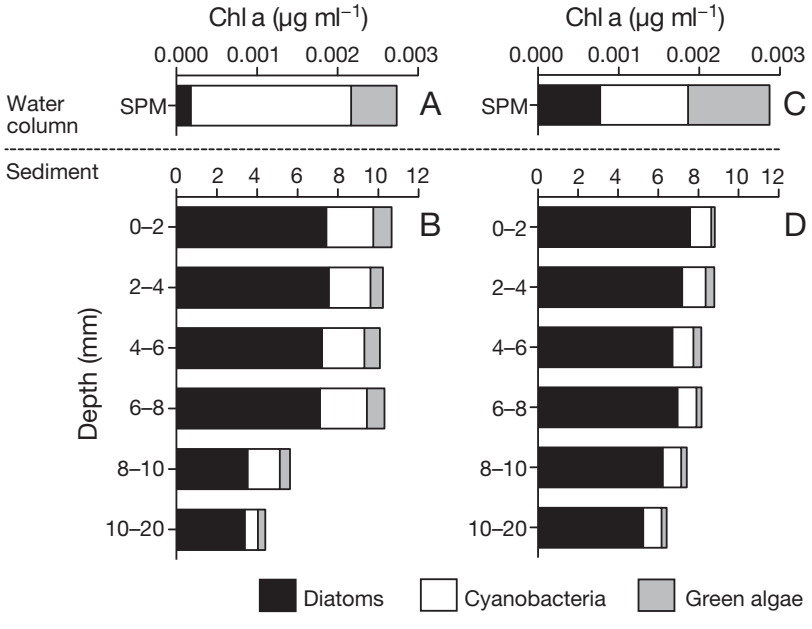

Fig. 1. Phytoplankton and microphytobenthos (MPB) composition estimated from CHEMTAX analysis of pigment data, in µg chl *a* ml^{-1} of seawater and sediment respectively. (A) seawater and (B) MPB before Expt 1, and (C) seawater and (D) MPB before Expt 2. SPM: suspended particulate matter

Benthic fauna included 12 meiofauna taxa and 3 macrofauna species, and the assemblages were different at the 2 sampling times. For Expt 1 (Table 1), meiofauna in the top 1 cm layer was dominated by the harpacticoid copepod species *Paraleptastacus spinicauda* (~53 × 10^3 ind. m^{-2}) and ostracods (~48 × 10^3 ind. m^{-2}), although the latter showed high patchiness. The harpacticoid copepod community appeared to be diverse (from microscopic observations), but density, biomass and isotopic data were estimated only for *P. spinicauda* and *Huntemania jadensis* as the other unidentified individuals were scarce and present irregularly, precluding isotope analysis. Nematodes showed the third highest densities at the sediment sur-

Table 1. ^{13}C-phytodetritus addition experiment (Expt 1). Meiofauna and macrofauna individual weight and densities (mean ± SD). j: juveniles; ND: not detected

	Median weight ($\mu g\ C_{org}\ ind.^{-1}$)	Densities (10^3 ind. m^{-2}) 0–1 cm	1–2 cm	2–3 cm
Amphipods (j)	13.73	0.60 ± 0.70	0.50 ± 0.71	0.10 ± 0.32
Chironomids	1.93	13.44 ± 6.42	0.10 ± 0.32	0.20 ± 0.63
Hydrobia ulvae (j)	3.76	11.90 ± 8.96	2.90 ± 2.56	1.50 ± 2.07
Huntemania jadensis	0.81	28.14 ± 17.19	18.00 ± 9.30	16.33 ± 9.02
Halacarides	ND	2.40 ± 2.01	1.00 ± 1.05	3.30 ± 3.16
Mya arenaria (j)	1.84	14.78 ± 12.73	5.00 ± 2.12	5.11 ± 6.09
Mytilus sp. (j)	0.79	11.22 ± 11.08	12.22 ± 7.60	15.78 ± 10.43
Nauplii	ND	21.00 ± ND	7.00 ± ND	4.00 ± 2.00
Nematodes	0.17	43.90 ± 16.31	76.60 ± 25.46	89.50 ± 53.76
Oligochaetes	3.78	1.80 ± 2.25	9.50 ± 10.21	9.50 ± 7.25
Ostracods	0.13	46.80 ± 93.36	4.90 ± 12.83	0.80 ± 2.53
Paraleptastacus spinicauda	0.10	52.86 ± 30.98	27.50 ± 19.62	37.67 ± 41.59
Tardigrades	0.02	13.00 ± 16.00	38.80 ± 58.10	63.80 ± 105.76
Turbellaria	0.18	6.75 ± 4.27	5.00 ± 2.98	2.38 ± 3.81

	Mean weight ($mg\ C_{org}\ ind.^{-1}$)	Densities (ind. m^{-2}) 0–15 cm
Bathyporeia pilosa	0.28 ± 0.15	167.55 ± 107.07
Nereis diversicolor	3.26 ± 1.07	156.77 ± 78.79

face with ~44 × 10^3 ind. m^{-2}, and their numbers increased with depth to 90 × 10^3 ind. m^{-2} in the 2–3 cm layer. Other significant contributors to the meiobenthic community were chironomid larvae, and juveniles of *Mytilus* sp., *Mya arenaria* and amphipods. These 3 taxonomic groups were included in the meiofauna as they satisfied the size criterion (<1 mm). For Expt 1, macrofauna in the whole 0–15 cm depth was limited to large individuals of the polychaete *Nereis diversicolor* (~157 ind. m^{-2}) and the amphipod *Bathyporeia pilosa* (~168 ind. m^{-2}), although the latter was present only at the sediment surface. In Expt 2, the meiofauna distribution was different with fewer taxonomic groups (Table 2): chironomid larvae and oligochaetes were not present anymore, and nematodes were the most important taxon (~122 × 10^3 ind. m^{-2}) and showed a clear vertical distribution, with small individuals at the sediment surface and increasingly bigger ones in the deeper layers. Due to low densities of juveniles

Table 2. Microphytobenthos ^{13}C-labelling experiment (Expt 2). Meiofauna and macrofauna individual weight and densities (mean ± SD). j: juveniles; ND: not detected

	Median weight ($\mu g\ C_{org}\ ind.^{-1}$)	Densities (10^3 ind. m^{-2}) 0–1 cm	1–2 cm	2–3 cm
Amphipods (j)	7.32	1.50 ± 0.71		
Bivalves (j)	2.71	3.83 ± 1.17	0.17 ± 0.41	1.00 ± 1.55
Hydrobia ulvae (j)	7.54	1.60 ± 0.89		
Huntemania jadensis	0.53	12.00 ± 3.67		
Nematodes	0.15, 0.19, 0.29[a]	121.83 ± 77.97	127.33 ± 70.92	35.00 ± 25.47
Paraleptastacus spinicauda	0.08	26.00 ± 17.17		
Tardigrades	ND	30.40 ± 14.94	6.17 ± 9.60	

	Mean weight ($mg\ C_{org}\ ind.^{-1}$)	Densities (ind. m^{-2}) 0–15 cm
Bathyporeia pilosa	0.22 ± 0.02	105.82 ± 127.04
Gammarus spp.	0.17 ± 0.05	423.28 ± 236.50
Nereis diversicolor	0.38 ± 0.30	261.02 ± 124.21

[a]Individual weight of nematodes is provided for all 3 layers (0–1, 1–2 and 2–3 cm respectively)

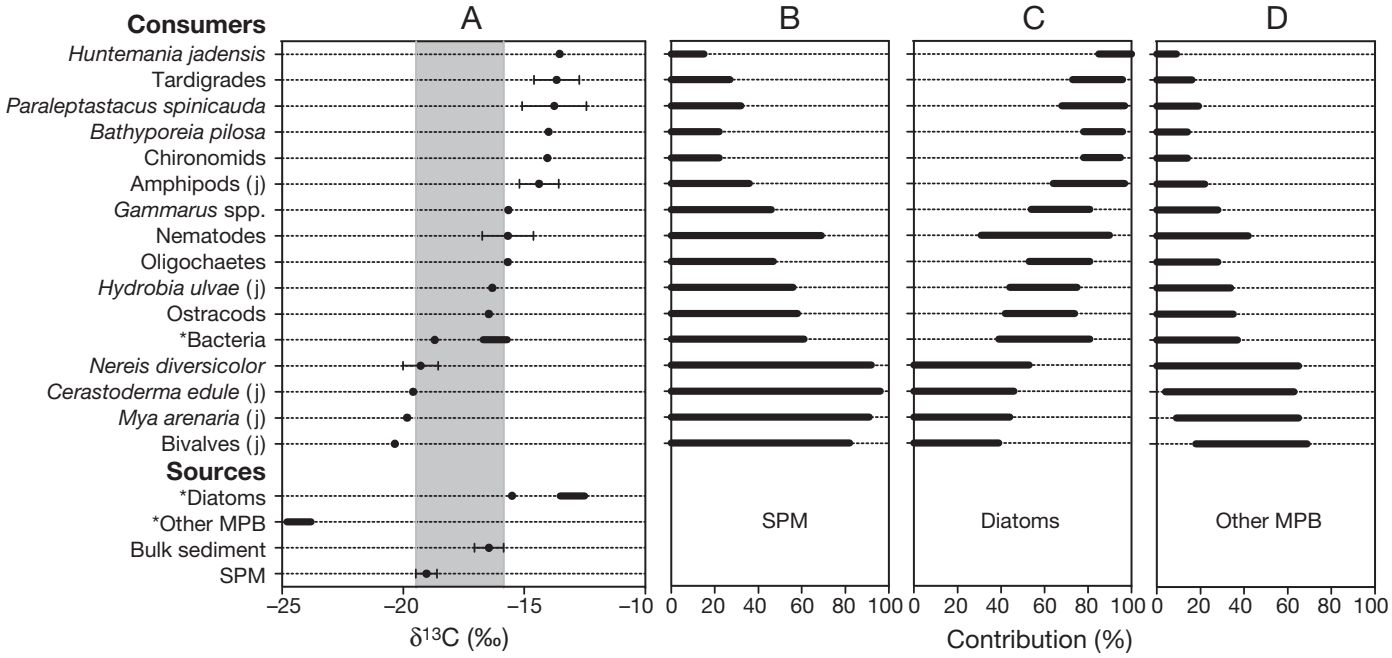

Fig. 2. (A) Stable isotope signatures of benthic consumers and food sources (mean ± SD, n = 2 if applicable). Grey area: includes all consumers for which the diet could be resolved by a 2-source (benthic vs. pelagic) mixing model. (B,C,D) Results of a 3-source mixing model, distinguishing between (B) suspended particulate matter (SPM), (C) diatoms and (D) other microphytobenthos (MPB). Ranges of solutions were calculated by using min.–max. $\delta^{13}C$ values of sources and consumers (when replicates were available). *: $\delta^{13}C$ values estimated from PLFA using a correction of 2–3‰ to account for the carbon depletion of PLFA relative to that of the biomass (for diatoms and bacteria); other MPB stable isotope signature estimated from diatoms and bulk sediment $\delta^{13}C$ mass-balance equation ($\delta^{13}C_{Bulk\ sediment} = 0.69 \times \delta^{13}C_{Diatoms} + 0.31 \times \delta^{13}C_{Other\ MPB}$). j: juveniles

of *M. arenaria* and *Mytilus* sp., all individuals from both taxa were pooled into one 'bivalves' group for stable isotope analysis. Macrofauna composition was different from Expt 1 as well, with significantly smaller individuals of *N. diversicolor* and high densities of *Gammarus* spp. (~423 ind. m^{-2}) in Expt 2.

The sediment also contained a fraction of heterotrophic bacteria whose biomass was estimated from PLFA concentrations. The biomass of heterotrophic bacteria accounted for the largest fraction of the heterotrophic organisms with 407 ± 63 mg C m^{-2} (mean ± SD, n = 10) and 380 ± 55 mg C m^{-2} (mean ± SD, n = 6) at the first and second sampling, respectively.

Natural abundance carbon isotope signatures

Natural stable isotopic signatures of macrofauna and meiofauna showed a broad range of $\delta^{13}C$ values from –13.5‰ for *Huntemania jadensis* to –20.4‰ for juveniles of bivalves (Fig. 2A). $\delta^{13}C$ values of lumped compartments such as bulk sediments (–16.5 ± 0.6 ‰) and suspended particulate matter (SPM; –19 ± 0.4 ‰) are traditionally considered proxies for the $\delta^{13}C$ of potential food sources MPB and phytoplankton, respectively. Most of the isotopic signatures of the taxonomic groups analysed were outside this range (Fig. 2A, grey area). Using these 2 proxies would only allow determining the diet of some nematodes, *H. ulvae*, ostracods, bacteria and *Nereis diversicolor*. However, PLFA analysis offered additional resolution and allowed us to estimate the $\delta^{13}C$ value of diatoms, which dominated the MPB. From the isotopic signature of the diatom-specific PLFA (C20:5ω3) and using a correction of 2 to 3‰ to account for the carbon depletion of PLFA relative to that of their biomass (Boschker et al. 1999, 2005, Hayes 2001, Evrard et al. 2010), we derived a $\delta^{13}C$ value for diatoms ranging from –13.5 to –12.5‰. That value was consistent with the highest value found for consumers. Similarly, we also estimated the bacteria ^{13}C natural abundance from the weighted average bacterial-specific PLFA $\delta^{13}C$ (–18.7 ‰) and derived a $\delta^{13}C$ value between –16.7 and –15.7‰. Unfortunately, the resolution of the chromatograms did not allow distinguishing the peaks of C18:3ω3 and C18:4ω3 PLFAs which are characteristic of cyanobacteria and green algae and present in the MPB. How-

ever, based on the $\delta^{13}C$ value of benthic diatoms and using a mass balance calculation, we can estimate a $\delta^{13}C$ value for other primary producers (i.e. cyanobacteria and green algae combined). Assuming that bulk sediment is mostly made up of MPB or MPB-derived material (detritus, extracellular polymeric substances) and that MPB comprises 69% diatoms, 31% of other MPB (i.e. 23% cyanobacteria + 8% green algae), we can write

$$\delta^{13}C_{Bulk\ sediment} = 0.69 \times \delta^{13}C_{Diatoms} + 0.31 \times \delta^{13}C_{Other\ MPB}$$

where $\delta^{13}C_{Diatoms} = \delta^{13}C_{C20:5\omega3} + 2.5‰$. Following this equation, the estimated $\delta^{13}C$ value of other MPB (cyanobacteria and green algae) was between –24.8 and –23.8‰ (Fig. 2A). These more depleted end-members were consistent with the $\delta^{13}C$ values of the group of suspension feeders (juveniles of bivalves and *N. diversicolor*) that were lower than that of SPM.

The results of the 2-source mixing model combining SPM and bulk sediment as food sources are not shown because the contributions of SPM and bulk sediment can be grasped from Fig. 2A (grey area). In addition, it would not have allowed for resolving the diet of all taxa. The reliance of each consumer on SPM, benthic diatoms or other MPB, or a combination of the 3 sources was estimated from a 3-source mixing model (Fig. 2B,C,D). The mixing model resolved the diet of all taxa. Benthic diatoms contributed most to the diet of most consumers (Fig. 2B). Other MPB and/or SPM contributed most to the diet of *Nereis diversicolor* and bivalves (Fig. 2C,D). Altogether these contributions permitted us to distinguish 3 different groups of fauna: deposit feeders depending mainly on benthic diatoms, with high $\delta^{13}C$ values (> –15‰); suspension/filter feeders depending mainly on phytoplankton and/or a fraction of the MPB (cyanobacteria and green algae), with low $\delta^{13}C$ values (<–19‰); and non-selective deposit feeders with intermediate $\delta^{13}C$ values relying on multiple resources.

Expt 1: ^{13}C-phytodetritus addition

Phytodetritus sedimentation occurred rapidly and bulk sediment incorporation of ^{13}C (I_{Sed}) was maximum at the sediment surface 1 h after ^{13}C-labelled diatoms were added (t = 0; 17.7 mg ^{13}C m^{-2}; Fig 3A). I_{Sed} in surface sediment (0–1 cm) decreased until t = 24 h and then stayed roughly unchanged until the end of the experiment at t = 72 h, with an average of 4.7 ± 0.5 mg ^{13}C m^{-2}. I_{Sed} in deeper layers (1–2 and 2–3 cm) was maximum at t = 12 h. Although the variability of I_{Sed} was higher in the deeper layers, these delayed maxima are consistent with a slow burial of phytodetritus through advection in permeable sediments.

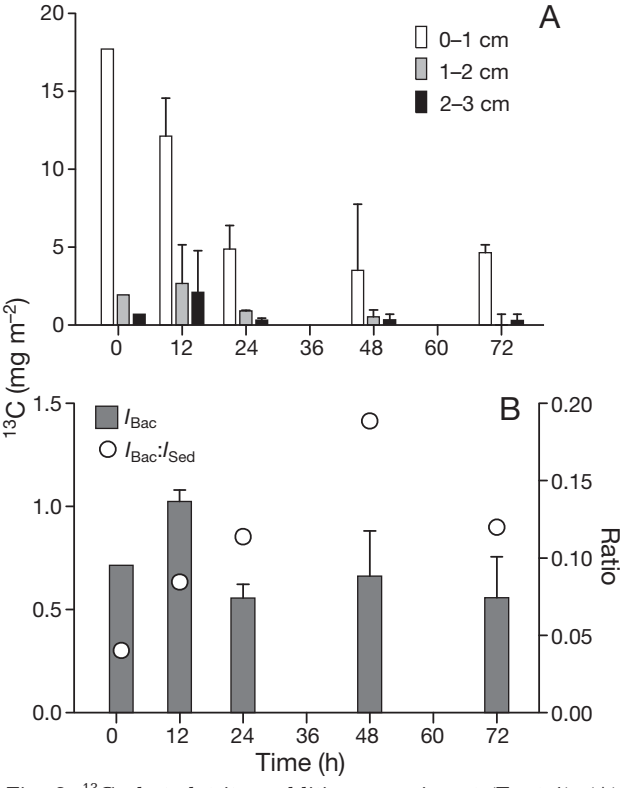

Fig. 3. ^{13}C-phytodetritus addition experiment (Expt 1). (A) Label incorporation (I) into the top three 1-cm layers of bulk sediment (in mg ^{13}C m^{-2}). (B) Label incorporation into bacteria in the top 1 cm layer (I_{Bac} in mg ^{13}C m^{-2}, on the left y-axis) and Bacteria:Sediment ^{13}C-incorporation ratio (I_{Bac}:I_{Sed}, on the right y-axis). Data: mean ± SD, with n = 2 where available

^{13}C-incorporation into bacteria (I_{Bac}) at the sediment surface (0–1 cm) was rapid and reached a maximum at t = 12 h (1.03 ± 0.05 mg ^{13}C m^{-2}; Fig 3B). Despite a rather uniform pattern of ^{13}C-incorporation over time, there was steady incorporation into bacteria over the course of the experiment as the values of the I_{Bac}:I_{Sed} ratio in the 0–1 cm layer increased over time (Fig. 3B). The ratio increased over time to 0.19 at t = 48 h suggesting that about 19% of the added phytodetritus was transferred to bacteria at that time.

Meiofauna was rapidly labelled as illustrated by the levels of enrichment measured (Fig. 4A). Chironomid larvae were the most enriched taxon with a maximum δ^E value of 266.7‰ (n = 1) at t = 48 h. Their high enrichment was consistent with the typical green colour of their gut content observed under the

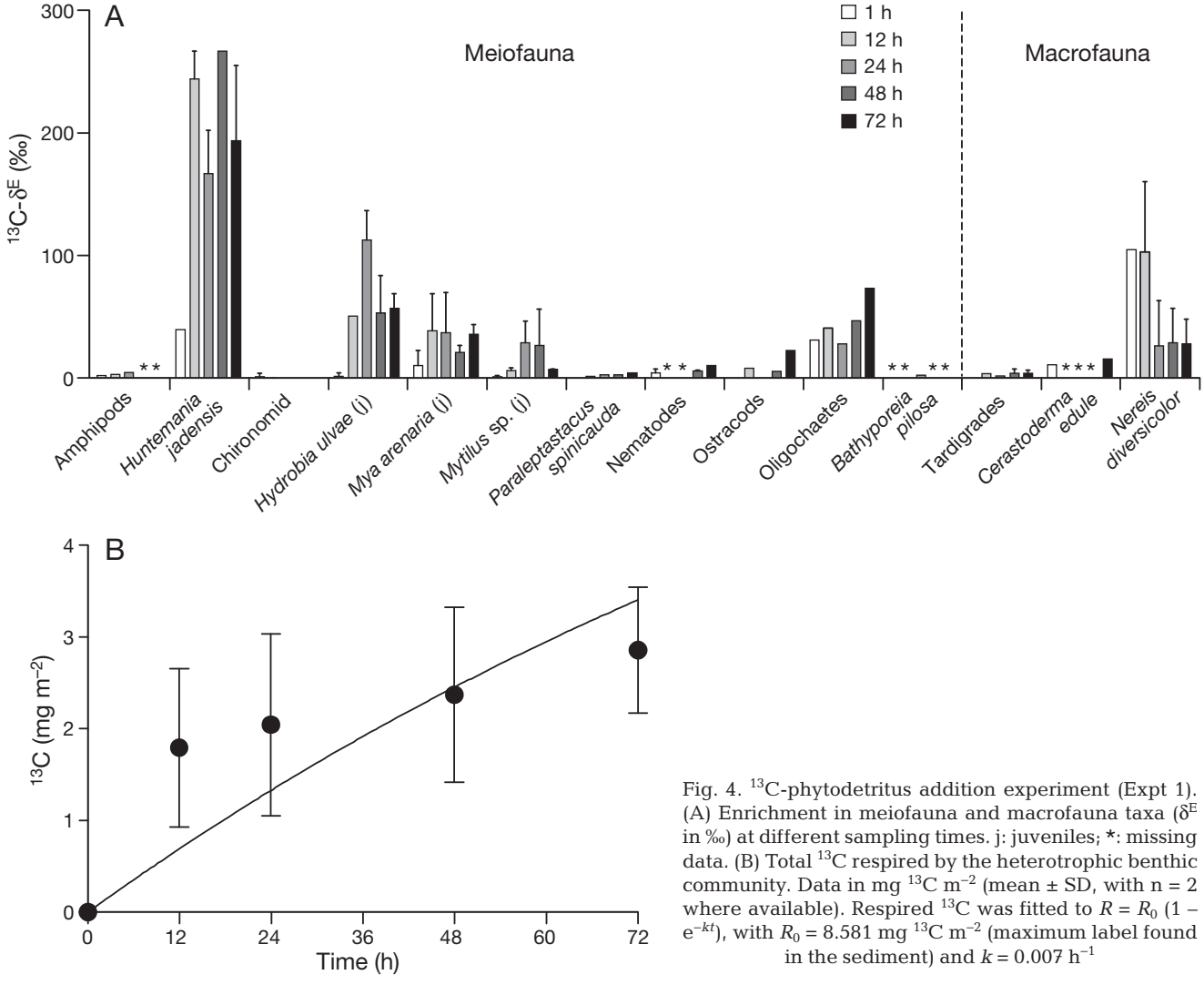

Fig. 4. ^{13}C-phytodetritus addition experiment (Expt 1). (A) Enrichment in meiofauna and macrofauna taxa (δ^{E} in ‰) at different sampling times. j: juveniles; *: missing data. (B) Total ^{13}C respired by the heterotrophic benthic community. Data in mg ^{13}C m^{-2} (mean ± SD, with n = 2 where available). Respired ^{13}C was fitted to $R = R_0$ (1 – e^{-kt}), with R_0 = 8.581 mg ^{13}C m^{-2} (maximum label found in the sediment) and k = 0.007 h^{-1}

microscope at the time of sorting. Juveniles of the gastropod *Hydrobia ulvae* and the copepod *Paraleptastacus spinicauda* were also significantly enriched, reaching maximum δ^{E} values of 112.8 ± 24.1‰ and 73.2‰ at t = 24 and 72 h, respectively. Juveniles of the bivalves *Mya arenaria* and *Mytilus* sp. were also clearly enriched. Remaining meiofauna taxa were only slightly enriched. Note that the patchy distribution of scarcely distributed taxa did not always allow replicating or measuring their enrichment. Enrichment of the large copepod *Huntemania jadensis* was estimated at each sampling but was negligible. Finally, nematodes showed gradually increasing but limited enrichment (4.3‰ at t = 72 h).

Contrary to the meiofauna, the macrofauna showed labelling patterns similar to that of the bulk sediment with highest δ^{E} values right after the pulse of labelled phytodetritus (Fig. 4A). The suspension feeder *Nereis diversicolor* was the most enriched species with values greater than 100‰ at t = 1 and 12 h and between 25 and 30‰ from t = 24 h onwards. The amphipod *Bathyporeia pilosa* showed very little enrichment with a maximum of ~4‰ at t = 72 h. Individuals of *Cerastoderma edule*, which were found only at t = 1 and 72 h, showed enrichments of 11‰ and 15.7‰ respectively.

Total respired ^{13}C by heterotrophs, estimated from ^{13}C-DIC accumulation in the dark was 2.9 ± 0.7 mg ^{13}C m^{-2} at t = 72 h (Fig. 4B). This represented about 16% of the total label found in the sediment at the start of the experiment (t = 1 h). Total respired ^{13}C dynamic was fitted to $R = R_0(1 - e^{-kt})$, where R_0 = 8.6 mg m^{-2} was fixed (the average sediment incorporation over the course of the experiment) and k = 0.007 h^{-1} estimated; k is the first-order respiration rate of the added phytodetritus.

Expt 2: ^{13}C pulse-chase labelling

^{13}C-incorporation by MPB (I_{MPB}) through photosynthesis was rapid and significant in the 0–1 cm layer of sediment and very low in the deeper layers (Fig. 5A). Although all cores were flushed twice after the labelling period of 8 h, surface I_{Sed} was highest at t = 24 h with a maximum of 8.9 mg ^{13}C m^{-2}. ^{13}C-incorporation in the sediment was fitted to $I = I_0(1 - e^{-kt})$, with I_0 = 7.01 mg m^{-2} and k = 0.155 h^{-1}. The pattern of I_{Sed} was consistent with that of the ^{13}C-enrichment into the diatom-specific PLFA (C20:5ω3).

Contrary to Expt 1, ^{13}C-incorporation into PLFA$_{bacsp}$ showed a linear increase from the start of the experiment until t = 96 h, suggesting a direct dependence of bacteria on recently fixed carbon (Fig. 5B). Transfer of label from MPB to bacteria was confirmed from the I_{Bac}:I_{Sed} ratio (Fig. 5B), which showed a steady increase over the course of the experiment. At t = 96 h, the ratio was 0.02, suggesting that about 2% of the ^{13}C fixed by MPB was incorporated into bacteria.

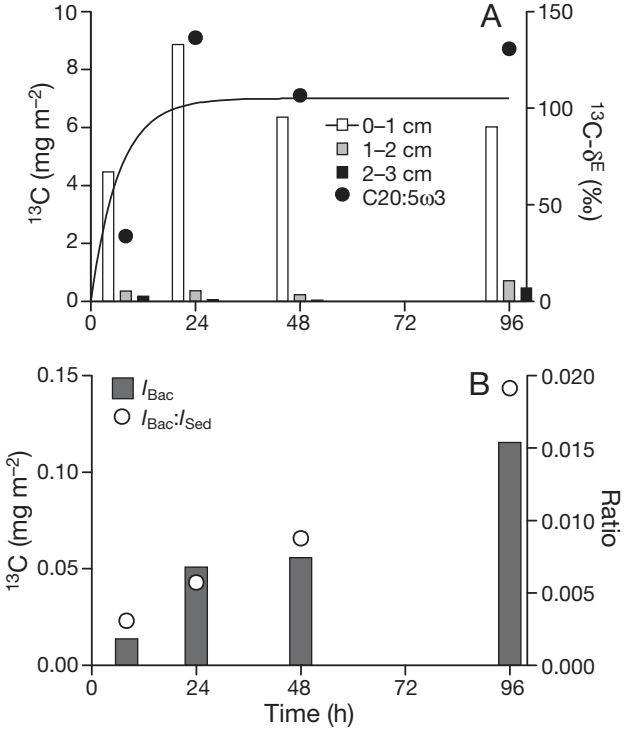

Fig. 5. Microphytobenthos ^{13}C-labelling experiment (Expt 2). (A) Label incorporation into the top three 1-cm layers of bulk sediment (in mg ^{13}C m^{-2}, on the left y-axis) and diatom-specific phospholipid-derived fatty acid enrichment (C20:5ω3 PLFA, δ^E in ‰) on the right y-axis. ^{13}C-incorporation in the top 1 cm layer was fitted to $I = I_0 (1 - e^{-kt})$, with I_0 = 7.01 mg ^{13}C m^{-2} and k = 0.155 h^{-1}. (B) Label incorporation into bacteria in the top 1 cm layer (I_{Bac} in mg ^{13}C m^{-2}, on the left y-axis) and Bacteria:Sediment ^{13}C-incorporation ratio (I_{Bac}:I_{Sed}, on the right y-axis)

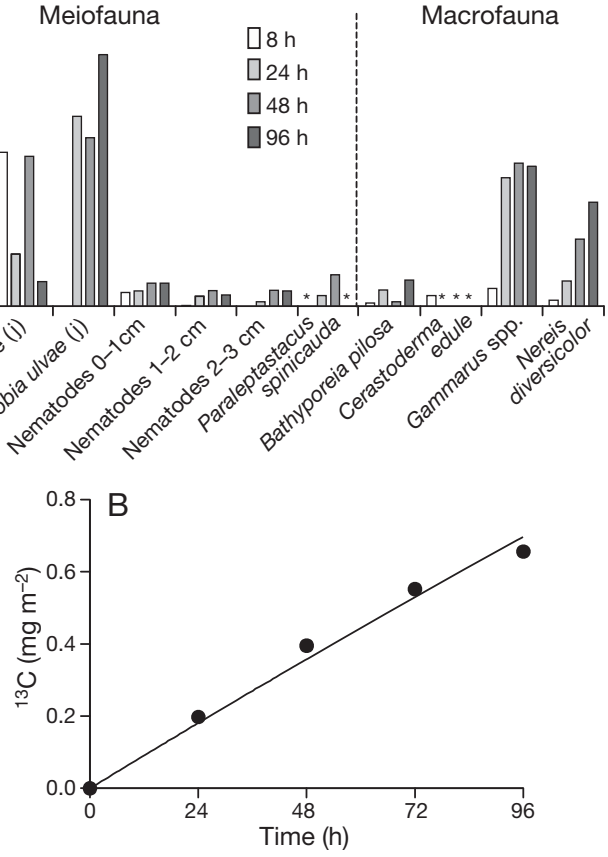

Fig. 6. Microphytobenthos ^{13}C-labelling experiment (Expt 2). (A) Meiofauna and macrofauna enrichment (δ^E in ‰); j: juveniles; *: missing data. (B) Total ^{13}C respired by the heterotrophic benthic community (mg ^{13}C m^{-2}). Respired ^{13}C was fitted to $R = R_0 (1 - e^{-kt})$, with R_0 = 7.01 mg ^{13}C m^{-2} (maximum label found in the sediment) and k = 0.001 h^{-1}

Meiofauna labelling was clear and showed enrichment patterns that contrasted with Expt 1 (Fig. 6A). Juveniles of *Hydrobia ulvae* showed the highest ^{13}C-enrichment, with a maximum δ^E value of 128.6‰ at t = 96 h. Juveniles of amphipods for which enrichment could only be assessed at t = 24 h were also significantly labelled (122‰). Juveniles of bivalves were also gradually enriched, however to a lower extent. *Huntemania jadensis* showed a very contrasting response to ^{13}C-enrichment compared to Expt 1, with high δ^E values from the beginning of the experiment (78.6‰). In contrast, the other copepod species *Paraleptastacus spinicauda* which could only be measured at t = 24, 48 and 96 h showed relatively lower enrichment with maximum δ^E value of 16.1‰

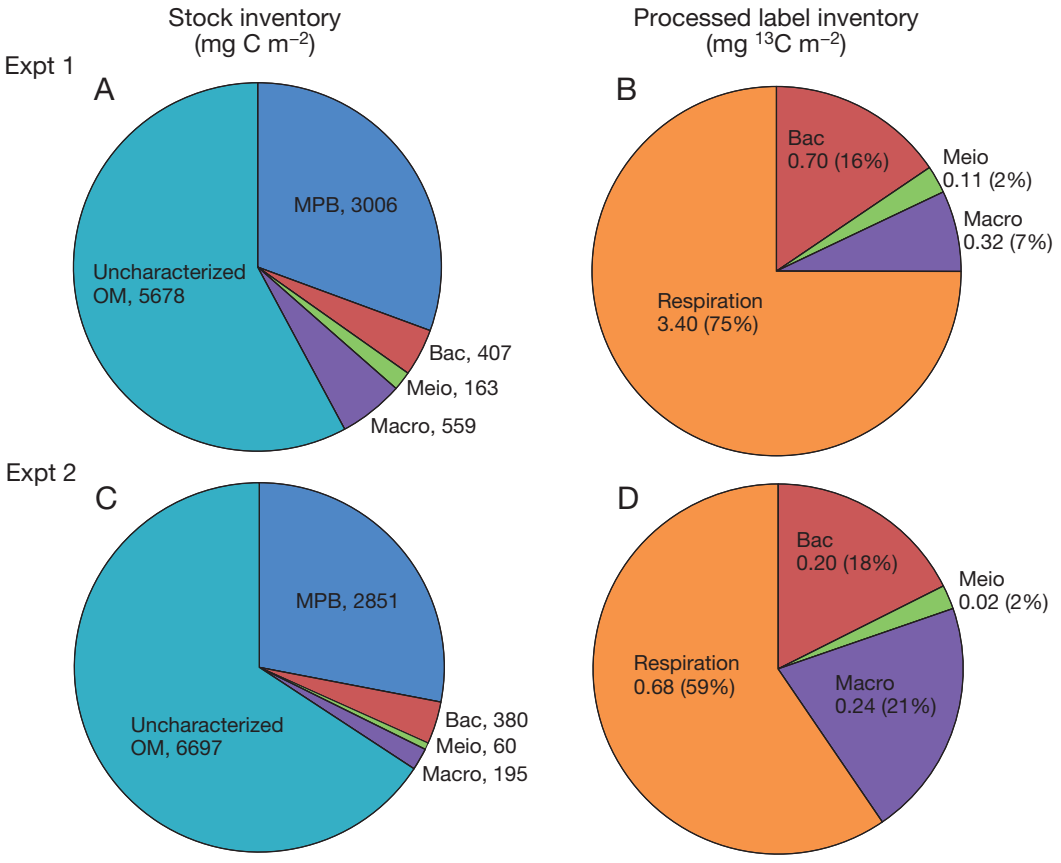

Fig. 7. (A) Stock inventories (mg C m^{-2}) and (B) processed label inventories (mg ^{13}C m^{-2}) for the ^{13}C-phytodetritus addition experiment (Expt 1) and (C) stock inventories and (D) processed label inventories for the MPB ^{13}C-labelling experiment (Expt 2), in the 0–1 cm layer. Label inventories correspond to the total metabolised ^{13}C (incorporation and respiration) at the end of the experiments. Note: macrofauna data are likely to be overestimated as the *Nereis diversicolor* biomass and label incorporation correspond to the whole 0–15 cm depth. MPB: microphytobenthos; Bac: bacteria; Meio/Macro: meio/macrofauna; OM: organic matter

at t = 48 h. Nematodes were present in sufficient numbers to assess the vertical distribution of ^{13}C-enrichment. Smaller individuals were found in the top 1 cm of sediment and showed higher enrichment than the larger individuals found in deeper layers (1–2 and 2–3 cm). However, their enrichment remained limited compared to other taxa, as illustrated earlier (Expt 1), with δ^E values increasing to a maximum of ~12‰ at the sediment surface and ~8‰ in the deeper layers.

Macrofauna ^{13}C-enrichment patterns were also different from that of Expt 2. *Gammarus* spp., which were not present in Expt 1, showed the highest ^{13}C-enrichments with maximum δ^E value of 73.1‰ at t = 48 h. Individuals of the other amphipod species *Bathyporeia pilosa* were slightly more enriched than in Expt 1 (13.3‰ at t = 96 h). Individuals of *Nereis diversicolor* were smaller than those found in Expt 1. Their ^{13}C-enrichment increased linearly over time, reaching a maximum δ^E value of 53.1‰ at t = 96 h.

Total respired ^{13}C by heterotrophs, estimated from ^{13}C-DIC incubations in the dark was 0.66 mg ^{13}C m^{-2} at t = 96 h. This corresponds to ~7.3% of the label fixed considering maximum bulk I_{Sed} (at t = 24 h). Total respired ^{13}C dynamic was fitted to $R = R_0(1 - e^{-kt})$, where R_0 = 7.67 mg m^{-2} was fixed (R_0 = bulk sediment I_0 + respired ^{13}C) and k = 0.001 h^{-1} estimated. At t = 96 h, R = 0.68 mg ^{13}C m^{-2}.

Inventories

Inventories of C_{org} stocks and total ^{13}C metabolized by heterotrophs were summarized for each experiment (Fig. 7). Total sediment C_{org} (including macrofauna) and MPB C_{org} (estimated from chl *a*) were similar before each experiment with 9813 and 3006 mg m^{-2} respectively before Expt 1, and 10183 and 2851 mg m^{-2} respectively before Expt 2 (Fig. 7A,C). Uncharacterized C_{org} comprised the major fraction of

C_{org} stocks. The remaining identified compartment, which accounted for most of the sediment C_{org} was bacteria, with 407 and 380 mg m^{-2} in Expt 1 and Expt 2 respectively. Both meiofauna and macrofauna showed significantly lower biomasses in Expt 2.

At the end of both experiments, a large fraction of the ^{13}C present in the sediment had not been consumed. About 3.6 and 6.8 mg ^{13}C m^{-2} remained untouched at the end of Expt 1 and Expt 2, respectively, representing 44 and 86% of total added label (Total ^{13}C = bulk sediment ^{13}C + macrofauna ^{13}C + respired ^{13}C). The relative incorporation of ^{13}C into the different heterotrophic compartments reflected more or less that of their biomasses (Fig. 7). The fraction of total label incorporation into bacteria and meiofauna was similar between experiments. However, while macrofauna label incorporation was only 7% of total label incorporation inventories in Expt 1, this fraction was tripled in Expt 2 (21%). In both experiments, the largest fraction of the consumed label was respired. However, while 75% of consumed labelled-phytodetritus was respired in Expt 1, only 59% of consumed labelled-MPB was respired in Expt 2.

DISCUSSION

The present study combines a natural abundance stable isotope approach with dedicated experiments in which either phytodetritus or microphytobenthos have been labelled with ^{13}C. This enabled the identification of the various trophic interactions in a C_{org}-poor subtidal sandy sediment and assessment of the relative significance and fate of autochthonous and allochthonous food sources. Before discussing the results in detail, it is important to address the different methodological choices made.

Although the labelled phytodetritus addition experiment (Expt 1) as well as the pulse-chase microphytobenthos labelling experiment (Expt 2) could have been carried out *in situ*, they were carried out in the laboratory so as to minimise risks of technical problems, such as loss of material and disruption of the experimental setting by currents and waves, and to facilitate periodical sampling. The conditions of the laboratory experiments were set to mimic as closely as possible the conditions in the field. The stirring in the chambers induced advective porewater flow through the upper layers of the sediment as produced by bottom currents in the field. This is critical for the natural metabolism of permeable sublittoral sands because it dominates the solute exchange between the sediment and the overlying water column (Huettel et al. 1998, Huettel & Rusch 2000). Replicate incubations were used for Expt 1 but that was logistically not possible for Expt 2. Our approach was based on balancing maximal resolution in food web compartments and reproducing natural flow conditions on the one hand and replication on the other hand. Although Expt 2 was not replicated, the time series over 4 days showed systematic patterns and allowed capturing most of the dynamics of the processes involved.

It is also important to mention that for logistic and technical reasons, the 2 experiments could not be carried out simultaneously but were separated by 4 d. Unfortunately, sediment characteristics, in terms of faunal composition and density, differed due to a storm that reworked the whole sediment bed in between the 2 experiments. Repetitive sediment reworking is however a natural process that shapes the biogeochemistry and ecology of benthic communities in shallow sandy, permeable sediments.

Sediment composition

With 0.06 ± 0.02% C for the uppermost cm, the C_{org} content of Hel sediment (9255 mg C m^{-2} and 9988 mg C m^{-2} for Expt 1 and Expt 2 respectively) was typical of C_{org}-poor sandy subtidal sediments (Dauwe et al. 1998). This characteristic was not affected by the storm between Expt 1 and Expt 2 nor linked to seasonal conditions as similar observations were made during the following spring, with values of 0.05 ± 0.01% C (V. Evrard unpubl. data). These values were significantly lower than those found in intertidal mudflat areas (Herman et al. 2000) and also lower than those of other shallow sandy subtidal areas (Sundback et al. 1996, Evrard et al. 2010).

MPB, comprising a heterogeneous mixture of diatoms, cyanobacteria and green algae, made up ~30% of total C_{org} present in the sediment with ~3000 mg C m^{-2}. Consistent with the lower values found for total C_{org} in the sediment, MPB biomass data were significantly lower than those reported for sheltered silty intertidal areas (Sundback et al. 1991, Underwood & Kromkamp 1999, Middelburg et al. 2000, Cook et al. 2004) and other studies in subtidal sandy sediments (Sundback et al. 1991, Evrard et al. 2008). Nevertheless, MPB constitutes an important resource for benthic consumers.

Contrary to the MPB, which usually recovers rapidly from reworking of the sediment, the whole heterotrophic compartment was significantly affected by

the storm event that separated the 2 experiments. Among heterotrophic compartments, bacterial biomass was high with 407 and 380 mg C m^{-2} in Expts 1 and 2, respectively, representing 36 and 60% of the heterotrophic biomass. These values are lower than that reported for a North Sea sandy sediment site where bacteria biomass was ~1760 mg C m^{-2} (Evrard et al. 2010). Heterotroph relative fractions in Expt 1 and Expt 2 were 36:50:14 and 60:31:9, respectively (%bacteria:%macrofauna:%meiofauna). The data presented here contrast with most previous studies, which have emphasised the dominance of meiofauna over macrofauna in sandy sediments (Gerlach 1971, Koop & Griffiths 1982, Heip et al. 1992, Urban-Malinga & Moens 2006). Although amphipods were usually found at the sediment surface, the macrofauna's relative biomass fraction is likely to have been overestimated, as it comprised the top 15 cm of sediment while biomasses for the other compartments correspond to the top 1 cm of sediment. This was particularly relevant to Expt 1 where very large individuals of *Nereis diversicolor* were found throughout the whole 0–15 cm depth.

Taxonomic investigation of the different compartments revealed interesting points. Macrofauna density was dominated by amphipods (*Bathyporeia pilosa* and *Gammarus* sp.) in Expt 1 and Expt 2 respectively. Amphipods are typical for highly dynamic environments. However, the facultative filter-feeding polychaete *Nereis diversicolor* showed the highest biomass. This species is able to sustain its growth with both phytoplankton and MPB (Vedel & Riisgard 1993, Smith et al. 1996). In Expt 1, the meiofauna compartment was clearly dominated by juvenile forms of macrofauna species or temporary meiofauna (i.e. *Hydrobia ulvae*, *Mya arenaria*, *Mytilus* sp. and chironomid larvae). Despite the sediment reworking due to the storm, permanent meiofauna (mainly nematodes and harpacticoid copepods) recovered quickly. This is in agreement with similar studies examining the effect of physical disturbance in sandy sediments (Wulff et al. 1997, Szymelfenig et al. 2006). Surprisingly, harpacticoid copepods that were represented by 2 main species (*Huntemania jadensis* and *Paraleptastacus spinicauda*) in the surface layer showed higher densities and biomass than nematodes, which are often regarded as the most dominant taxon in terms of densities and biomass, regardless of the ecosystem (Heip et al. 1985, Huys et al. 1992). Although nematode assemblages in sandy sediments would deserve a quantitative study at the species level because of their high diversity (Gheskiere et al. 2005), this was not possible here because of their low densities.

Planktonic vs. benthic sources

Studies of benthic food webs typically partition the input of resources into benthic (e.g. MPB, macroalgae, marine macrophytes) and pelagic (e.g. phytoplankton) (Fry & Sherr 1988, Heip & Craeymeersch 1995, Herman et al. 2000, Carman & Fry 2002, Maddi et al. 2006). This partitioning between benthic and pelagic sources in food web studies has emerged because a clear distinction can often be made between the stable isotope signature of suspended particulate matter (SPM) and that of the surficial bulk sediment (i.e. proxies for phytoplankton and MPB respectively, France 1995).

Data from natural ^{13}C stable isotope signatures reveal that MPB clearly played a central role in the benthic food web studied here (Fig. 2). Nevertheless, due to the overlapping of SPM and other MPB (cyanobacteria and green algae) ^{13}C-depleted isotopic signatures, it remains impossible to clearly conclude whether ^{13}C-depleted heterotrophs rely exclusively on either MPB or SPM, or partly on 2 or 3 sources. Benthic diatoms are suggested to be the preferred C source for benthic fauna (Maria et al. 2011).

Harpacticoid copepods *Huntemania jadensis* and *Paraleptastacus spinicauda* had high ^{13}C natural abundance, indicating that they relied substantially on benthic diatoms. However, MPB grazing is often species-specific and density-dependent (De Troch et al. 2005) and thus favours resource partitioning strategies. This is particularly important for dominant species. Our dual experimental approach confirmed this hypothesis as the 2 copepod species responded very differently to the 2 labelling treatments. In Expt 1, *P. spinicauda* showed high affinity for labelled phytodetritus, but *H. jadensis* had negligible ^{13}C-enrichment. This contrasted significantly with Expt 2, in which *H. jadensis* was significantly more labelled than *P. spinicauda*. These findings confirm the potential importance of food quality and palatability to the diet of harpacticoid copepods (Cnudde et al. 2011). To our knowledge, this is one of the few studies documenting resource partitioning between co-occurring species of harpacticoid copepods (Carman & Thistle 1985, Pace & Carman 1996) and it is the first study to illustrate resource partitioning between *H. jadensis* and *P. spinicauda*.

Despite not being the most abundant, chironomid larvae were the most labelled taxon of the meiofauna and also showed the highest total incorporation. On the basis of 11% ^{13}C-labelling of the phytodetritus pool, we derived from the maximum incorporation at t = 48 h (0.074 mg ^{13}C m^{-2}) a total uptake of 0.672 mg

C_{org} m^{-2} of phytodetritus in 2 d (Evrard 2007), which represents ~3% of their biomass. Unfortunately, no chironomids were present in Expt 2 and we could not compare treatments. However, they are significant MPB consumers (sometimes the most important grazer among meiofauna; Pinckney et al. 2003) and they can have a top-down effect on the resource (Goldfinch & Carman 2000).

^{13}C natural abundance of juveniles of *Hydrobia ulvae* was intermediate, indicative of a non-selective diet on MPB and consistent with the feeding preferences of adult forms (Fenchel et al. 1975). In addition, Expt 1 provided evidence that phytodetritus might also significantly contribute to the diet of *H. ulvae*, which to our knowledge has never been shown before. Juveniles of bivalves (i.e. *Mytilus* sp. and *Mya arenaria*) showed natural abundance ^{13}C values typical of suspension feeders. This was confirmed by the important uptake of labelled phytodetritus in Expt 1. However, the bivalves were also enriched in Expt 2, suggesting that following resuspension MPB is also an important direct or indirect part of their diet. Sauriau & Kang (2000) also found that more than 70% of cockle growth (*Cerastoderma edule*) was derived from MPB, with highest proportions for juveniles. Moreover, Rossi et al. (2004) reported significant contributions of MPB to the diet of juvenile *Macoma balthica*.

Although ubiquitous, nematodes were present only in limited numbers in our study, contrasting with previous observations of increased diversities and densities in coarser sediments (Gheskiere et al. 2005). Nematodes showed very limited enrichment in Expt 1 although this enrichment was consistent throughout the time of the experiment (Fig. 4A). The enrichment was more pronounced in all 3 sediment layers in Expt 2 (Fig. 6A). Most nematodes are non-selective deposit-feeders (Gheskiere et al. 2004) using labile organic matter (Moens et al. 2002). Our results suggest that phytodetritus or MPB was not readily assimilated and that nematodes might depend on microbenthic organisms that rely on MPB or phytodetritus. Nematodes' very low but steady enrichment increase could be indicative of a higher trophic position or consumption of older reworked material. It is also important to note that during Expt 2, there was an apparent size-class stratification of the nematode community distribution, with small individuals living at the sediment surface and large individuals in the deeper layers (Table 2). Specific observation at the same sampling site and during the same period by Urban-Malinga et al. (2006) revealed a mixed community of non-selective deposit feeders and predators. The nematode community of this C_{org}-poor sandy sediment showed a strong contrast in terms of biomass, densities and carbon uptake with that of C_{org}-rich North Sea sandy sediment studied by Evrard et al. (2010) and Urban-Malinga et al. (2006).

The macrofauna community was mainly dominated by the amphipods *Bathyporeia pilosa* and *Gammarus* spp., although the latter was present only in Expt 2. Their enrichments were generally lower than those of the meiofauna but, due to their significant biomass, their contribution to ^{13}C incorporation and remineralisation was important. *Nereis diversicolor* was highly enriched in ^{13}C in both experiments, suggesting that this suspension feeder is not limited to phytoplankton or particulate detrital resources and that MPB can constitute an important fraction of its diet as well.

Bacterial labelling was significant and I_{Bac} after 24 h accounted for 62% of total heterotrophic community uptake in Expt 1 and 43% in Expt 2. Our results for Expt 1 are in good agreement with Buhring et al. (2006) who found in a similar labelled-phytodetritus addition experiment that up to 62% was incorporated into bacteria. In Expt 1, where we administered labelled phytodetritus, at t = 48h, almost 20% of total label fixation in bulk sediment was actually present in the bacterial compartment. The same ratio in Expt 2, in which we labelled MPB, was smaller by one order of magnitude. However, assuming that bacteria relied principally on labile C_{org} such as extracellular polymeric substances (EPS) (Middelburg et al. 2000, Evrard et al. 2008), we need to take into account the significant dilution of label in the substrate (i.e. the total ^{13}C found within the pool of freshly produced ^{13}C-labelled organic matter and the stock unlabelled though readily available organic matter). If EPS production is proportional to the uncharacterized fraction (66%) found in the stock (MPB + EPS = bulk sediment – bacteria – meiofauna), we can derive the newly produced ^{13}C-EPS over the course of the labelling period from the ^{13}C inventories. If total ^{13}C is 7.93 mg ^{13}C m^{-2} (bulk sediment ^{13}C + macrofauna ^{13}C + respired ^{13}C), ^{13}C-EPS is 0.66 × 7.93 = 5.24 mg ^{13}C m^{-2}. Considering that DIC was ^{13}C-labelled at 6.7%, total EPS production was therefore 5.24/0.067 = 78.16 mg C_{org} m^{-2}. If EPS standing stock was 6697 mg C_{org} m^{-2}, then the final dilution of ^{13}C in the total EPS pool is 5.24/(6697+78.16) = 0.0008 (i.e. 0.08%). Assuming that bacteria relied non-specifically on both newly produced ^{13}C-labelled EPS and unlabelled stock EPS, we can estimate that total C assimilation by bacteria at the end of the experiment

was 0.20/0.0008 = 261.87 mg C_{org} m^{-2} (~65 mg C_{org} m^{-2} d^{-1}). This is close to the lower limit found in an intertidal sand flat in the North Sea (Rusch et al. 2001). This suggests that about 55% of the MPB production during the 8 h of the labelling period (7.93/0.067 = 118.42 mg C_{org} m^{-2}) was assimilated by bacteria. Although this consumption was likely overestimated as we calculated the dilution with respect to the entire uncharacterized C_{org} pool, it is still significantly more important in comparison to the 20% phytodetritus assimilation by bacteria after 72 h in Expt 1, equivalent to 6.36 mg C_{org} m^{-2} (0.2 mg ^{13}C, considering 11% labelling).

The ^{13}C-dilution into freshly produced labelled MPB and stock MPB can be calculated the same way and yields a ^{13}C-dilution in the total MPB pool of 0.09%. Therefore, assuming that all invertebrates relied non-specifically on both newly produced ^{13}C-labelled MPB and unlabelled stock MPB, all invertebrates assimilated 0.26/0.0009 = 282.36 mg C_{org} m^{-2}. This is again more important than the total incorporation of 3.91 mg C_{org} m^{-2} from phytodetritus in Expt 1 (0.43 mg ^{13}C, considering 11% labelling). This corresponds to ~71 mg C_{org} m^{-2} d^{-1} and 60% of the MPB production during the 8 h of the labelling period.

In both tracer experiments, most of the ^{13}C was recovered in the DIC pool indicating that respiration was the largest sink for labelled carbon, consistent with observations of Moodley et al. (2000, 2005). Altogether, the total C_{org} processed by heterotrophs in Expt 2 (incorporation and respiration) was 1425 mg C_{org} m^{-2} considering a 0.08% dilution of label. However, this dilution factor may be overestimated as it takes into account the whole C_{org} pool in the sediment (i.e. the MPB on the one hand and the uncharacterized fraction which is comprised mostly of EPS on the other hand). If MPB was the only C_{org}-pool considered for the dilution calculation, the total C_{org} processed by heterotrophs in Expt 2 would still be 427 mg C_{org} m^{-2} (with 0.27% ^{13}C-dilution). This lower estimate remains one order of magnitude higher than the estimated C_{org} processed from phytodetritus in Expt 1 (41 mg C_{org} m^{-2}, considering 11% ^{13}C-dilution). Finally, the overall benthic community growth efficiency, i.e. the ratio of $I_{heterotrophs}/(I_{heterotrophs}$ + Respired $^{13}C)$, showed significant difference between treatments. The labelled-phytodetritus addition experiment yielded a 25% growth efficiency, consistent with observations by Moodley et al. (2002), while the MPB labelling experiment yielded a 40% growth efficiency.

CONCLUSIONS

Our results show that food web in this C_{org}-poor subtidal sandy sediment is supported by both phytodetritus deposition and MPB production. Although our experiments were run in benthic chambers mimicking the flow condition found in the field, the extrapolation of our results to *in situ* condition should be done cautiously.

Despite the difference in faunal assemblages between Expts 1 and 2, MPB recovered rapidly and dominated as the food source to most organisms. The rapid response of most taxa to a phytodetritus pulse indicated that organisms in these dynamic environments can also utilise organic matter from the water column during depositional events (such as a spring phytoplankton bloom). Compound-specific isotope analysis was essential to infer this from stable isotope natural abundance data. Deliberate ^{13}C-tracer manipulation of MPB and phytodetritus not only confirmed inferences from natural stable isotope abundance, but also allowed higher resolution on how consumers depend on these 2 carbon inputs and how in certain cases 2 coexisting copepod species rely differently on either sources.

Acknowledgements. This research was supported by the European Union (COSA project, EVK#3-CT-2002-00076) and the Netherlands Organisation for Scientific Research (PIONIER 833.02.002). M. Houtekamer, P. van Breugel and P. van Rijswijk are thanked for analytical support. We warmly thank all COSA team members for providing advice, logistic support and a stimulating environment. We are grateful to the editor R. Kiene and 4 anonymous reviewers for their constructive feedback that helped improve this manuscript. This is NIOO-KNAW publication number 5234.

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Editorial responsibility: Ronald Kiene, Mobile, Alabama, USA

Submitted: September 27, 2011; Accepted: February 15, 2012
Proofs received from author(s): May 13, 2012