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Carbon and nitrogen flows through the benthic food web of a photic subtidal sandy sediment

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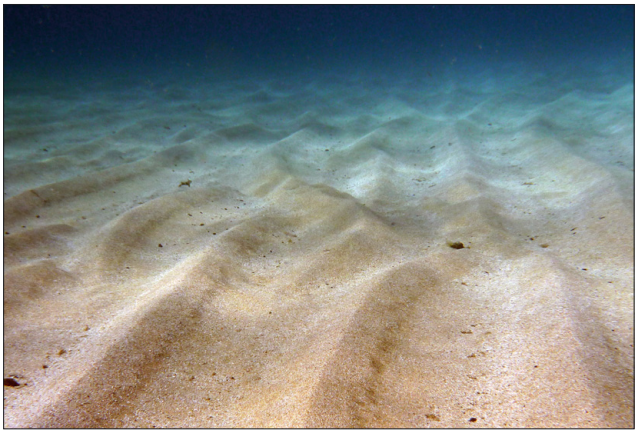
ABSTRACT: Carbon and nitrogen flows within the food web of a subtidal sandy sediment were studied using stable isotope natural abundances and tracer addition. Natural abundances of ^{13}C and ^{15}N stable isotopes of the consumers and their potential benthic and pelagic resources were measured. $\delta^{13}C$ data revealed that consumers did not feed on the bulk microphytobenthos (MPB) but rather were selective in their food uptake, preferring either benthic diatoms (–16‰), or benthic cyanobacteria (–20‰). MPB was labelled through a pulse-chase experiment with ^{13}C-bicarbonate and ^{15}N-nitrate. The fate of MPB was followed in the different heterotrophic compartments. Transfer of ^{13}C and ^{15}N to consumers was fast, although only a small fraction of total label was transferred to the heterotrophic compartments within the 4 d of the experiment. Heterotrophic bacteria were responsible for most of the total heterotrophic incorporation of ^{13}C. Within the metazoan community, the incorporation of ^{13}C by the meiofauna was more than 2-fold that of the macrofauna, despite a significantly lower biomass. The dual labelling also revealed differential feeding or assimilation strategies in meio- and macrofauna. The low ^{13}C:^{15}N ratios of the meiofauna (the smaller organisms) seemed to indicate that they preferentially assimilated N or specifically grazed on N-rich resources. However, the macrofauna (larger organisms) seemed to feed on bulk sediment, consistent with high ^{13}C:^{15}N ratios. This dual approach, which combined natural abundance and a pulse-chase addition of stable isotopes, revealed crucial information on the key role of MPB in structuring benthic communities in sandy sediments.



Sand ripples created by wave oscillations; although they appear to be unvegetated, sandy sediments harbour a very diverse flora and fauna

Photo: V. Evrard

KEY WORDS: Stable isotopes · Food web · ^{13}C · ^{15}N · Microphytobenthos · Meiofauna · Macrofauna · Bacteria · PLFA

INTRODUCTION

Although often appearing to be bare and unvegetated, sandy sediments in intertidal and shallow-water marine environments often harbour an abundant and diverse flora and fauna. The microphytobenthos (MPB),

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which comprises microscopic photosynthetic organisms (e.g. diatoms and cyanobacteria) living at the sediment surface, is highly dependent on light availability (Barranguet et al. 1998). About 33% of the continental shelf receives enough light to sustain a positive net community production (Gattuso et al. 2006), suggesting that benthic primary production contributes significantly to global shelf production and thus to global marine production. Together with phytoplankton, MPB represents the principal direct or indirect source of food for various heterotrophic organisms living in sediments (Heip et al. 1995). MPB can directly sustain grazers, deposit feeders, and, through resuspension, suspension feeders (Cahoon 1999, Herman et al. 2000, Middelburg et al. 2000). MPB also allocates part of the photosynthesis to the production of carbohydrates (Smith & Underwood 1998, Goto et al. 1999) in different forms but mainly as extracellular polymeric substances (EPS) that are available to bacteria and deposit feeders (Cahoon 1999, Goto et al. 2001). Thus, being at the base of the benthic food web, MPB indirectly represents a substantial source of energy for higher trophic levels.

The recognition of the important contribution of MPB to coastal primary production and its role in food webs (Middelburg et al. 2000) has initiated many research efforts. However, there are still many unresolved issues. First, coastal food web studies have often been confined to accreting silty and muddy sediments (e.g. depositional shelf sediments, estuaries, or coastal lagoons) and more specifically to the intertidal area (Heip et al. 1995, Middelburg et al. 2000, Carman & Fry 2002), leaving sandy, permeable sediments poorly documented (Sundbäck et al. 1996, Buhring et al. 2006). This is unfortunate, since sandy sediments represent about 70% of continental shelf sediments globally (Emery 1968). In contrast to fine-grained sediments, where mineralization of fresh organic matter and grazing occurs mainly within the top millimetres of sediment and solute transfer is diffusion-limited, grazing and mineralization in permeable sediments reach much deeper layers, and solute transfer is strongly enhanced through pore water advection (Huettel & Rusch 2000, Rusch & Huettel 2000). A second issue is that most (benthic) food web studies have been limited to one size class (microbenthos, meio-, or macrofauna). The smaller, most abundant organisms at the base of the food web (i.e. the microbial domain) are either ignored or not resolved and lumped with detritus into the bulk sediment compartment. Since many studies have reported a dependency of metazoans on algae, bacteria, and detritus (Herman et al. 2000, Middelburg et al. 2000, van Oevelen et al. 2006), it is critical that we fully assess the role of the microbial component in the transfer and flow of elements in benthic food webs. Because of the complexity of trophic interactions, fully quantifying the microbial component can help to clearly delineate detrivorous, bacterivorous, and herbivorous food webs in sediments.

^{13}C and ^{15}N stable isotopes provide time-integrated measures of both food sources and trophic level necessary to address this food web complexity (Fry & Sherr 1984, Vander Zanden & Rasmussen 1999, Herman et al. 2000). Nitrogen isotopes are primarily used to estimate trophic position since consumers are normally enriched with ^{15}N by 2 to 4‰ relative to their diets, while carbon isotopes are normally used to evaluate the primary sources of carbon for an organism. The assumptions and limitations in using stable carbon isotopes in food web studies have been discussed at length (e.g. Fry & Sherr 1988, Herman et al. 2000, Post 2002): trophic fractionation, the variability in food sources signatures, and/or the overlap of stable isotopic signatures of potential food sources often make assessment of trophic relationships uncertain. While ^{13}C natural abundance signatures of the bulk MPB and phytoplankton are often distinct (France 1995), it can be difficult to clearly elucidate the feeding behaviour of some heterotrophic organisms, as both pelagic and benthic microalgal communities comprise a heterogeneous mixture of primary producers with a range of different isotopic signatures. For a given system, it is therefore essential to adequately resolve the isotopic signature at the base of the food web. Phillips & Gregg (2003) and Lubetkin & Simenstad (2004) advance the use of isotope mixing models to deal with multiple sources and uncertainty in source partitioning based on natural abundance stable isotope data. An alternative, efficient way to overcome the problem of overlapping isotope signatures of potential food resources is to combine a natural abundance stable isotope investigation with a deliberate tracer stable isotope addition experiment (Herman et al. 2000, Galván et al. 2008). Studies during the last decade have shown that it is possible to selectively label different food sources (phytodetritus: Blair et al. 1996; MPB: Middelburg et al. 2000; and bacteria: van Oevelen et al. 2006) and follow their fate within the benthic food web. Moreover, through the use of compound-specific isotope analysis, it is now possible to include and resolve the microbial compartment of food webs (Middelburg et al. 2000, Boschker & Middelburg 2002).

In the present study, we combine a natural abundance stable isotope approach with a dual stable isotope pulse-chase experiment. Freshly sampled cores of sandy sediment were incubated in the laboratory and its MPB was labelled with ^{13}C-bicarbonate and ^{15}N-nitrate. In a complimentary study, Evrard et al. (2008) reported in detail the flow of carbon and nitrogen, focusing on the interactions between phototrophic,

chemoautotrophic, and heterotrophic microbes. In this contribution, we trace the flows of carbon and nitrogen from the microbial compartment into meio- and macrofauna, and investigate the relative contributions of benthic diatoms, benthic cyanobacteria, and suspended matter (phytoplankton and detritus) to animals' diets.

MATERIALS AND METHODS

Study site and experimental setting. The study took place in List (Germany) on the island of Sylt in the Wadden Sea, in July 2004. The sampling site, the Hausstrand, was situated south of the harbour close to the site described earlier by de Beer et al. (2005). The area is exposed to the east and strongly influenced by north-south tidal currents parallel to the shore and wind-driven waves. The sediment is a silicate sand with a median grain size of 350 µm (M. Huettel unpubl. data), a porosity of 0.42 and organic carbon content of 0.21 % by weight, and a molar C:N ratio of 7.9 (Evrard 2007). At the time of sampling, bottom-water temperature was 15°C and contained ~5 µM dissolved inorganic nitrogen (Hedtkamp 2005).

At low tide, 5 sediment cores (15 cm high, 19 cm in diameter) with overlying water were retrieved from the shallow subtidal area (55° 0′ 47.46″ N, 8° 25′ 52.73″ E) at ~1.5 m depth and brought 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 by PVC lids and used as flux chambers. The upper transparent lid of the chambers supported an electric motor that rotated a 15 cm diameter transparent disc stirring the water above the sediment cores. The discs' stirring speed in the chambers was set to 40 rpm, which generated a pressure gradient of 0.2 Pa cm^{-1} at the sediment–water interface between the wall and the center, causing advective exchange of porewater and overlying water (Huettel & Gust 1992). All chambers were immersed in a 200 l filtered seawater tank maintained at *in situ* temperature (15°C) and constantly aerated. Initially, chambers' upper lids were elevated about 1 cm above the chambers' edge to allow exchange between the water in each chamber and the aerated water of the tank. Water circulation 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 acclimate for 24 h prior to the start of the experiment. In addition to the sediment cores, triplicate samples of suspended particulate matter (SPM) were collected for C and N stable isotope analysis by filtering 2 l seawater from the sampling site onto pre-combusted GF/F filters, and were stored at –20°C until freeze-drying and analysis.

Labelling and incubations. On the following day, one of the chambers was sampled to provide background values (t = 0) for the different analyses described hereafter. The remaining chambers were closed and received a pulse of 0.85 mmol $NaH^{13}CO_3$ (~11 mg ^{13}C-DIC), equivalent to a 10% ^{13}C-labelling of the DIC pool (~1950 µmol l^{-1}), and 0.17 mmol $Na^{15}NO_3$ (~2.5 mg ^{15}N), corresponding to ~95% labelling of the DIN pool (<5 µmol l^{-1}). The label was dissolved in 5 ml seawater from the tank and was added to the stirred, well-mixed water column of the chamber. The cores were illuminated by artificial light providing a homogenous irradiance of 185 µmol quanta m^{-2} s^{-1} at the sediment surface, which corresponded roughly to the average daily irradiance observed in the field at the sediment surface for that period (F. Wenzhöfer unpubl. data). The MPB was exposed to label for a period of 9 h with light. Then the light was switched off, and the water column of the 5 cores was flushed twice to remove labelled bicarbonate and nitrate. Flushing was performed by gently siphoning the water above the sediment cores and replacing it with fresh 100 µm filtered 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). When in the dark, the cores were always kept open and immersed in the tank to allow aeration and mixing of the water within the whole system. A 09:15 h light:dark cycle was maintained during the 4 d of the experiment.

On each day (t = 0, 1, 2, 3, and 4 d) total respired ^{13}C was estimated from changes in ^{13}C-DIC concentrations over a 4 h period. Four h prior to every illumination period, each chamber was closed, and a small amount of its overlying water was sampled into a 12 ml headspace vial to which 12 µl HgCl was added to preserve the sample. After 4 h incubation in the dark, and just before turning on the light, another water sample was taken and the concentration difference between those 2 points allowed estimating ^{13}C respiration. Integration over the entire duration of the experiment permitted estimation of total respired ^{13}C.

Immediately after each period of illumination, one chamber was taken out of the tank and its sediment core sampled. Sampling was done by taking 5 small subcores (3.56 cm inner diameter, area ~10 cm^2) from each core. The 3 subcores were sliced horizontally 0–1, 1–2, 2–3, 3–4, 4–5, and 5–10 cm for C, N, and phospholipid-derived fatty acids (PLFA) analysis. The same layers were pooled, homogenized, and freeze-dried.

One subcore was sliced the same way and kept for meiofauna analysis. The remaining subcore was sliced 0–2, 2–4, 4–6, 6–8, 8–10, and 10–20 mm, freeze-dried, and used for analysis of pigment concentrations (results reported in Evrard et al. 2008). Sediment samples for C, N, PLFA, and meiofauna were stored in a freezer at –20°C until freeze-drying and/or analysis. Sediment samples for pigments were stored at –80°C.

Fauna sampling. ***Macrofauna:*** On the same day as the field sampling of the sediment cores, 2 extra cores of same dimensions (15 cm height, 19 cm diameter) were pooled into a large bucket, and then the sediment was carefully sifted on a 1 mm mesh size sieve. Retained macrofauna was handpicked, pooled into a large container with seawater, and gently cleaned to remove mucus, faeces, and particles. Animals were sorted to species level into different glass vials and stored in the freezer. Macrofauna from the different labelled cores, sampled at Day 1, 2, 3, and 4, was extracted the same way from the remaining sediment of each large core. All animals were dried individually in an oven for 48 h at 60°C, and their dry weight was estimated. For each core, macrofauna individuals belonging to the same species were pooled together and ground to a fine homogenous powder, which was analyzed for organic C and N contents, and isotopic signatures. Samples for organic C were all acidified to remove all inorganic C contamination.

Meiofauna: Animals were neither stained nor fixed to avoid addition of exogenous C that could have contaminated the samples with a different isotopic signature. In this procedure, frozen sediment samples were thawed at room temperature and thoroughly rinsed with distilled water on a 38 µm sieve. Meiofauna was extracted from sediment with colloidal silica (Ludox® HS 40; DuPont) with a density of 1.31 g cm^{-3} following the protocol proposed by Burgess (2001). In brief, the sediment sample is washed with Ludox® into a 50 ml disposable polypropylene centrifuge tube. The tube is capped and thoroughly mixed using a vortex mixer at a gradually decreasing speed and finally centrifuged for 5 min at 900 × *g*. The supernatant is rinsed again with distilled water in the sieve and finally poured into a Petri dish for counting and picking. The sediment pellets were set aside for verification of its remaining content. The animals were sorted to major taxonomic levels and counted under a stereo-microscope. They were finally transferred to Sn cups for C and N stable isotope analysis. However, meiofauna samples with a $CaCO_3$ shell (gastropods, ostracods, and foraminifers) were transferred to Ag cups and acidified (10% HCl) to remove any inorganic C. Individual biomasses of meiofauna taxa were estimated by integrating C and N peaks from the chromatograms of the stable isotope analysis.

Due to the large amount of material needed for one ^{15}N analysis (e.g. >10 ind. for juvenile gastropods, >50 ind. for copepods, >100 ind. for nematodes, >200 ind. for tardigrades), and because priority was given to ^{13}C analysis for meiofauna, only a few taxa could be analyzed for their ^{15}N content.

Analyses. Small fractions of the freeze-dried sediment samples were ground in agate mortars to obtain a homogeneous and fine powder and acidified (10% HCl) to remove any inorganic C, including any trace of label. Organic carbon and nitrogen content and isotopic composition (^{13}C and ^{15}N) of sediment, SPM, macrofauna, and meiofauna were measured using a Carlo Erba/Fisons/Interscience elemental analyser coupled online via a conflo interface to a Finnigan Delta S isotope ratio mass spectrometer.

PLFA for all layers down to 5 cm were extracted from approximately 6 g of sediment per layer, following the method of Boschker et al. (1999) and Middelburg et al. (2000), and their concentrations were determined by gas chromatograph-flame ionization detection (GC-FID). PLFA carbon isotopic composition was determined using a gas-chromatograph combustion-interface isotope ratio mass spectrometer (GC-c-IRMS). Bacterial and MPB carbon content in the sediment were estimated from the PLFA concentrations (for details see Evrard et al. 2008). Stable isotope data are expressed in the delta notation ($\delta^{13}C$ and $\delta^{15}N$) relative to the stable isotopic ratio of Vienna Pee Dee Belemnite standard (R_{VPDB} = 0.0111797) and the air standard (R_{Air} = 0.0036765), for carbon and nitrogen respectively. $\delta^{H}X = (R/R_{std} - 1) \times 1000$, where X is C or N and $R = {}^{H}X/{}^{L}X$ the heavy (H) to light (L) isotope ratio measured in the sample and in the standard (VPDB or air). Label uptake is reflected as enrichment in ^{13}C and ^{15}N and is presented in the δ^{E} notation of Maddi et al. (2006):

$$ {}^{H}X\delta^{E} = 1000 \times (R_s - R_b) / R_b = $$

$$ [(\delta^{H}X_s + 1000) / (\delta^{H}X_b + 1000) - 1] \times 1000 $$

where R_b is the isotope ratio in the background and R_s in the enriched sample. Positive values indicate that the organisms have acquired some of the introduced label. The incorporation as defined by Middelburg et al. (2000), $I = E \times Q$, is the total uptake of label expressed in mg ${}^{H}X\ m^{-2}$, where E is the atomic excess and Q a quantity (organic C, N or C-PLFA; i.e. a biomass in mg m^{-2}). The atomic excess, $E = F_s - F_b$, is the difference between the stable isotope fraction (F) of the enriched sample and of the background, with $F = {}^{H}X / ({}^{H}X + {}^{L}X) = R / (R + 1)$.

Data handling. ***Natural abundances and isotope-mixing model:*** 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 that of the consumer, 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 3 food sources or more and only one stable isotopic ratio (^{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 sequentially applied 4 isotopic mixing models with increasing number of food sources and with and without variability in isotopic ratios accounted for (1) a 2-food source mixing model using the mean values of food source and consumer composition, (2) a 2-food source isotope mixing model based on the ranges (min. – max.) of food source and consumer isotopic signatures, (3) a 3-food source mixing model using mean values, and (4) a 3-food source model using ranges of food sources and consumers.

For 2 and 3 food sources, the linear model to solve for is:

$$\delta_M^C = p_1\delta_1^C + p_2\delta_2^C \quad \text{and} \quad \delta_M^C = p_1\delta_1^C + p_2\delta_2^C + p_3\delta_3^C$$
$$1 = p_1 + p_2 \qquad\qquad 1 = p_1 + p_2 + p_3$$

where δ_M^C and δ_i^C are the carbon stable isotopic $\delta^{13}C$ values of the consumer and source i respectively, and p_i (≥ 0) is the relative proportion of food item i in the diet of consumer M. The first model with 2 equations for 2 unknowns has one solution or is infeasible (no solution) and is solved by matrix inversion. The second model with 2 equations for 3 unknowns either leads to a range of solutions, or is infeasible; this model can be solved using linear programming techniques. The modelling was done in the open-source software R (R Development Core Team 2007), using package limSolve (Soetaert et al. 2008).

For those simulations where ranges of isotopic signatures were taken into account, the model was solved using all possible combinations of minimum and maximum values of food source and consumer values. This resulted in estimated ranges of the contribution of food sources to the consumer's diet. Note that the obtained contributions can be direct, i.e. when the consumer feeds on the primary food source, and indirect when the consumer preys on another consumer relying on the primary food source.

Deliberate tracer experiment: In the absence of prior knowledge about the relevant time scale of carbon and nitrogen transfer from MPB to meio- and macrofauna consumers in subtidal sandy sediments, we adopted an experimental design that was intended to follow ^{13}C and ^{15}N over a period of 4 d through a time series of label enrichment and incorporation in the different biological compartments. However, label incorporation into all biological compartments was rapid, and there were no significant temporal trends in the labeling pattern over the timeframe of the experiment (Evrard 2007). The variability within the time series could be associated with any biological and/or experimental parameter. The data were therefore pooled and used as replicates so as to increase the robustness of our inferences. All data are presented as mean values of the replicates, and error bars indicate standard deviations (SD).

RESULTS

Benthos composition

Although meiofauna was sampled down to 15 cm in the sediment, we report only the upper 3 cm layers because the decrease of abundance with depth precluded isotope analyses further down (Fig. 1A). Nematodes and harpacticoid copepods were the 2 most abundant taxa with about 641 ± 203 × 10^3 and 390 ±

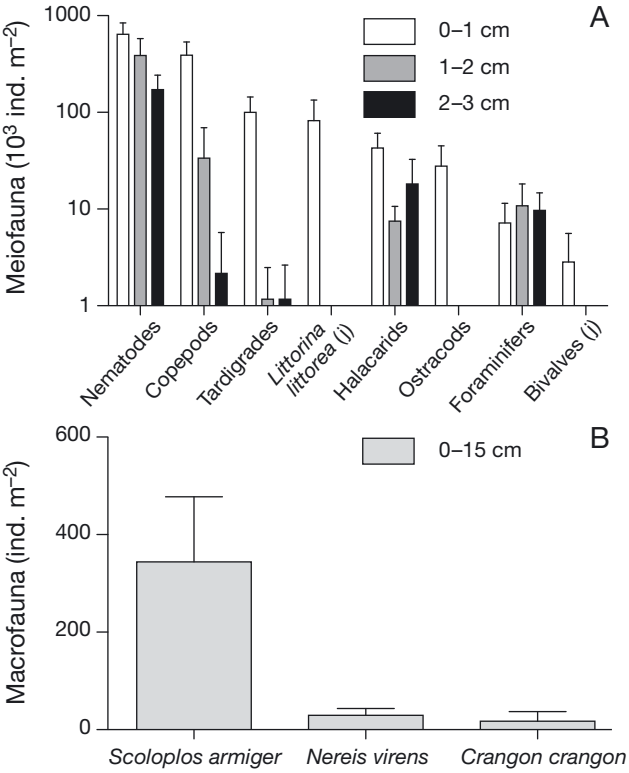

Fig. 1. Benthic metazoan densities (mean ± SD, n = 6). (A) meiofauna, including juvenile (j) forms of macrofauna satisfying the size criteria (<1 mm), for the top 3 × 1 cm layers; (B) macrofauna for the whole sediment core (15 cm). NB: *Crangon crangon* was only found at the sediment surface

146×10^3 ind. m^{-2} respectively within the first 1 cm layer. Tardigrades were the third most abundant taxon with approximately $100 \pm 44 \times 10^3$ ind. m^{-2}. With the exception of nematodes and copepods, all taxa were mainly restricted to the uppermost 1 cm of the sediment. Although adult gastropods and bivalves are part of the macrofauna, juvenile individuals (j) of the gastropod *Littorina littorea* and some unidentified species of bivalves were included in the meiofauna compartment as they satisfied the size-class criterion (passing through a sieve with a mesh size of 1 mm).

The macrofauna consisted of 2 species of deposit feeding polychaetes and 1 species of decapod predator (Fig. 1B). The polychaete *Scoloplos armiger* was the most abundant species with about 344 ± 134 ind. m^{-2}. The other polychaete present in the study, *Nereis virens*, was far less abundant with approximately 29 ± 14 ind. m^{-2}. Some young and small individuals of the brown shrimp *Crangon crangon* were observed in 3 out of 6 cores corresponding to an abundance of 18 ± 19 ind. m^{-2}. As *C. crangon* is a highly motile epifaunal species, its estimated abundance should be taken with caution as shrimps might have escaped during sampling.

The horizontal distribution of fauna was very patchy as reflected in macrofauna and meiofauna densities (Fig. 1). The coefficient of variation (standard deviation /mean) was always higher than 0.25, on average ~0.60. The biomass of the fauna was calculated as the product of the taxon's individual mean dry weight multiplied by its abundance and is presented in terms of C and N in the top 3 cm (Table 1). Because of high variability in individual weight due to some outliers, nematodes, copepods, and *Littorina littorea* biomasses were calculated based on median dry weight. Nematodes and juveniles of *L. littorea*, followed by copepods, contributed most to the total biomass of the meiofauna compartment. Other species represented only a very small fraction of the total faunal biomass. Meiofauna biomass was ~661 mg C m^{-2} and macrofauna ~478 mg C m^{-2} (with total polychaete biomass divided by 5 to scale to the upper 3 cm of sediment). Altogether, the meiofauna accounted for about 60 % of the total metazoan biomass.

MPB composition and biomass were assessed from pigment concentrations and revealed the presence of diatoms, cyanobacteria, and a negligible fraction of haptophytes (Evrard et al. 2008). These results were further confirmed with the PLFA composition of the bulk sediment. The algal-specific PLFA C20:5ω3 (diatoms) was among the major PLFA compounds present in the sediment. In addition, since the presence of green algae could not be detected (absence of chl *b*; Evrard et al. 2008), C18:3ω3 and C18:4ω3 PLFAs were attributed to cyanobacteria and therefore used as a proxy for their isotopic signatures. MPB biomass estimated from PLFA was about 17 g C_{org} m^{-2} for the top 2 cm of sediment, based on a C_{PLFA}:C_{MPB} conversion factor of 0.053 (Evrard et al. 2008). Bacterial biomass, estimated from the bacterial-specific PLFA (iC14:0, iC15:0, aiC15:0, and iC16:0), was 3.8 g C_{org} m^{-2} for the top 2 cm of sediment.

Natural abundance of stable isotopes

Microbial compartment

Natural stable isotopic signatures indicated that MPB (composed primarily of diatoms and cyanobacteria) and phytoplankton (measured as suspended par-

Table 1. Organic C and N biomass expressed in mg m^{-2} (mean ± SD, n = 6) and average C:N ratio (mol:mol, where available) for the different components of the sediment. Bulk sediment includes all components excluding macrofauna (which was extracted prior to sediment component analysis); the uncharacterized fraction is the difference between the bulk sediment and the sum of the known components. *Crangon crangon* individuals were only found at the sediment surface. MPB: microphytobenthos; –: unavailable data

	C_{org}			N			C:N
	0–1 cm	0–2 cm	0–3 cm	0–1 cm	0–2 cm	0–3 cm	
Bulk sediment	28794.9 ± 2667.3	28523.5 ± 3795.6	27534.5 ± 2555.3	4238.6 ± 373.6	4110.9 ± 693.8	4061.4 ± 581.0	8.0
MPB	12081.0 ± 3846.0	12756.6 ± 3430.4	11459.4 ± 4632.9	–	–	–	–
Bacteria	1756.8 ± 422.9	2058.3 ± 484.8	2134.9 ± 553.5	–	–	–	–
Littorea littorea (j)	247.1 ± 157.5			56.0 ± 35.7			5.1
Nematodes	130.9 ± 41.5	85.9 ± 42.5	40.8 ± 16.4	27.0 ± 8.6	18.2 ± 9.0	9.6 ± 3.9	5.4
Copepods	109.8 ± 41.1	9.5 ± 10.1	0.6 ± 1.0	14.4 ± 5.4	1.2 ± 1.3	0.1 ± 0.1	8.9
Halacarids	12.6 ± 6.1	2.3 ± 1.0	5.7 ± 4.6	–	–	–	–
Tardigrades	6.8 ± 2.8	0.1 ± 0.1	0.1 ± 0.1	–	–	–	–
Ostracods	5.1 ± 2.5	–	–	–	–	–	
Foraminifera	1.8 ± 0.9	1.7 ± 0.2	3.8 ± 3.5	–	–	–	–
Uncharacterized	12198.8 ± 6135.4	13899.6 ± 3267.8	12868.4 ± 5319.2	–	–	–	–
Macrofauna		0–15 cm			0–15 cm		
Scoloplos armiger		2098.6 ± 816.1			463.4 ± 180.2		5.3
Nereis virens		205.1 ± 100.5			51.9 ± 25.4		4.6
Crangon crangon		16.8 ± 18.4			3.9 ± 4.3		5.0

ticulate matter, SPM) were the 2 major resources for the benthic food web (Fig. 2). The biomarker information obtained from PLFA analysis allowed us to characterize natural abundance $\delta^{13}C$ values of the different constituents of the microbial compartment from their specific PLFA (20:5ω3 for diatoms, C18:3ω3 and C18:4ω3 for cyanobacteria, and iC15:0 and aiC15:0 for heterotrophic bacteria; see Evrard et al. 2008). However, $\delta^{13}C$ values of lipids, including PLFA, are depleted relative to total cell biomass $\delta^{13}C$ (Hayes 2001), and a correction is needed to obtain cell biomass $\delta^{13}C$ values from PLFA $\delta^{13}C$. This correction factor is usually about 3‰ (Boschker et al. 1999, 2005, Hayes 2001). If we assume that MPB and MPB-derived organic material (including EPS and phytodetritus) made up the bulk sediment organic matter in this sandy sediment (Evrard et al. 2008), we independently estimate a correction factor of ~2.8‰; i.e. $\delta^{13}C_{Bulk\ sediment} = 0.66\ \delta^{13}C_{Diatoms} + 0.33\ \delta^{13}C_{Cyanobacteria}$, because diatoms and cyanobacteria contributed 66 and 33% to MPB (Evrard et al. 2008). Based on the weighted average of their specific PLFA, diatoms, cyanobacteria, and heterotrophic bacteria were estimated to have $\delta^{13}C$ values of –16.3 ± 1.4‰, –19.9 ± 2.8‰, and –16.3 ± 0.5‰, respectively (± SD, n = 3). The $\delta^{13}C$ of SPM (–20.7 ± 0.7‰), a proxy for phytoplankton, was similar to that of benthic cyanobacteria. $\delta^{15}N$ of SPM and bulk sediment were similar with 11.7 ± 0.8 and 11.9 ± 1.9‰, respectively (n = 3; background replicates). Unfortunately, we lack biomarkers that would allow us to distinguish $\delta^{15}N$ of diatoms and cyanobacteria.

Meiofauna

Most taxa present in the study had natural abundance $\delta^{13}C$ values within the range of the values of the different food sources, suggesting no clear dependency on either source but rather a heterogeneous diet (Fig. 2). Juvenile *Littorina littorea* and ostracods were most ^{13}C-depleted, suggesting a reliance on settled SPM and/or cyanobacteria. Copepods had rather low $\delta^{15}N$ values, while the $\delta^{15}N$ of nematodes and juveniles of *L. littorea* were high, similar to those of the bulk sediment and SPM.

Macrofauna

The polychaetes *Scoloplos armiger* and *Nereis virens* and the shrimp *Crangon crangon* had natural abundance $\delta^{13}C$ values ranging from –15.5 to –13.1‰, suggesting

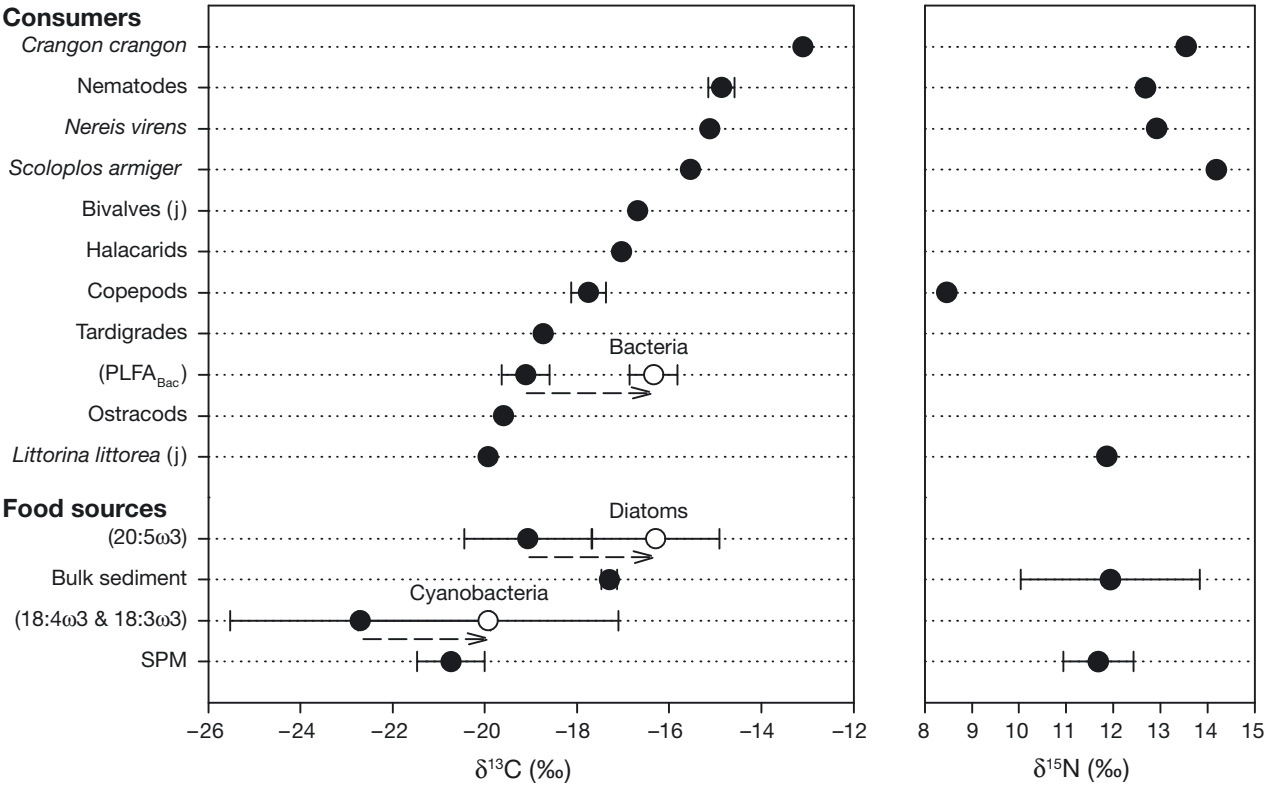

Fig. 2. Stable isotope background $\delta^{13}C$ and $\delta^{15}N$ values (where available) of the benthic components (mean ± SD, n = 3 if applicable, n = 1 otherwise). Arrows represent the shift of $\delta^{13}C$ values to account for fractionation between phospholipid-derived fattyacids (PLFA) and biomass. j: juvenile; $PLFA_{Bac}$: bacterial PLFA (iC14:0, iC15:0, aiC15:0, and iC16:0). SPM: suspended particulate matter

that most of their carbon eventually came from diatoms. Macrofauna species had natural abundance $\delta^{15}N$ values slightly higher than that of the main substrates.

The relative contributions of the different potential food sources to the diet of the various consumers were estimated using 4 isotopic mixing models with 2 or 3 food sources and with or without variability in $\delta^{13}C$ accounted for (see 'Materials and methods: Data handling'). First we considered 2 food sources: ^{13}C of surface bulk sediment (proxy for MPB) and ^{13}C of SPM (proxy for phytoplankton) (Fig. 3A,B). Mathematically, the contribution of these 2 sources can be uniquely determined based on 1 isotopic measurement. However, based on the mean $\delta^{13}C$ values only (i.e. ignoring the variability of the stable isotopic signatures of the consumers and that of their food sources), the contribution of these food sources could be resolved for only 4 consumer taxa. Juvenile *Littorina littorea* were mostly feeding on SPM (0.77) and less on MPB (0.23). The opposite pattern was found for copepods whose diet is composed primarily of MPB (0.87) and less of SPM (0.13). Ostracods and tardigrades, however, showed intermediate patterns. Second, uncertainty was accounted for in the isotope-mixing model, which yielded ranges of food source contribution for these 4 consumers, but still not allowing for the determination of the diet of the other consumers (Fig. 3A,B). Third, we considered a 3-source mixing model with diatoms and cyanobacteria identified (from PLFA stable isotopic signatures) as 2 distinct food sources within the MPB (Fig. 3C,D,E). With only one isotopic element and 3 potential food sources (cyanobacteria, diatoms, and SPM), the isotope mixing model could only resolve the diet of consumers within ranges, because it is mathematically un-

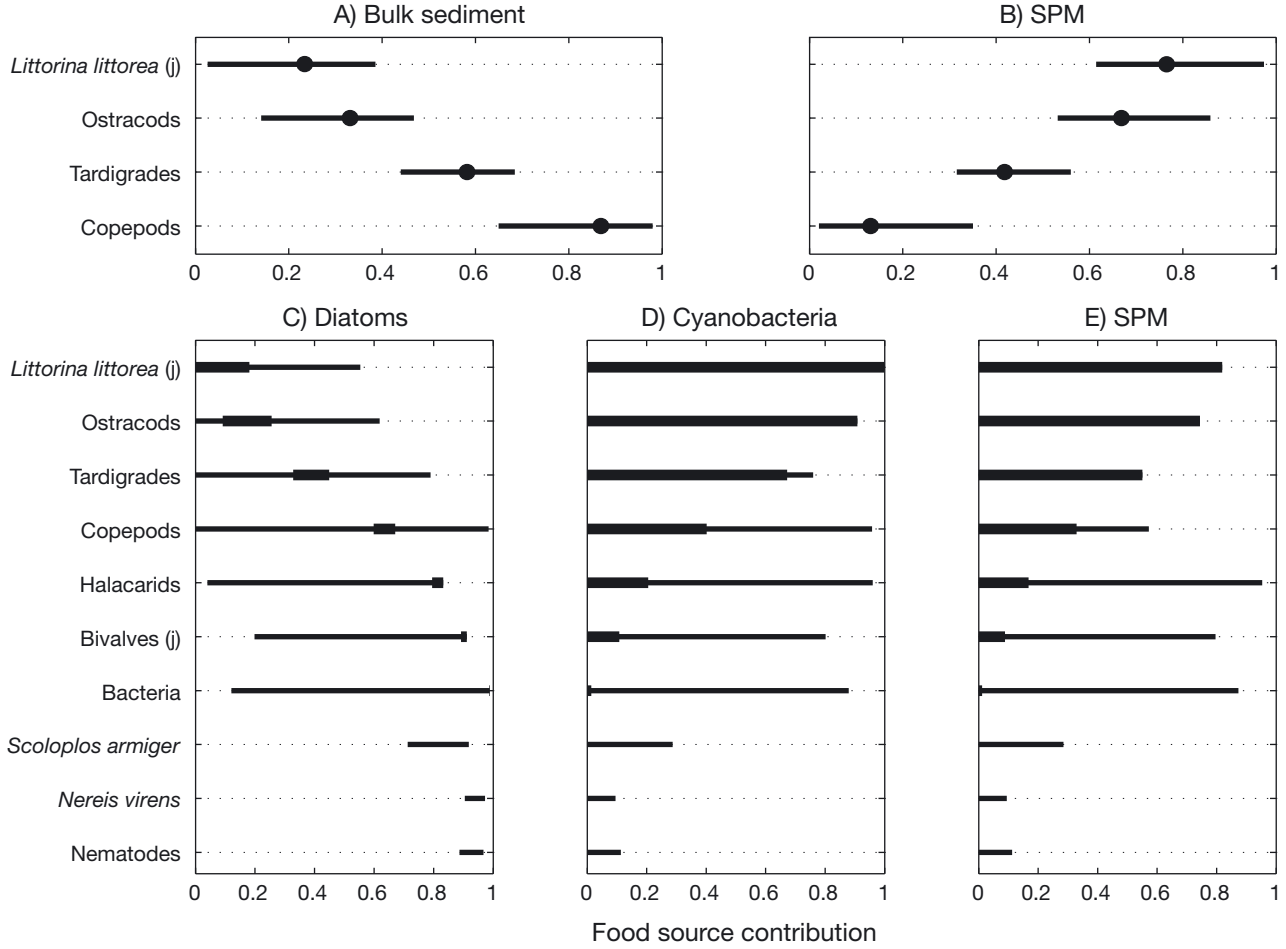

Fig. 3. Food source contributions to the diet of the heterotrophs expressed as relative fractions (no unit). (A,B) Two-source mixing model, including (A) benthic and (B) pelagic sources (dots: single solution based on mean isotopic values; horizontal line: ranges estimated based on min. – max. values of isotopic composition). The 2-source mixing model could only explain the diet of 4 taxa; therefore, the other taxa are omitted here. (C,D,E) Results of 3-source mixing model, distinguishing between diatoms and cyanobacteria (comprising the microphytobenthos, MPB), and suspended particulate matter (SPM) $\delta^{13}C$ values (thick bars: solution for ranges of contributions, based on the average $\delta^{13}C$ values of food sources and consumers; thin bars: ranges of contributions obtained by taking into account the ranges of food sources and consumers $\delta^{13}C$ values). NB: *Crangon crangon* was omitted since its diet could not be determined from either model. j: juvenile

derdetermined (Phillips & Gregg 2003, Lubetkin & Simenstad 2004). In this case, the mixing model allowed estimation of the contribution of the different food sources to the diet of all the different taxa except *Crangon crangon*. In addition, various consumers showed more complex dependencies on the 3 potential food sources (Fig. 3C–E). Due to their similar $\delta^{13}C$ values, the mixing model revealed that SPM and cyanobacteria contributed equally to the diet of most taxa, both with and without including $\delta^{13}C$ ranges in the calculations. The taxa *L. littorea*, Ostracoda, Tardigrada, Copepoda, Halacarida, and Bivalvia (from top to bottom in Fig. 3C) showed increasing dependence on diatoms and conversely for cyanobacteria and SPM (Fig. 3D,E). This 3-food source model allowed inferences about the diet of most consumers based on their average $\delta^{13}C$ values, but not for bacteria, nematodes, and macrofauna species. All consumers except *C. crangon* could be resolved with calculations that included ranges of food sources and consumers isotopic composition. Using the fourth approach (3 food sources and variability included in the isotope mixing model) bacteria were estimated to depend on all 3 food sources with a slightly higher reliance on diatoms. *Scoloplos armiger, Nereis virens,* and the nematodes relied preferentially on diatoms.

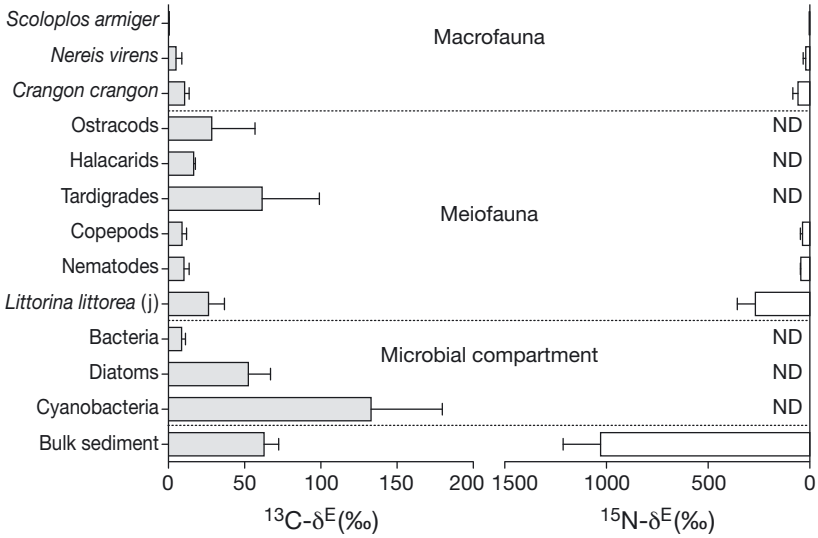

Fig. 4. Benthic ^{13}C and ^{15}N enrichment (δ^E; mean ± SD, n = 4), respectively, on the primary and secondary *y*-axis (where available), as estimated from PLFA for the microbial compartment (bacteria, diatoms, and cyanobacteria) and from bulk organic material for the sediment, the macrofauna, and the meiofauna. Note: hard-shelled Foraminifera and juvenile bivalves were not present in sufficient numbers to allow measurements of isotopic enrichment. ND: no data; j: juvenile

Stable isotope addition experiment

Average MPB isotopic enrichment was significant with a pronounced difference between diatoms and cyanobacteria (Fig. 4). Enrichment values derived from PLFA data revealed that cyanobacteria (133 ± 47‰) were almost 3× more enriched in ^{13}C than diatoms (52 ± 15‰), implying that cyanobacteria were capable of fixing almost 3× more C than diatoms should their biomasses be equal. Bacterial enrichment was low compared to that of MPB (9 ± 3‰). As a result, bulk sediment had intermediate ^{13}C-δ^E values with respect to the MPB (63 ± 9‰). The MPB ^{15}N-enrichment (^{15}N-δ^E) could not specifically be estimated, but bulk sediment enrichment was 1028 ± 184‰.

Macrofauna ^{13}C and ^{15}N enrichments were significant given that analytical uncertainty and background natural variability are on the order of 1‰ (Figs. 2 & 4). The predatory shrimp *Crangon crangon* was the most enriched macrofauna species. *Nereis virens*, an omnivorous surface deposit-feeder, was as expected significantly more enriched than *Scoloplos armiger*, a subsurface deposit-feeder.

Meiofauna enrichment was significant for all taxa and generally higher than that of the macrofauna (Fig. 4). In addition to their higher δ^E, species with the smaller individuals (ostracods and tardigrades) also showed large variability in their enrichment (reflected by the SD). The limited biomass of tardigrades, halacarids, and ostracods recovered did not provide enough material for ^{15}N-δ^E measurements. Copepods, juvenile *Littorina littorea,* and nematodes were strongly enriched in ^{13}C, especially compared to the polychaetes.

Total C and N incorporation

Total organic C and N pools (bulk sediment) for the 0–1 cm layer were about 29 and 4 g m^{-2}, respectively and slightly decreased with depth, with a average molar C:N ratio of 8.0 (Table 1). For the same layer, MPB and bacteria (based on PLFA) and meiofauna accounted for about 41.8, 6.1, and 1.8% of the total carbon pool, respectively. As macrofauna was extracted from the sediment prior to bulk sediment analysis and because this was done over the whole 0–15 cm layer, macrofauna (which contributed 2320 mg C m^{-2}) could not be related to the 0–1 cm layer. The sum of all living compartments accounted for about 49.7% of the bulk sediment organic carbon pool (macrofauna not included), suggesting that detritus and EPS could con-

tribute 50.3% to the total sedimentary organic carbon (the uncharacterized fraction). Nitrogen content was inferred for macrofauna and a few meiofauna taxa. Most taxa of the metazoan community had a C:N ratio of about 5, however copepods had a C:N ratio of 8.9.

MPB organic ^{13}C incorporation for the upper 1 cm layer, estimated from PLFA, was high, with an incorporation of 8.6 ± 1.3 mg ^{13}C m^{-2} (Fig. 5A). However, as the label incorporation of the bulk sediment for the same layer was twice as high, it seems that a major part of the fixed carbon was excreted (uncharacterized fraction, 10.7 ± 1.7 mg ^{13}C m^{-2}). Bulk sediment ^{15}N incorporation for the upper cm layer was significant, with 16.2 ± 2.9 mg ^{15}N m^{-2} (Fig. 5B). The molar C:N ratio of incorporation, estimated considering a 10% ^{13}C-labelling of the dissolved inorganic carbon pool and a 95% ^{15}N-labelling of the N pool, was high (~13) and consistent, with a high production of carbon-rich extracellular material (e.g. carbohydrates).

Bacterial ^{13}C incorporation for the upper 1 cm layer estimated from PLFA (Fig. 5A), was the highest among heterotrophic organisms, with 0.164 ± 0.049 mg ^{13}C m^{-2}. The sum of the meiofauna ^{13}C incorporation for the same sediment layer was 0.104 ± 0.040 mg ^{13}C m^{-2}; juvenile *Littorina littorea* accounted for the largest fraction (0.070 ± 0.028 mg ^{13}C m^{-2}). ^{15}N incorporation was only available for a limited number of taxa (Fig. 5B) and revealed that juveniles of *L. littorea* contributed most (5.52 ± 1.84 mg ^{15}N m^{-2}). Nematodes and copepods also contributed to label incorporation, although to a lesser extent. The molar C:N ratios of incorporation for the meiofauna were low (Fig. 5A), suggesting that meiofauna assimilated N preferentially or grazed on N-rich resources. ^{13}C and ^{15}N incorporation by macrofauna was limited. The sum of macrofauna ^{13}C incorporation for the whole 15 cm depth of sediment (0.033 ± 0.020 mg ^{13}C m^{-2}) was about a third that of the meiofauna for the 0–1 cm sediment layer. In contrast to meiofauna, macrofauna C:N ratios of incorporation were very high, implying that macrofauna preferentially assimilated C or ingested material with a high C:N ratio. Finally, total ^{13}C respired by the whole heterotrophic community estimated from ^{13}C-DIC incorporation measurements integrated over the 4 d period was 3 mg m^{-2}. This corresponds to ~14% of the total ^{13}C incorporation (respired ^{13}C + 0–1 cm bulk sediment ^{13}C incorporation; Fig. 5A).

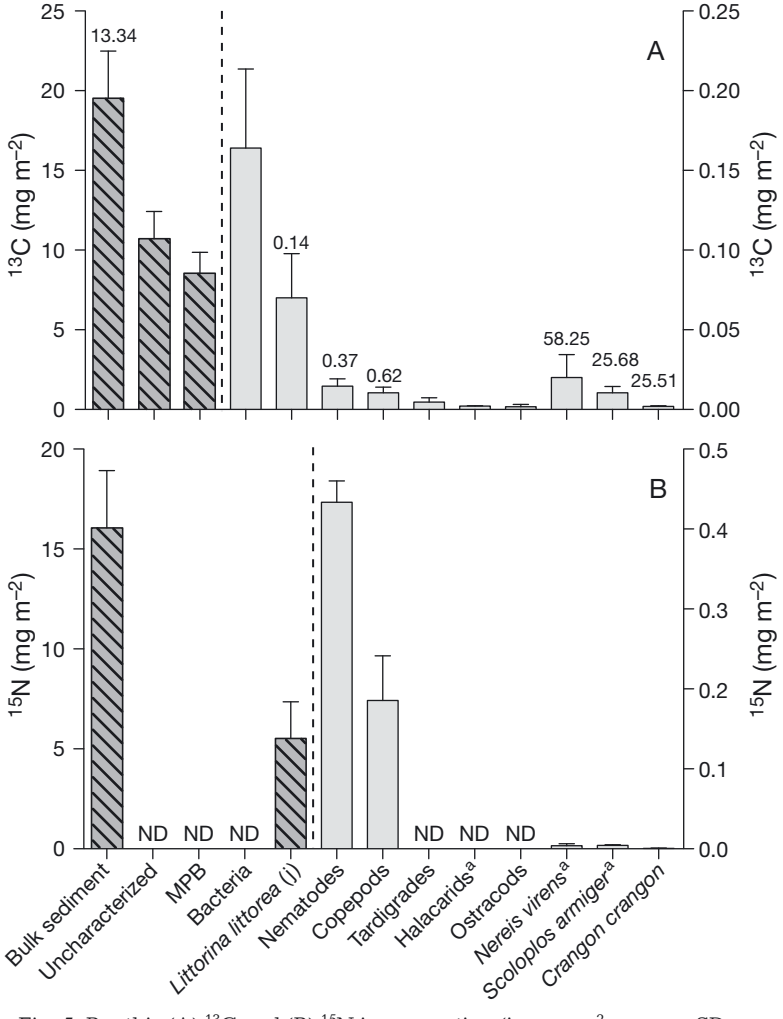

Fig. 5. Benthic (A) ^{13}C and (B) ^{15}N incorporation (in mg m^{-2}; mean ± SD, n = 4 or [a]n = 3) in the different benthic organisms or compartments for the top 1 cm layer. The hatched bars refer to the left *y*-axis and the plain bars to the right one. When ^{15}N-data were available, the C:N ratio of incorporation is reported above the bars in (A). MPB: microphytobenthos; ND: no data

DISCUSSION

This food web study demonstrates the key role of MPB as a resource for benthic heterotrophic organisms in a photic subtidal sandy sediment. In a complimentary study focussing on the microbial interactions, Evrard et al. (2008) showed rapid transfer of ^{13}C and ^{15}N label from dissolved inorganic pools to the MPB and that a large fraction of the primary production was allocated to the exudation of EPS. EPS represented the main source of energy for the heterotrophic bacteria that were gradually enriched during the course of the experiment. In the present study, we put emphasis on the metazoan community inhabiting this subtidal sandy sediment and uncover the relative contributions of the different heterotrophic compartments to the processing of fresh organic matter.

The low stock and high turnover of organic matter combined with high temporal and spatial variability of benthic communities and biogeochemical processes (Boudreau et al. 2001) complicate the study of sandy sediment especially in dynamic settings. Our understanding of carbon and nitrogen flows within sandy sediments is therefore very limited compared to that in silty and muddy sediments. We have adopted 2 complementary approaches: a natural abundance stable isotope study and deliberate tracer study, each with its strengths and weaknesses. The natural abundance stable isotope approach is most commonly used and has the following advantages: (1) that no incubations with potentially disturbed environmental conditions are required and (2) that it integrates carbon flows over longer periods of time. However, it requires that potential food sources are adequately sampled and differ significantly in isotope signatures (Mutchler et al. 2004). The deliberate tracer approach does not have this limitation because potential food sources can be labelled uniquely and sufficiently to resolve the problem of overlapping end-member values. However, tracer addition experiments require (1) isolation of the ecosystem to be studied and (2) prior knowledge of carbon and nitrogen flow dynamics of the system.

Our deliberate tracer experiment was conducted in the laboratory rather than *in situ* in order to allow maximum control and recovery of different parameters investigated. Contrary to tidal flats where sampling plots can be easily delimited and enriched with a stable isotope tracer during air exposure (Middelburg et al. 2000, van Oevelen et al. 2006), the same approach in subtidal areas is more complex. The only way to perform a pulse-chase experiment *in situ* in a subtidal area would have been through the use of benthic chambers anchored to the sediment. The complete recovery of biological, sediment, and water samples would have been logistically impractical in an environment often subject to rough wave conditions. In the laboratory incubations, temperature, light regime, and advective flow were set to mimic the natural conditions and moderate boundary flow conditions.

Deliberate tracer approaches involve incubations of limited duration. These experiments therefore provide only information on contemporary organic sources, e.g. MPB production that has occurred prior to incubations will not be traced. Experimental duration of tracer studies is the most critical issue because tracer added in inorganic form has to be taken by primary producers and then be transferred to consumers. No or very limited label will be found in consumers when incubations are too short (no label transfered yet) or too long (dilution and recycling of label). Without prior knowledge on carbon or nitrogen flow in permeable sandy sediment we therefore adopted a time-course experimental setup similar to that used in studies of fine-grained sediments (Middelburg et al. 2000). However, label processing in these sandy sediments was more rapid and the time series of label of enrichment more erratic (Evrard 2007), indicating that future studies should sample at higher temporal resolution and allow for more replication.

Benthic community composition

PLFA analysis, supported by pigment analysis (Evrard et al. 2008), proved to be a good approach in determining MPB composition. In that complimentary study (op. cit.), diatoms (~60%) and cyanobacteria (~30%) dominated MPB, and we found a small contribution (~10%) of haptophytes to MPB. The haptophyte contribution was attributed to a recent phytoplankton settlement and can therefore be neglected in the interpretation of tracer ^{13}C and ^{15}N flows.

Our macro- and meiofauna census showed that taxonomic diversity, densities, and biomass were much lower than those reported earlier for a contiguous site (Armonies & Reise 2000). In this previous study, 14 meio- and macrofaunal taxa were recorded in a compilation of data spanning a 30 yr period. The present study revealed only 8 higher meiofauna taxa and few macrofauna species. Interestingly, whereas Armonies & Reise (2000) found Turbellaria to be the most abundant taxon over the years of survey, they were not observed in our study at the time of sampling. Parallel investigation of the meiofauna at the same date and location on fixed and stained samples supported our results and didn't reveal the presence of Turbellaria (L. Kotwicki pers. comm.). In contrast, our study highlighted the predominance of copepods, nematodes, and juveniles of the gastropod *Littorina littorea* both in terms of densities and biomass. However, the presence of *L. littorea* in the meiofauna should be regarded as a transient phenomenon, as their presence as interstitial fauna is only temporary, possibly just following larval recruitment (Saier 2000). From this, it is clear that permeable sediments are highly heterogeneous environments where drastic changes can occur in faunal assemblages. Nevertheless, our study supported earlier observations of a limited contribution of macrofauna relative to meiofauna in terms of densities and biomass at the same site (Armonies & Reise 2000).

Trophic interactions

Although phytoplankton, MPB, macroalgae, and other plants can all serve as a resource for benthic consumers, many coastal benthic food web studies distin-

guish between (phyto)plankton on the one hand and benthic primary producers on the other hand (Fry & Sherr 1988, Heip et al. 1995, Herman et al. 2000). This simple partitioning into pelagic and benthic resources has been widely used because of the clear difference found between the stable isotope signatures of SPM and those of surficial sediment, proxies for phytoplankton and MPB, respectively (France 1995). In the present study, food sources for benthic consumers were first considered based on these 2 distinct pools (Fig. 3A,B): a pelagic ^{13}C-depleted one (ca. –21‰) and a heavier benthic one (ca. –17‰). This first approach fell short, as it didn't allow an explanation of the diet of most consumers that had δ^{13}C values beyond these 2 traditional end-members. The PLFA show that surface sediment hosts 2 communities of primary producers, cyanobacteria and diatoms, with a significant difference between the δ^{13}C values of cyanobacteria (ca. –20‰) and diatoms (ca. –16‰). Considering 3 different food sources (plankton, benthic diatoms, and benthic cyanobacteria) and isotopic variability of these food sources and consumers rather than a plankton and single MPB compartment with mean values, allowed the resolution of the main food source for all taxa/species except *Crangon crangon*, but at the expense of increased uncertainty for animals' diet estimations with food sources of similar δ^{13}C values (Fig. 3C–E). As an independent validation, the use of a deliberate tracer further constrains the benthic food sources.

Meiofauna and macrofauna stable isotope analysis exhibited a broad range of δ^{13}C values indicating variable dependence of consumers on available resources. *Crangon crangon* was the only animal for which its diet could not be resolved from the 3-source analysis based on its natural abundance isotope signature. Young individuals of *C. crangon* are typical predators of meiofauna (Oh et al. 2001, Feller 2006). The combined natural abundance and tracer data suggest that *C. crangon* feeds either directly on MPB or indirectly via consumption of rapidly growing (tracer-rich) nematodes or copepods that depend on diatoms. However, the ^{15}N natural abundance data (Fig. 2) did not enable us to confirm *C. crangon*'s predatory behavior, as the differences between its δ^{15}N value (13.6‰) and those of its potential prey were not consistent with the typical 3.5‰ shift between predators and prey (e.g. Post 2002). The 2 macrofauna deposit feeders, *Nereis virens* and *Scoloplos armiger*, had similar δ^{13}C values, within the range of those of the diatoms, implying that they either feed on diatoms, their by-products (detritus or EPS), or organisms grazing on diatoms. Nematodes, halacarids and copepods showed δ^{13}C values close to that of diatoms (ca. –16‰) or bulk sediment (ca. –17‰), suggesting a direct dependence (for nematodes) or a mixed diet mainly composed of diatoms for halacarids and copepods (see Fig. 3C). Tardigrades, ostracods, and juveniles of *Littorina littorea* showed δ^{13}C values similar to that of cyanobacteria (ca. –20‰) and/or SPM (phytoplankton, ca. –21‰). Juvenile bivalves, which are suspension feeders at the sediment–water interface, had a δ^{13}C value similar to that of benthic diatoms. Our findings support previous observations of the significant reliance of juvenile bivalves on MPB (Sauriau & Kang 2000, Rossi et al. 2004). Halacarid δ^{13}C signature also suggested a strong dependence on diatoms, in line with the brown spots in the guts of the animals observed during sorting (V. Evrard pers. obs.) and also previous studies that addressed their diet (Bartsch 2004).

The combined deliberate-tracer PLFA biomarker approach helped further delineate the diet of juveniles of *Littorina littorea*, ostracods, and halacarids. Although the natural stable isotopic signatures of these consumers were similar to those of cyanobacteria and SPM, their high tracer enrichment values suggest that MPB represented the major part of their diet. This also might imply that these organisms graze selectively on benthic cyanobacteria. Several studies have illustrated the significance of phytoplankton to the diet of the meiofauna (Buffan-Dubau & Carman 2000, Carman & Fry 2002, Maddi et al. 2006), while our study shows that MPB rather than phytoplankton may have been the main resource for meiofauna. Given the short duration of our tracer experiment, it is unclear whether the inferred limited benthic–pelagic coupling was a permanent or temporal phenomenon. High phytoplankton biomass and advective flows are critical factors in entrainment and trapping of phytoplankton in sandy, permeable sediments (Huettel & Rusch 2000, Huettel et al. 2007), a prerequisite for phytoplankton to become a significant food source for small infaunal consumers. These conditions were probably not met at the time of the experiment, i.e. summer season, because the pigment composition of sediments and particles in the water column were distinct. The pigment spectrum of the sediment revealed that it was largely dominated by diatoms and cyanobacteria, and was free of chlorophytes (Evrard et al. 2008); conversely, phytoplankton was dominated by diatoms and chlorophytes, with very little evidence of cyanobacteria (V. Evrard unpubl. data).

C and N flows to consumers

The ^{13}C and ^{15}N pulse chase experiment allowed direct assessment of C and N flows from the MPB to the different heterotrophic compartments of the sediment. Label incorporation by MPB was rapid (Evrard et al. 2008), consistent with observations in intertidal flats

with fine sediments (Underwood & Kromkamp 1999, Middelburg et al. 2000, Cook et al. 2004). Turnover time, calculated as the ratio of MPB biomass (12 081 mg m^{-2}; see Table 1) to primary production (~195 mg considering 10% ^{13}C-labelling; see the incorporation in bulk sediment in Fig. 5A), was 62 d. This is well above what has been observed (2 to 44 d) in subtidal, permeable sediments by Sundbäck et al. (1996), suggesting nutrient-limited conditions at the time of the experiment (Evrard et al. 2008). This is consistent with the very high (~55%) excretion of recently fixed carbon (EPS production), which is a significant food source for bacteria and also for non-selective deposit feeders (Middelburg et al. 2000, Goto et al. 2001).

To consider the potential interactions between the various consumers and their different food sources, we need to account for the label dilution in the standing stock of organic matter. Tracer level of ^{13}C remained very low (~0.1%) compared to that of ^{15}N (~0.4%). Subsurface deposit feeders (*Scoloplos armiger* and possibly a fraction of the nematode community) showed lower ^{13}C enrichment than surface deposit feeders (*Nereis virens* and possibly some nematodes) suggesting that they fed on bulk organic matter from the sediment. The higher ^{13}C enrichment of juveniles of *Littorina littorea*, ostracods, and tardigrades compared to that of the other metazoans confirmed their preference for cyanobacteria rather than SPM (phytoplankton). The combined biomarker and stable isotope approach showed similar $\delta^{13}C$ values for *L. littorea* and both SPM and cyanobacteria (suggesting that both phytoplankton and MPB could be a food source), but the consecutive label enrichment of *L. littorea* implies that the potential contribution of phytoplankton to its diet is rather low. Surprisingly, juveniles of *L. littorea* also showed extremely high ^{15}N enrichments compared to that of other animals, suggesting that cyanobacteria were possibly more ^{15}N-enriched than diatoms. Unfortunately, there are no ^{15}N-δ^E data for cyanobacteria, ostracods, or tardigrades to support this assumption.

Both approaches used here (natural stable isotope abundance and enrichment experiment) revealed that meiofauna relies primarily on MPB, while the readily available standing stock of EPS represented a significant C source for bacteria and deposit-feeding macrofauna. To fully grasp the relative contributions of the different heterotrophic compartments, it is important to bear in mind that, in addition to the label dilution in the resources, enrichment levels of heterotrophs were tightly coupled to their respective expected turnover; i.e. the faster turnover of smaller organisms feeding on highly enriched and specific food was consistent with their higher enrichment, while the slower turnover of larger organisms feeding on non-specific bulk organic matter (diluted label) was consistent with their lower enrichment.

The recovery of ^{13}C label in total organic matter, MPB, heterotrophic bacteria, meio-, and macrofauna allow for the elucidation of the relative importance of size classes and for the construction of mass balances (Fig. 6). These budgets are approximate because of high variability due to spatial heterogeneity (average

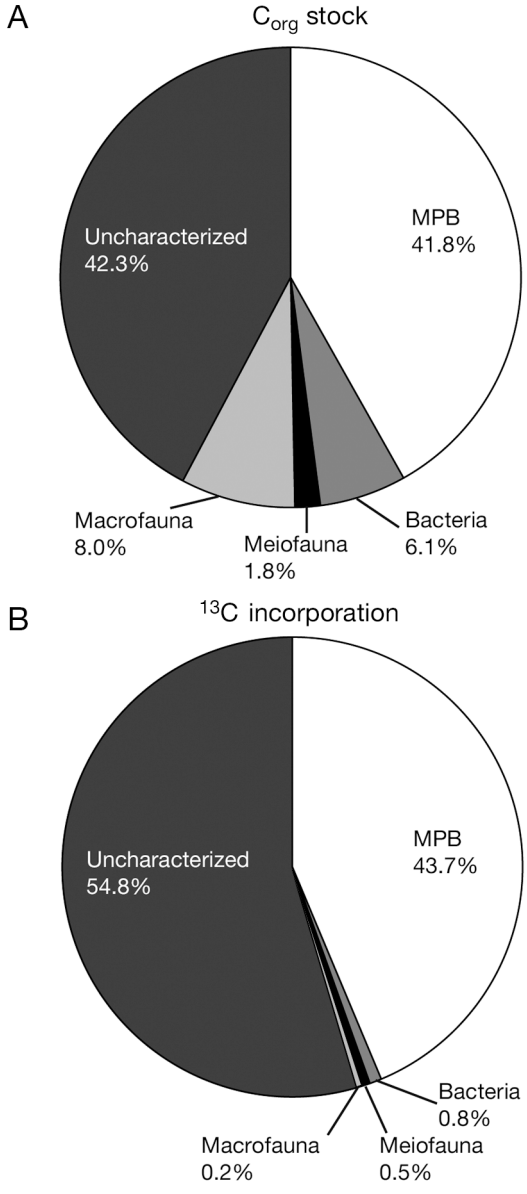

Fig. 6. (A) Importance of the different organic fractions in the sediment (as fractions of the total organic carbon) and (B) their respective ^{13}C incorporations (as fractions of the total organic ^{13}C incorporation). The uncharacterized fraction is calculated as the difference between the bulk sediment and the sum of all known components (for stock or incorporation). Data are for the top 1 cm layer for all the compartments except the macrofauna (top 15 cm). The macrofaunal contribution is therefore overestimated. MPB: microphytobenthos

coefficient of variation was 0.35) and because of uncertainties inherent to the use of conversion coefficients (e.g. from PLFA concentration to biomass). Consequently, the quantitative findings should be interpreted with caution. First, it is important to note that very little label (<2%) was actually transferred to the different heterotrophic compartments for both C and N, with the exception of bacteria and juveniles of *Littorina littorea*. Most of the label was still in MPB and the uncharacterized pool (EPS and detritus) at the end of the experiment (Evrard 2007), i.e. after 4 d, consistent with the estimated turnover of 62 d and observations by Middelburg et al. (2000). In addition, the design of the experiment did not allow us to make a distinction between ingested and assimilated C and N (Herman et al. 2000). Therefore, incorporation values might be slightly overestimated, particularly for larger organisms with slower metabolism, but this has little influence on the general findings of the present study. Second, enrichment data showed that all compartments were significantly labelled and that the amount of label transferred to the different heterotrophic compartments (bacteria > meiofauna ≫ macrofauna; Fig. 6B) didn't follow the biomass patterns (bacteria > macrofauna > meiofauna; Fig. 6A). Macrofauna stock and label incorporation estimates are for 0–15 cm, while those for the other compartments are based on the upper 1 cm only. Macrofauna biomass and uptake thus represent overestimates. There are few studies available for comparison, as most are qualitative or cover only one size class. Middelburg et al. (2000) studied the fate of intertidal MPB carbon and reported that label transfer from MPB to consumers followed more or less the biomass pattern in silty sediments (bacteria > macrofauna > meiofauna). Moodley et al. (2005) and Woulds et al. (2007) investigated the fate of phytodetritus in ocean margin sediments and reported that label transfer followed biomass patterns, although deviations were reported for individual species/taxa. Although Sundbäck et al. (1996) did not include the macrofauna in their study, they also found that heterotrophic bacteria and meiofauna contributed proportionally to MPB-derived carbon processing in subtidal sandy sediments. Third, our labelling study revealed a significant contribution of meiofauna to carbon flow, similar to that of bacteria. This high contribution of meiofauna to carbon flow in sandy sediments has often been implied from its high densities and fast turnover (Fenchel 1969, Kuipers et al. 1981), but has hardly been quantified, because quantitative and comprehensive data on carbon flow in sandy sediment have been lacking so far. Such a high relative contribution of meiofauna has been reported for sediment underlying low-oxygen bottom waters (Woulds et al. 2007) and deep-sea sediments (Moodley et al. 2002). However, in these studies foraminifera accounted for most of the meiofaunal uptake, whereas metazoans appear to dominate in sandy coastal sediments.

While carbon transfer from MPB to meiofauna has now been studied in a number of tidal flats (Middelburg et al. 2000, Moens et al. 2002), there are very few studies on nitrogen transfer from benthic microbes to meiofauna in photic sediments (Carman & Fry 2002, Veuger et al. 2007). Nitrogen incorporation can be attributed to MPB as well as to heterotrophic bacteria, but MPB likely dominate nitrogen uptake (Evrard et al. 2008). In the present study, the C:N ratio of total incorporation was high (~13), which is consistent for a sediment in which a large fraction of the fixed carbon is exudated as EPS (Evrard et al. 2008). However, the 3 meiofauna taxa for which data were available showed a preferential incorporation of N relative to C. Although we have no data on the C:N ratio of the microbes consumed by these meiofauna taxa, it is likely these had a much lower C:N ratio (4 to 7), closer to the Redfield ratio. Surprisingly, juveniles of *Littorina littorea* showed extremely high amounts of ^{15}N incorporation. Considering they primarily grazed on cyanobacteria, this merely suggests that cyanobacteria were more ^{15}N-enriched than diatoms. Norton et al. (1990) showed that *L. littorea* are selective feeders with the ability to select their food after it has been ingested and spit out the rest. Our results are further supported by Sommer (2001) who showed that *L. littorea* juveniles have high requirements for N relative to C, and that, in N-limited conditions, they will exclusively rely on cyanobacteria, which have higher N content. Contrary to meiofauna, which appears to be dependent on nitrogen-rich material (bacteria, MPB) and acquires more nitrogen via selective feeding, macrofauna taxa showed high C:N ratios of incorporation consistent with their feeding behaviour (non-selective deposit feeders and predator) and their food source, which includes mainly EPS (^{13}C-rich and ^{15}N-poor).

Sandy permeable sediments represent the most common habitat on continental shelves (Boudreau et al. 2001, Hall 2002), and about one third of the continental shelf area receives enough light for MPB growth (Gattuso et al. 2006). Through the investigation of stable isotope natural abundance, a dual-tracer enrichment experiment and a close look at microbial biomarkers, we have documented key aspects of the role of MPB in structuring benthic food webs in photic subtidal sandy sediments. The combination of stable isotope natural abundance with enrichment experiment has proven very valuable to the study of food webs (Herman et al. 2000, Galván et al. 2008). However, the resolution added by the use of microbial biomarkers to clearly distinguish components within the MPB was important to disentangle the complexity of the food

web. To our knowledge, the present study is the first to provide such a level of resolution and, furthermore, in a semi-quantitative way. However, we remain cautious regarding generalizations of our findings, and further investigation on larger spatial and temporal scales are needed to elucidate the pathways of carbon and nitrogen in photic sandy sublittoral zones. Nevertheless, the combination of methods and analyses of the different faunal components revealed that the hidden green garden of sandy subtidal sediments is pivotal to metazoans, in particular for the meiofauna, living in these permeable and dynamic sediments.

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