Temporal dynamics in a shallow coastal benthic food web: Insights from fatty acid biomarkers and their stable isotopes

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ABSTRACT

We investigated the temporal variation of pelagic and benthic food sources in the diet of benthic taxa at a depositional site in the Southern Bight of the North Sea by means of fatty acid (FA) biomarkers and compound-specific stable isotope analysis (CSIA). The taxa were the non-selective deposit feeding nematodes (*Sabatieria* spp. and 'other nematodes'), and three dominant macrobenthic species: two true suspension-deposit feeders (the bivalve *Abra alba* and the tube dwelling polychaete *Owenia fusiformis*) and the suspected predatory mud-dwelling anemone *Sagartia* sp. These species make up on average 16% (*Abra alba*), 17% (*Sagartia* sp.) and 20% (*Owenia fusiformis*) of the biomass in the *Abra alba–Kurtiella bidentata* community in this area. Phytoplankton dynamics in the suspended particulate matter of the water column as inferred from cell counts, chlorophyll-*a* and organic carbon content were clearly visible in sediment and animal FA abundance as well, whereas phytodetritus dynamics in the sediment FA composition were less clear, probably due to patchy distribution or stripping of FA by macrofauna. Nematodes appeared to assimilate mainly Polyunsaturated Fatty Acids (PUFAs) from their sedimentary environment and were further non-selectively accumulating more (*Sabatieria* spp.) or less ('other nematodes') FA from the deposited phytodetritus. In contrast, *Abra alba* FA composition was consistent with a diatom-dominated diet and consumption of *Phaeocystis* was observed in *Owenia fusiformis,* whereas *Sagartia* sp. showed evidence of a predatory behaviour. While the total FA content in *Owenia fusiformis* remained constant throughout the year, *Sagartia* sp. doubled and *Abra alba* increased its FA level more than 10-fold in response to the organic matter deposition from the phytoplankton bloom. This leads to the conclusion that there is no resource partitioning between non-selective deposit feeding nematodes and the suspension-deposit feeding macrobenthic organisms, suggesting they belong to separate parts of the benthic food web.

1. Introduction

Owing to intensive primary production and shallow water depth, a close coupling generally exists between the pelagic and benthic compartments in coastal ecosystems (e.g. Marcus and Boero, 1998). Benthic organisms living in the aphotic subtidal zone are strongly dependent on the organic matter that is produced in the surface waters during the annual spring phytoplankton bloom (Graf, 1992). Although the bulk of phytoplankton production is mineralized in the water column by micro-zooplankton and bacteria (Rousseau et al., 2000), about a quarter is deposited on the sediment of the Belgian part of the North Sea (BPNS), 65% of it being *Phaeocystis* colonies (Lancelot et al., 2005). By cutting up and breaking apart the algal material during ingestion and defaecation, sediment-dwelling organisms facilitate microbial decomposition of the organic matter (Boon and Duineveld, 2012; Rhoads, 1974; Van Nugteren et al., 2009) to the essential nutrients that are then released back to the water column to fuel the next phytoplankton bloom. During this process, benthic organisms transform the pelagically produced organic matter and facilitate assimilation by

other benthos that is not in direct contact with the water column (Braeckman et al., 2011; Levin et al., 1997; Van Nugteren et al., 2009), or might impose interference (i.e. direct, with interactions among organisms) or exploitative competition (i.e. indirect, by limiting resources for the other organisms) on the smaller competitors (Braeckman et al., 2011; Nascimento et al., 2011; Van Colen et al., 2009).

Many functional groups of benthic organisms are involved in this processing of organic matter. Suspension-deposit feeders and filter feeders like bivalves, sessile polychaetes and sea anemones are thought to directly rely on the pelagically produced organic matter which they catch from the water, but might also show trophic plasticity according to the seasonal variation in available food sources (Okamura, 1990; Levinton, 1991; Dubois et al., 2007; Kuerten et al., 2013a,b). Other organisms take up food after it has been deposited on and/or incorporated into the sediment. In coastal food webs, nematodes are believed to play a crucial role in processing sedimentary organic matter, through stimulation of bacterial breakdown of fresh organic matter (Nascimento et al., 2012), their intermediate trophic position (between bacteria/detritus/microalgae and macrofauna/megafauna) and high metabolic and reproductive rates, in conjunction with their ubiquity and high standing stock (Giere, 2009).

However, nematode diet and food processing pathways are under debate. Nematode diet appears to be very difficult to unravel: originally, the morphology of the buccal cavity was used to distinguish different feeding types (Wieser, 1953). However, *in situ* observations (Guilini et al., 2013, 2010; Moens and Vincx, 1997; Pape et al., 2013) tend to contradict this classification. In addition, pulse-chase experiments with labelled material (DOC, bacteria, algae) show very little uptake of fresh organic matter by nematodes. Only between 0 and 5% of their daily carbon needs appear to be fulfilled by such labile food sources (Braeckman et al., 2011; Franco et al., 2008a; Gontikaki et al., 2011; Guilini et al., 2010), with a single exception of up to 120% by bacterivorous deep sea seamount nematodes (Pape et al., 2013). Although the daily carbon requirements of nematodes might be overestimated (Braeckman et al., 2013), the enigma of the extremely limited uptake of algal or bacterial material in these pulse-chase studies remains. In contrast, some coastal field studies tend to suggest a direct dependence on primary produced material, as upon arrival of the phytoplankton bloom in spring, nematode abundances drastically increase in number (Franco et al., 2008b; Vanaverbeke et al., 2004), indicating their affinity for the fresh algal material. Other shallow-water studies, e.g., Braeckman et al. (2011) showed a lack of competition between suspension-deposit feeding macrofauna and a nematode community mainly composed of non-selective deposit feeders, suggesting that the first ones feed on fresh organic matter, while the latter feed on detritus. Nematode dependence on (semi-labile) detritus has also been suggested for deep-sea nematodes (Gontikaki et al., 2011; Guilini et al., 2013; van Oevelen et al., 2012; Veit-Köhler et al., 2013).

To unravel the links between the pelagic and benthic realm, and as such diet sources of sediment-dwelling organisms, biomarkers can be helpful as tracers. Lipid biomarkers are frequently used in trophic ecology (see reviews of Sargent and Whittle, 1981; Dalsgaard et al., 2003; Budge et al., 2006; Parrish, 2013). Polar lipids ([PL], phospholipid-derived fatty acids [PLFA] and glycolipids) are important constituents of cell membranes (Dalsgaard et al., 2003). Some PLFA are specific for bacteria and phytoplankton (e.g. Viso and Marty, 1993; Dunstan et al., 1993) and are turned over within days after cell death, which makes them good biomarkers for living biomass (Boschker and Middelburg, 2002). Since it is energetically more efficient to incorporate FA and to catabolize dietary carbohydrates, and because some essential

PUFAs like eicosapentaenoic acid (EPA; 20:5ω3) and docosahexaenoic acid (DHA; 22:6ω3) cannot be synthesized *de novo* by most animals, FA from the diet are partly routed intact or with minor modification into consumer tissues (Ruess et al., 2005). Therefore, the neutral lipid derived FA [NLFA] found in the reserve lipids of the primary consumers are to a great extent left unmodified during trophic transfer (Caramujo et al., 2008), which makes them useful in identifying food sources and feeding modes on the short-term. However, the PLFA fraction (cell membranes) of consumers is genetically predetermined (Kuerten et al., 2013a) and more under metabolic control and only provide long-term dietary information (Koussoroplis et al., 2010). Since no single FA can be assigned uniquely to any one species and not even to any single group of phytoplankton or bacteria (Dalsgaard et al., 2003), a combination of FA biomarkers and the stable isotope signature of these FA might help in the diet characterization. This compound-specific isotope analysis (CSIA) of FA can provide important insights into the biogeochemical conditions under which carbon fixation occurred (Bieger et al., 1997), and can be used to distinguish possible sources of organic matter in the diet of organisms (Budge et al., 2008; De Kluijver et al., 2012; Middelburg, 2014; Ramos et al., 2003; Van den Meersche et al., 2009; Van Gaever et al., 2009). As such, carbon from a FA of a certain food source is incorporated into the tissue of consumers such that the carbon isotopic ratio ($^{13}C:^{12}C$) generally reflects that of the diet. However, the isotopic composition of individual FA in a consumer can differ from the FA in its diet according to the isotopic fractionation accompanying metabolism and biosynthesis in the animals (Murphy and Abrajano, 1994; Ruess et al., 2005; Treignier et al., 2009). This combined approach of lipid analysis with CSIA has been used to distinguish species diet sources but there are only few studies investigating temporal dynamics (Braeckman et al., 2012; Budge et al., 2008; Gladyshev et al., 2012; Kuerten et al., 2013a, 2013b).

In this study, we investigated the temporal variation in the importance of benthic and pelagic food sources for selected nematode taxa and three dominant macrobenthic species in the *Abra alba-Kurtiella bidentata* community (Van Hoey et al., 2004) at a depositional, fine–sandy site in the Southern Bight of the North Sea by means of FA biomarkers and their stable carbon isotopes (CSIA). The area is characterized by strong tidal currents ($1–2 \text{ m s}^{-1}$) and is located in a high turbidity field (Fettweis and Van den Eynde, 2003). These conditions give rise to high organic matter input (van Oevelen et al., 2009) fueling the *Abra alba-Kurtiella bidentata* community, considered the most diverse and productive macrobenthic community in the Belgian part of the North Sea (Van Hoey et al., 2004). The tube-dwelling polychaete *Owenia fusiformis* (Hartmann-Schröder, 1996) and bivalve *Abra alba* (Dame, 2011) are suspension-deposit feeders feeding on sediment detritus and micro-organisms suspended in the water column. *Sagartia* spp. are known as suspension-deposit feeding anemones with an omnivore (Schaal et al., 2008) to a likely predatory behavior (van Oevelen et al., 2009). These species make up on average 16% (*Abra alba*), 17% (*Sagartia* sp.) and 20% (*Owenia fusiformis*) of the biomass in the *Abra alba–Kurtiella bidentata* community in this area (data from Braeckman et al., 2014). The nematode genus *Sabatieria* are non-selective deposit feeders (Wieser, 1953), that show opportunistic feeding responses to phytoplankton sedimentation (Franco et al., 2008a). The 'other nematode' group in this study mainly comprised smaller nematodes: bacterivores, detritus feeders and non-selective deposit feeders (Steyaert, 2003; Vanaverbeke et al., 2011), but few predators. Our field study on the temporal variation in the diet of several members of the benthic food-web investigates their role in linking the pelagic and the benthic realm. We tested whether (1) the temporal succession in phytoplankton composition could be traced with biomarkers and their compound-

specific stable isotopes, (2) the diet composition of the benthic organisms could be determined, (3) the temporal variation in the relative abundance of pelagic and benthic food sources in the diet of these species could be assessed, and whether (4) there is resource partitioning between nematodes and the selected macrobenthic species.

2. Material and methods

2.1. Study site and sampling

From February to September 2010, the suspended matter in the water column (SPM) and sediment from a fine sandy coastal station in the Belgian part of the North Sea (51°9.50′N, 2°37.82′E, 15.5 m depth, 3 km offshore) were sampled 6 times (Table 1) with respectively a Niskin bottle and a Reineck Boxcorer from the RV Zeeleeuw.

We sampled the pelagic food sources by means of a 10 l Niskin bottle, 3 m below the surface and filtered between 150 mL (bloom period) and 1200 mL (post-bloom period) of these seston samples (without sieving) on pre-combusted GF/F glass microfiber filters for pigment and lipid analysis. Data on phytoplankton composition and abundance were retrieved from the closest monitoring station, 7 km east (51°10.19′N, 2°42.94′E, 12 m depth, 1.5 km offshore). The Belgian coastal area is spatially quite homogeneous in terms of phytoplankton composition (Rousseau et al., 2006). There might be a small temporal lag of about 1 month in phytoplankton bloom arrival between the south western area and the north eastern area of the Belgian part of the North Sea (Muylaert et al., 2006). However, since both the sampling and the monitoring station are situated in the south-western area, we are confident that these stations were at each sampling time similar in terms of phytoplankton composition. For the monitoring of phytoplankton composition, 1 l samples for phytoplankton counts were collected monthly or fortnightly (during the spring bloom) with a Niskin bottle; samples were fixed with a gluteraldehyde-lugol solution (final concentration 1%) and stored in the dark. In addition, live samples were collected for Phaeocystis colony counts (see below) and stored cool and dark until analysis. Counts of diatoms, dinoflagellates and noncolonial Phaeocystis cells were performed with a Zeiss Axiovert inverted microscope using the Utermöhl method (Hasle, 1978). For Phaeocystis colonies, within 24 h of sampling, live samples were put in sedimentation chambers and fixed with 2 or 3 drops of a gluteraldehyde-lugol solution. After >8 h of sedimentation >100 colonies were counted and sized. The number of cells in a colony was then estimated based on the average volume of a colony and a conversion factor proposed by Rousseau et al. (1990). The total Phaeocystis cell number is a sum of the cell numbers of both colonial and non-colonial cells.

Benthic food sources were sampled by means of three 8 ml (4 cm deep into the surface layer) sediment subsamples of a Reineck Box corer (0.018 m²) that was deployed several times. These subsamples were used for the determination of chlorophyll-

a, particulate organic carbon (POC) and lipid analysis of the sediment organic matter. Chlorophyll-a samples were stored at −80 °C until analysis.

Sediment samples (approx. 3 g of sediment) and SPM filters for lipid analysis were immediately transferred to extraction liquid (methanol/chloroform). Chlorophyll-a was determined by HPLC (Gilson) analysis according to Wright and Jeffrey (1997). Following acid treatment for removal of inorganic carbon, total organic C content was analysed with a FLASH 2000 NC Elemental Analyzer.

Nematodes − The sediment from the Reineck box corer was also subsampled with 4−5 3.6 cm ⌀ Perspex cores. Sediment collected on 8 sampling events (Table 1) was sliced in 1 cm sections from which only the 0−1 cm ("surface") and 3−4 cm ("deep") horizons were immediately frozen in liquid nitrogen and stored in the lab at −80 °C. In the laboratory, meiobenthos (>38 μm) was extracted with Ludox, using the well-rinsed supernatant after centrifugation (Heip et al., 1985). Nematodes were hand-picked with a fine needle under a stereomicroscope, cleared of adhering particles and rinsed with Milli-Q before being transferred to 2.5 ml GC vials that were frozen at −80 °C and subsequently freeze-dried. Since nematode biomass was small, we made sure to minimize the contamination risk. Therefore, all equipment was sterile. In addition, samples were ice-cooled to prevent degradation during the time-consuming processing. Samples were processed within 2 h and broken or leaking nematodes were not selected. Nematodes clearly belonging to the Sabatieria genus were picked separately from 'other nematodes'. Depending on the available biomass, one to three pseudoreplicates were gathered from each sampling occasion, containing between 75 and 244 nematodes.

Macrobenthos was sampled on 6 occasions (Table 1) using a Van Veen grab and sieved on board. For every sampling event, two adult specimens of Abra alba, Owenia fusiformis and Sagartia sp. (further referred to as 'Abra', 'Owenia' and 'Sagartia') were selected. On board and within at most 6 h, organisms were taken out of their shells or tubes and cleaned of debris; their tissue was blended to a slurry and transferred to extraction liquid (methanol/chloroform). Immediate elaboration of the samples (one specimen per sample) using slurries precluded dry weight estimation of the animals. Owenia was not present in the samples from February, while Abra was absent in March.

2.2. Fatty acid extraction and analysis

We used the polar lipids as biomarkers for food sources (algae and bacteria) in the SPM and sediment, and neutral lipids for tracing these food sources in the diet of macrobenthos. Due to limited biomass of nematodes, we used the total FA extract to trace their food sources.

The lipids were extracted from the suspended matter, sediment and macrobenthic slurries using a modified Bligh and Dyer method (Boschker, 2004; Boschker et al., 1999). In short, total lipid samples were extracted in chloroform−methanol−water (1:2:0.8/v:v:v) and fractionated on a heat-activated silicic acid column (Silica 60 Merck, Darmstadt, Germany) by sequential elution with chloroform, acetone and methanol to obtain two polarity fractions: neutral lipids containing the Triacyl-glycerol reserve lipids (NLFA) in the chloroform fraction and polar lipids containing mainly membrane lipids (PLFA) in the methanol fractions. The acetone fraction containing mostly pigments was not retained. Fatty acid methyl esters (FAME) were obtained from both polar and neutral lipid extracts using derivatisation by mild alkaline transmethylation. FAME concentrations were determined by gas chromatograph-combustion-interface-isotope ratio mass spectrometer (GC-c-IRMS, Hewlett Packard G1530 GC; polar BPX-70 column 50 m, 0.32 mm film 0.25 μm, SGE054607; Type-III combustion interface; Thermo

Table 1
Overview of sampling occasions and sampled food web compartments.

Date	Julian day	Sample
19/02/2010	49	SPM, sediment, nematodes, macrofauna (no Owenia)
15/03/2010	74	SPM, sediment, nematodes, macrofauna (no Abra)
30/03/2010	89	Nematodes
14/04/2010	104	SPM, sediment, nematodes, macrofauna
27/04/2010	117	SPM, sediment, nematodes, macrofauna
17/05/2010	137	Nematodes
22/06/2010	173	SPM, sediment, nematodes, macrofauna
20/09/2010	263	SPM, sediment, nematodes, macrofauna

Finnigan Delta-plus IRMS). Samples were injected in splitless mode and concentrations were calculated based on the added amount of the internal standard (19:0). The use of GC-c-IRMS for concentration measurement and CSIA implies a higher detection limit for individual compounds than when using GC–MS or GC-FID. Identification of FAME was based on the comparison of relative retention times and compared to those of internal standards (12:0, 16:0, 19:0) and FAME reference sediment mixtures which were also used to check the accuracy of the isotopic ratios determined by the GC-c-IRMS. Blanks and a standard mixture were measured regularly in between the samples to check for system stability and possible contamination (sd for 19:0 standard over all 142 samples: 0.67‰).

The modified Bligh and Dyer method could not be used for the nematode samples, because of their small biomass. For these samples, a modified Masood extraction protocol was used to extract total fatty acids (Van Gaever et al., 2009). In short, fatty acid extraction and preparation of methyl esters (FAME) were carried out according to Masood et al. (2005) with reagent volumes adapted for use in this 2.5 ml GC-vials using FAME C19:0 as internal standard to calculate the concentration of FAs. These individual samples were analyzed separately for their FA compositions employing a large volume splitless injection method on a Thermo Finnigan Trace Ultra GC with the following configuration: large volume liner with glass wool, pre-column deactivated silica 5 m × 0.53 μm and analytical column SGE BPX-70 50 m × 0.32 mm × 0.25 μm.

Stable carbon isotope data are expressed in the delta notation ($\delta^{13}C$) relative to Vienna Pee Dee Belemnite (V-PDB). The $\delta^{13}C$ of individual FA (PLFA, NLFA or Total) were calculated by correcting for methyl C atoms added during derivatisation following: $\delta^{13}C_{FA} = ((n + 1) \times \delta^{13}C_{FAME} - 1 \times \delta^{13}C_{Methanol})/n$, where n is the number of C atoms in the FA, $\delta^{13}C_{FAME}$ is the isotope ratio of measured methylated FA and $\delta^{13}C_{Methanol}$ is the isotopic ratio of the used methanol. For most of the FA we use shorthand notation of the form A:BωX, where A represents the number of carbon atoms, B gives the number of double bounds and X gives the position of the double bond closest to the terminal methyl group (Guckert et al., 1985). The common eicosapentaenoic acid (EPA; 20:5ω3) and docosahexaenoic acid (DHA; 22:6ω3) will also be presented with their common abbreviations. FA were allocated as biomarkers of food sources according to references listed in Table 2.

2.3. Data analysis

Results are expressed in terms of relative FA biomarker composition within the PLFA (SPM and sediment), TFA (nematodes) and NLFA (macrofauna) fraction. The temporal variations in the totals of pelagic and benthic food sources (PLFA), macrobenthic diet (NLFA) and structural FA (PLFA) and nematode TFA were explored

with univariate analyses, while their composition was explored with multivariate analyses (not for macrofauna PLFA since these do not reflect short-term diet). Multifactorial, fully crossed permutational ANOVAs (PERMANOVA) were carried out to test the effect of the fixed factors Species [Sp], Months [M] and for nematodes also sediment depth [D] on the relative abundance of all biomarkers (multivariate data with Bray–Curtis resemblance matrix; univariate data with Euclidean resemblance matrix). For nematodes, analyses on relative abundances were compared to tests on absolute abundances to estimate the effect of total abundances. Two sediment samples (1 in mid-March and 1 in mid-April) were not taken into account because they had very atypical, noisy FA profiles without clear peak separation that were not trustworthy. Also one *Owenia* sample (mid-March) and one *Abra alba* sample (September) were omitted because they contained hardly any FA. For analyses, the sum of all unidentified peaks was included as "unknowns". In case a significant effect of a factor was found, pair-wise tests within this factor were carried out, with p-values drawn from Monte Carlo [MC] permutations, because of the restricted number of possible permutations (Anderson and Robinson, 2003). The same analysis was performed only on $\delta^{13}C$ signatures of biomarker FA that were present in all samples, because absence of a biomarker FA cannot be simply be indicated by a "zero" $\delta^{13}C$ signature. This implied we had to pool all bacterial FA (ΣBacterial FA = 10Me16:0, 10Me17:0, 10Me18:0, i-14:0, ai-15:0, i-15:0/14:1ω5c, i-16:0, i-16:0/15:1ω5c, i-17:0, ai-17:0, 18:1ω7c) and all saturated fatty acids (ΣSFA = 13:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 23:0, 24:0). For this analysis, however, we used a Euclidean distance matrix that can cope with the negative signatures. Principal Coordinates Analysis [PCO] on the same resemblance matrices as were used for PERMANOVA analysis visualised those FA from NLFA and PLFA that correlated at least 75% with one of the first 2 PCO-axes (but further specified in the figure captions). All analyses were performed within PRIMER v6 with PERMANOVA add-on software (Anderson et al., 2008; Clarke and Gorley, 2006).

3. Results

3.1. Microscopic observations of phytoplankton in the water column

Diatom abundances were low in January–February, but peaked between the end of March (day 85) and the end of April (day 117) and a second time at the end of May (day 151). Dinoflagellates attained lower abundances with small blooms in mid-March (day 74) and mid-April (day 104) (Fig. 1A). The short *Phaeocystis* bloom occurred at the end of April (day 117) (Fig. 1B). These phytoplankton counts delineate clear periods with respect to the bloom: a pre-bloom period (February − Mid-March), a bloom period (Mid-

Table 2
Most important PLFA biomarkers in phytoplankton.

Polar Lipid Fatty Acid biomarker	Group	Reference
Short branched PL: i-14:0, i-15:0, ai-15:0, i-16:0, -i17:0, ai-17:0	Bacterial: Cytophaga − Flavobacteria and Gram-positive bacteria	Dalsgaard et al., 2003
18:1ω7c	Bacterial, mainly Gram-negative Proteobacteria	
16:1ω7c	Diatoms, bacteria	Dalsgaard et al., 2003
16:4ω1	Diatoms	Viso and Marty 1993; Dunstan et al., 1993
16:2, 16:3	Diatoms	Ahlgren et al., 1992; Dunstan et al., 1993; Volkman et al., 1989
20:5ω3 (EPA), 22:5ω3	Diatoms (Bacillariophyceae)	Volkman et al., 1989; Ahlgren et al., 1992; Dunstan et al., 1993
18:1ω9c	Phaeocystis	Al-Hasan et al., 1990; Claustre et al., 1990
High amounts of 18:5ω3 and 18-PUFA in general	Phaeocystis, dinoflagellates	Dalsgaard et al., 2003; Hamm and Rousseau 2003
22:6ω3 (DHA)	dinoflagellates, Phaeocystis	Dalsgaard et al., 2003
20:1ω9, 22:1ω11	Carnivorous feeding (directly or indirectly on copepods)	Budge et al., 2006; Dalsgaard et al., 2003; Mansour et al., 2005
High 18:1ω9/18:1ω7 and DHA/EPA ratio	Carnivorous feeding	Dalsgaard et al., 2003; Falk-Petersen et al., 1990; Graeve et al., 1997

March – June), and a post-bloom period (June–September).

3.2. Biochemical characterization of suspended matter in the water column and sediment
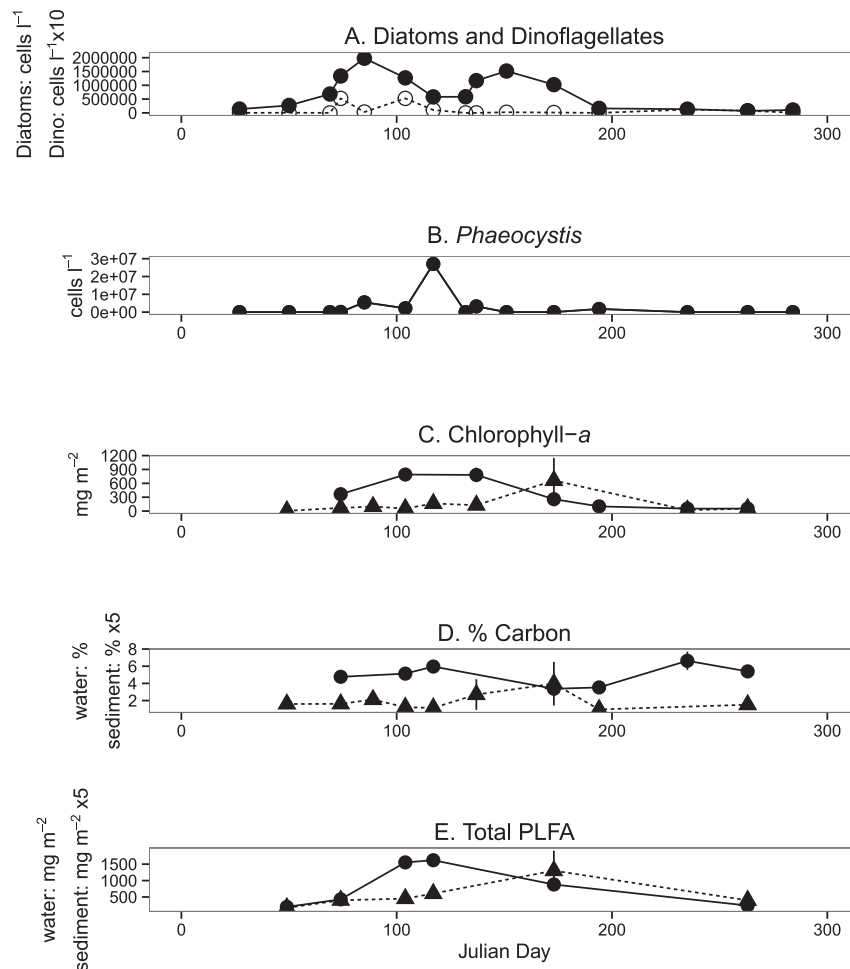
3.2.1. Chlorophyll-a and carbon content

The phytoplankton blooms were traced in SPM chl-*a*, which peaked in April, maintaining high concentrations until mid-May (day 137) (Fig. 1C). About one month later, a peak in chl-*a* in the sediment was observed (end of June; day 173). The chl-*a* concentrations in both SPM and sediment were strongly reduced by mid-July (day 194). These patterns were also mirrored in % organic carbon (Fig. 1D) and total PLFA fatty acid content (Fig. 1E; Table 3) of SPM and sediment with a strong distinguishable peak (pair-wise tests, p < 0.05), however with high variability in the magnitude of the sediment peak at the end of June (day 173).

3.2.2. Composition of fatty acids in phospholipid-derived fatty acids (PLFAs)

PLFA composition of the SPM in the water column (relative abundances) was characterised by low relative contributions of bacterial FAs (<10%) but high amounts of Polyunsaturated Fatty Acids (PUFAs; 51–65%; Table 1 Appendix). There was a clear temporal gradient in the FA composition of the SPM in the water column (Table 3; Fig. 2A). February and March were the only months

being similar in PLFA composition. PCO clearly displays this temporal pattern with a high percentage of the total variation explained by axis 1 (74.8%) separating April (bloom period) from February and March (pre-bloom period) with June and September (post-bloom period) in between (Fig. 2A). Biomarkers that correlated strongly with this time-separating 1st PCO axis were the *Phaeocystis* markers 18:1ω9c and 18-PUFAs (Table 2) on the one hand, and 16:2ω7 and 20:4ω3 on the other hand, all attaining higher concentrations during the bloom period in April. The pre- and post-bloom samples were characterised by higher amounts of the diatom markers 16:1ω7c and 20:5ω3 (Table 2) and an incompletely separable peak of 18:1ω9t/16:4ω1 and 16:3ω4.

Sediment PLFA composition (%) was more characterised by high concentrations of bacterial FA (26–34%) but lower amounts of PUFAs (13–25%; Table 1 Appendix). A temporal gradient was also present in the sedimentary PLFA composition, but to a lesser extent compared to water column SPM. The deposition of the phytoplankton bloom was not easily traceable in the FA composition (Table 3, Fig. 2B): jointly, the two PCO-axes explained 77.1% of the total explained variance and separated the pre-bloom samples of February from the later samples. The sediment samples obtained during the bloom in the water column were more variable in composition. However, only February differed in PLFA composition from the other months (except mid-April) as the relative abundances of both bacterial and diatom markers were lower in February
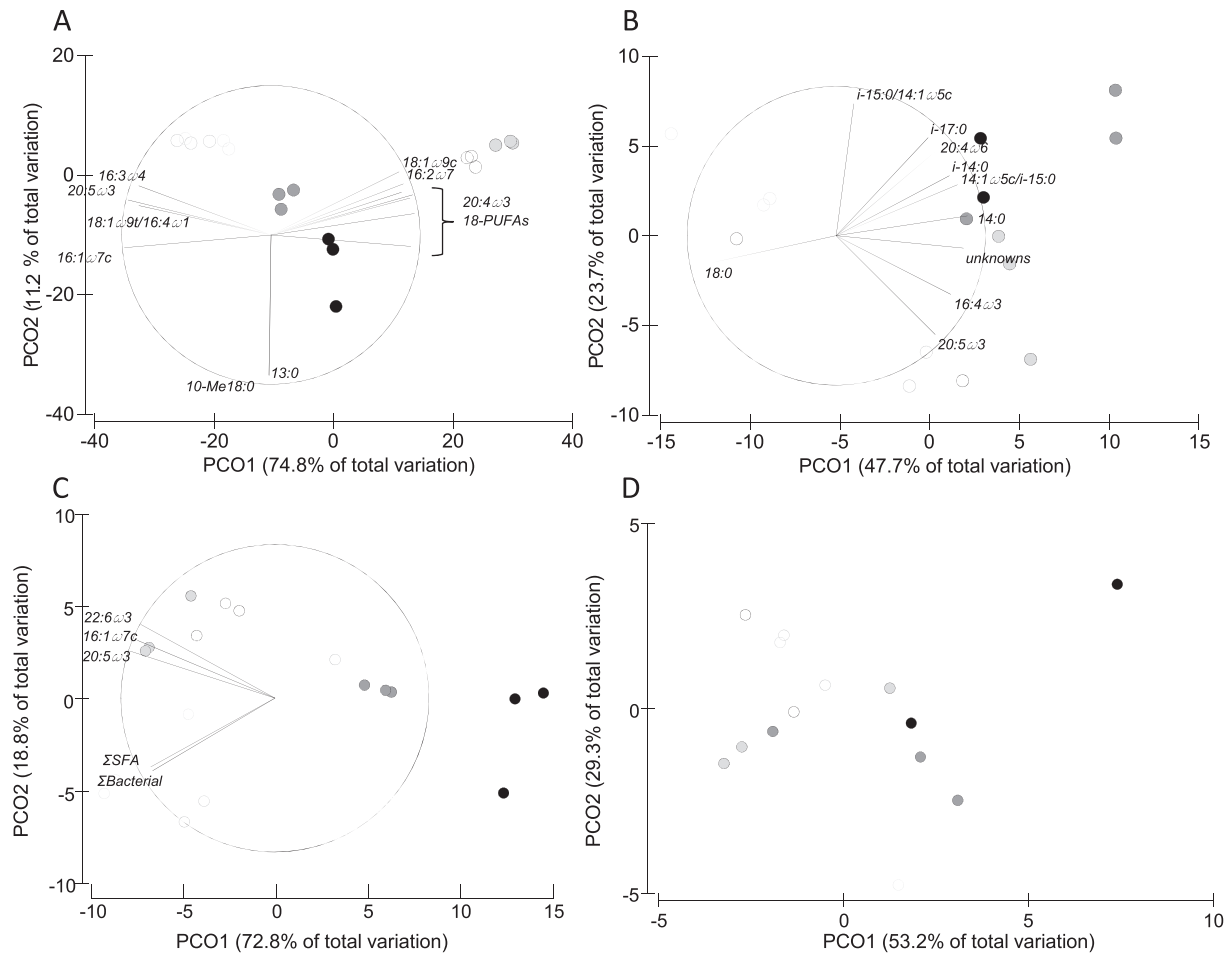

Fig. 1. Seasonal variation in A. Diatom (●) and Dinoflagellate (×10 for clarity purposes) (○) cell density in suspended matter; B. *Phaeocystis* cell density in suspended matter; C. Average ± SE (n = 3) integrated chlorophyll-*a* content in suspended matter ± SE (15.5 m) (●) and sediment (4 cm) (▲); D. Average ± SE (n = 3) % organic carbon in suspended matter (●) and in sediment (×5) (▲), and E. average ± SE (n = 3) integrated polar lipids in suspended matter (●) and sediment (×5 for clarity purposes) (▲).

Table 3
Results of PERMANOVA tests for differences in fatty acid abundance, composition and $\delta^{13}C$ signature in suspended matter in the water column (Polar Lipid fraction), sediment (Polar Lipid fraction), nematodes (total lipids) and macrofauna (Neutral Lipid fraction). Only factors and/or interactions significantly (at $p < 0.05$) distinguishing groups are displayed.

Compartment	Parameter	Factor	Pseudo-F	p (perm)
Water PLFA	Total abundance ($\mu g \, L^{-1}$)	Date	60.53	0.001
	Composition (%)	Date	30.05	0.001
	$\delta^{13}C$	Date	12.52	0.001
Sediment PLFA	Total ($\mu g \, g^{-1}$)	Date	3.19	0.027
	Composition (%)	Date	4.10	0.001
Nematodes TL	Total ($\mu g \, ind.^{-1}$)	Species × Date	3.57	0.013
		Depth	6.66	0.019
	Absolute concentration ($\mu g \, ind.^{-1}$)	Species × Depth	3.16	0.019
		Species × Date	2.36	0.003
	Composition (%)	Species	9.44	0.001
		Date	4.19	0.001
	$\delta^{13}C$	Species × Date	2.17	0.012
Macrofauna NLFA + PLFA	Total ($\mu g \, ind.^{-1}$)	Species × Date	4.45	0.027
	% NLFA of NLFA + PLFA	Species	3.19	0.049
		Date	4.00	0.048
Macrofauna NLFA	Total ($\mu g \, ind.^{-1}$)	Species × Date	4.68	0.016
	Composition (%)	Species × Date	2.04	0.006
	$\delta^{13}C$	Species	6.51	0.001
		Date	7.16	0.003

than in the other months (Fig. 2B). Relative PLFA composition of sediment was not different between the other months.

3.2.3. $\delta^{13}C$ signature of fatty acids in polar lipids

$\delta^{13}C$ signatures of bacterial and saturated FA, 20:5ω3 (EPA), 22:6ω3 (DHA) and 16:1ω7c in the suspended matter in the water column were strongly temporally structured (Table 3; Fig. 2C), with

Fig. 2. Principal Coordinates plots of lipid composition of suspended particulate matter in the water column (A: relative abundances; C: $\delta^{13}C$ signature) and sediment (B: relative abundances; D: $\delta^{13}C$ signature). Symbol colours represent the time course (18/2 ○ 15/3 ○ 14/4 ○ 27/4 ○ 22/6 ● 20/9 ●). Vectors represent specific FA biomarkers correlating >80% (composition in sediment) − 90% (composition SPM in water column) with the first 2 PCO axes or represent all selected biomarkers ($\delta^{13}C$ of FA in SPM in water column).

enriched values in bacterial and saturated FA in February and March and enriched values of EPA, DHA and 16:1ω7 during the bloom period (April) (Fig. 2C, Table 2 Appendix; Appendix Fig. 2). This temporal pattern was not found in δ13C signatures of the main biomarkers in the sediment (Fig. 2D). Therefore, no biomarker vectors are shown in Fig. 2D.

3.3. Lipid composition of nematodes

3.3.1. Composition of fatty acids in total lipids

Nematode FA profiles were composed of moderate amounts of bacterial FA (6–12%) and relatively high amounts of PUFAs (32–47%) and Saturated Fatty Acids (SFAs; 8–22%; Table 1 Appendix). DHA/EPA ratio was always >1, while 16:1ω7c/16:0 ratio remained <1, which in combination suggest a flagellate-based diet (Table 2). Absolute individual FA concentrations (μg ind.$^{-1}$) differed significantly among Species × Date and among Species × Depth (PERMANOVA: $p < 0.05$; Table 3; Fig. 3A). When relative concentrations (% ind.$^{-1}$) were considered, lipid composition differed only among Species (Table 3; Fig. 3B). This implies that nematode FA profiles differ in total concentration among depth layers and dates. In fact, 'other nematodes' had significantly higher FA concentrations in the surface layer (23.96 ± 2.00 μg ind.$^{-1}$) than in the deep layer (18.74 ± 1.58 μg ind.$^{-1}$) (Fig. 4; Table 3). 'Other nematodes' had also higher FA concentrations in February and March (pre-bloom period) as compared to April and later months (bloom and post-bloom period) (Fig. 4; Table 3). Upon bloom deposition, the average total FA concentration in *Sabatieria* nematodes doubled (Fig. 4), but due to a high variability among replicate samples, this difference was not detected by the statistical analysis. More than 60% of the explained variation in the total abundance FA profiles of the nematodes was accounted for by the distinction between *Sabatieria* and 'other nematodes' (Fig. 3A), which also indicates that *Sabatieria* have higher total FA concentrations per individual. Absolute FA concentrations per individual depend on the biomass and lipid content of an organism. However, this difference in lipid composition holds also with relative concentrations, but only 14% of the total explained variance is related to the difference between *Sabatieria* and 'other nematodes' (Fig. 3B). This difference was mainly made up by slightly higher abundances of EPA and DHA in *Sabatieria* (Fig. 3B, Appendix Fig. 1).

3.3.2. d13C signature of fatty acids in total lipids

Seven biomarkers were detected over the entire sampling period (ΣBacterial, ΣSFA, EPA, DHA, 16:1ω7c, 18:1ω9c, 18-PUFA; Table 2 Appendix) and could as such be compared with

PERMANOVA analysis. Jointly, the δ13C signature of these biomarkers differed among Species × Date (PERMANOVA: $p < 0.05$; Table 3, Fig. 5). Pair-wise comparison detected no temporal differences in δ13C of *Sabatieria* biomarkers, but some were detected in 'other nematodes', however unrelated to temporal phytoplankton dynamics. On species level, *Sabatieria* FA had significantly more enriched δ13C signatures than 'other nematodes' before the bloom (February, March) and more depleted signatures long after the bloom (September). During bloom and its deposition (April, May, June), δ13C signatures of the FA of both nematode groups converged (pairwise tests $p < 0.05$; Fig. 5).

3.4. Lipid composition of macrobenthos

3.4.1. Total FA in reserve lipids (NLFA) and structural lipids (PLFA)

The total concentration of extracted FA (NLFA + PLFA) per individual from the selected macrobenthic species followed a clear, and species-specific temporal pattern which is probably a combination of an increase in biomass and lipid content (PERMANOVA, Species × Date: $p < 0.05$; Table 3): *Sagartia* doubled its FA levels by mid-April, and by the end of April, *Abra* had even increased them more than 10-fold (pair-wise tests $p < 0.05$; Fig. 6). The FA concentrations in *Owenia* remained constant throughout the year (pair-wise tests $p > 0.05$). The proportion of reserve lipids in the total amount of extracted FA was more or less coupled to this temporal pattern (Table 3), with higher NLFA proportions around mid-April and lower in September (pairwise tests $p < 0.05$). *Owenia* also had a significantly higher proportion of NLFA than *Sagartia* (pairwise tests $p < 0.05$).

3.4.2. Composition of fatty acids in reserve lipids (NLFA)

Macrofauna reserve lipids were characterised by moderate amounts of bacterial FA (0–14%) and a wide range of SFA (18–50%) and PUFA concentrations (21–53%) (Table 1 Appendix). The standardized FA concentrations of macrofauna differed among Species × Date (PERMANOVA: $p < 0.05$; Table 3; Fig. 7A). While the FA composition of the different species was significantly different (pair-wise test, $p < 0.05$), the FA composition remained constant throughout the sampling period ($p > 0.05$ for all investigated dates within species). *Abra* and *Sagartia* had similar FA profiles at the end of April and September, but differed on all other dates. *Owenia* differed in FA profile from *Abra alba* and *Sagartia* only in mid-April (pair-wise tests: $p < 0.05$). *Sagartia* FA profiles clustered clearly apart from *Abra* and *Owenia* profiles (PCO; Fig. 7A). *Sagartia* was characterized by higher relative abundances of longer chain PUFAs such as DHA, 22:5ω3 (Fig. 7A), a DHA/EPA ratio mostly >1

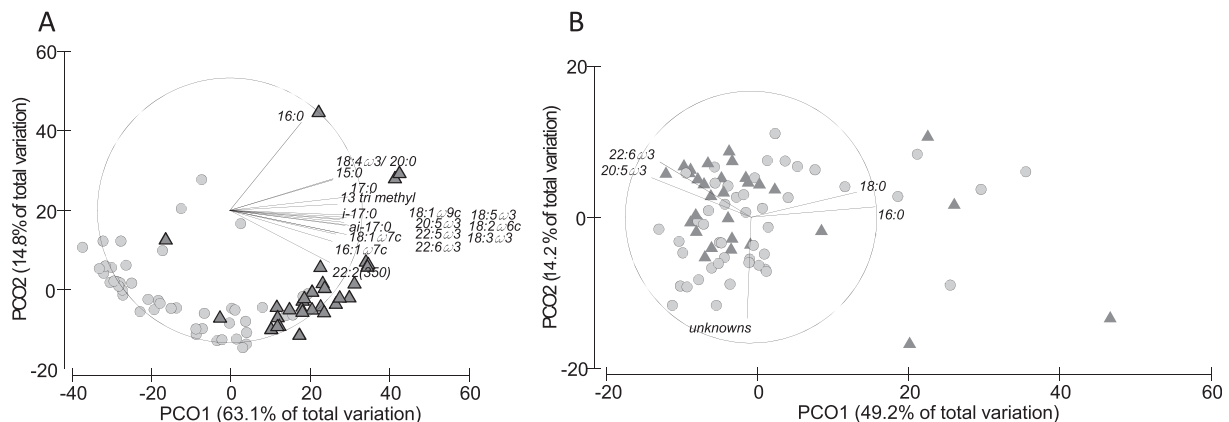**Fig. 3.** PCO analysis of total (A) and relative (B) abundance of fatty acids in *Sabatieria* spp. (△) and 'other nematodes' (○). Vectors represent specific FA biomarkers correlating >75% (relative) − 85% (total) with the first 2 PCO axes.
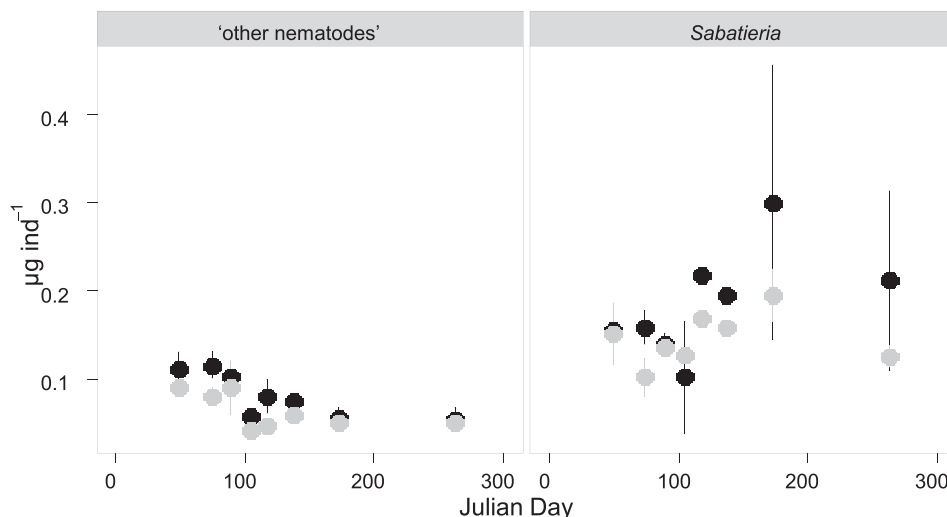
Fig. 4. Total Fatty Acids (μg ind.⁻¹) in 'other nematodes' and *Sabatieria* at the surface (0—1 cm; ●) and in deep sediment (3—4 cm; ●).

(Table 1 Appendix), indicative of a flagellate component in the diet (Table 2), and 2—5% 20:1ω9c (Table 1 Appendix), a biomarker for copepods (Table 2), all pointing to predation. *Abra* samples demonstrated higher percentages of 16-PUFAs, a 16:1ω7c/16:0 ratio mostly >1 and DHA/EPA ratio mostly <1 (Table 1 Appendix), evidence of a diatom-based diet (Table 2). *Owenia* showed higher relative contributions of 18:1ω9c (Figure 7A) and 18-PUFAs (Fig. 1 Appendix) (*Phaeocystis* marker; Table 2) and a DHA/EPA and 16:1ω7c/16:0 ratio both <1, indicating a diet that is neither composed of diatoms nor flagellates (Table 2).

3.4.3. δ¹³C signature of fatty acids in reserve lipids

The 5 biomarkers present at all sampling events in the macrobenthic individuals differed among Species and among Dates (PERMANOVA: p < 0.05; Table 3, Fig. 7B). *Sagartia* had significantly more depleted δ¹³C signatures of these biomarkers than *Abra* and *Owenia* (pair-wise tests p < 0.05, Table 2 Appendix; Appendix Fig. 2). The δ¹³C signature of the bacterial and saturated FA in all investigated species became gradually more depleted towards the

Fig. 5. PCO analysis of δ¹³C signatures of selected nematode fatty acid biomarkers. *Sabatieria* spp. (△) and 'other nematodes' (○). Colour codes: 18/2 □ 15/3 ▨ 30/3 ▧ 14/4 ▨ 27/4 ▨ 17/5 ▨ 22/6 ▨ 20/9 ▨). All biomarkers included in the analysis are shown as vectors.

end of June, whereas EPA and DHA in macrofaunal NLFA were more enriched during this bloom deposition period (pair-wise tests p < 0.05).

4. Discussion

The temporal succession in phytoplankton composition could clearly be traced using FA biomarkers and their compound-specific stable isotopes. The FA composition of nematodes appeared to be uncoupled from the temporal dynamics in primary production, while FA abundance of the selected macrobenthic suspension-deposit feeders depended strongly on the pelagically produced organic matter. The relative abundance of pelagic and benthic food sources in the FA composition of these suspension-feeders was less clear. Attributing PLFA biomarkers to unique phytoplankton taxa is not straightforward since there is no single marker for a single plankton group (Parrish et al., 2000). In addition, it is also challenging to undoubtedly interpret the FA profiles of the studied invertebrates. In the first place because we were bound to use different lipid fractions for nematodes (total FA) as for macrobenthos (NLFA). The NLFA fraction of the macrobenthos is expected to give an insight into the diet preferences, without being influenced by metabolic control or genetic predetermination. The TL fraction extracted from the nematodes is a mixture of reserve lipids (NLFA) and membrane lipids (PLFA), of which the latter are genetically predetermined (Kuerten et al., 2013a) and more under metabolic control (Koussoroplis et al., 2010). Therefore these PLFA are geographically and seasonally set (Ventrella et al., 2008) and might blur patterns in the more diet-reflecting reserve lipids of the nematodes. This would explain the less evident temporal patterns in nematode FA composition as opposed to macrobenthic FA composition. Secondly, the applicability of FA biomarkers to assess patterns in the diet of higher trophic level organisms and its temporal dynamics is constrained by the degree to which they alter their FA signature through *de novo* biosynthesis and breakdown (oxidation) of dietary FA (Caramujo et al., 2008). Since no feeding experiments were conducted (McLeod et al., 2013), differences in species or season specific physiology cannot be ruled out completely when interpreting differences in FA composition between invertebrate taxa. Keeping these considerations in mind, we will discuss various characteristics of the benthic food web. We compare these to the patterns observed in bulk δ¹³C in SPM, sediment and macrobenthos (van Oevelen et al., 2009) and nematodes

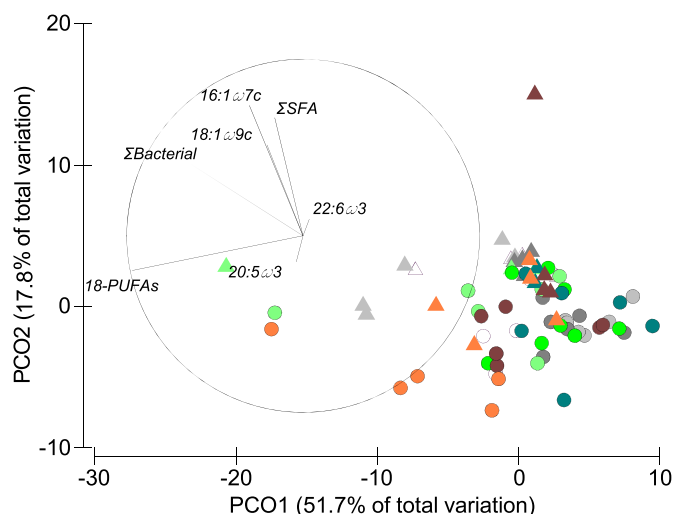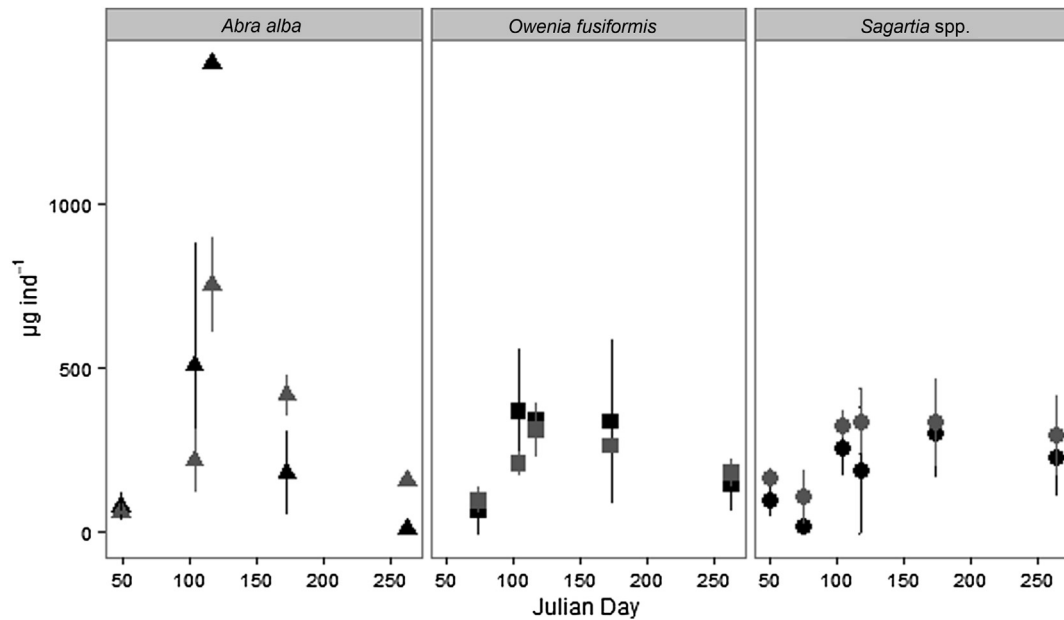**Fig. 6.** Total FA in neutral lipid (■) and polar lipid (■) fraction in *Abra alba*, *Owenia fusiformis* and *Sagartia* sp.

(Franco et al., 2008b) and organic matter mineralization (Provoost et al., 2013) at a very similar study site within a few 100 m from our station and FA composition accompanied by CSIA in SPM, sediment and polychaetes at a comparable depositional site 20 km to the east (Braeckman et al., 2012). Finally, we address the possibility of resource partitioning between the different metazoan components of the benthic food web.

4.1. Temporal dynamics in SPM in the water column and sediment

The expected temporal dynamics in phytoplankton in the Belgian part of the North Sea (Rousseau et al., 2002) were clearly observed in the suspended matter in the water. An early diatom bloom was observed in March and was reflected in higher EPA, 16:1ω7c and 16:3ω4 concentrations. The main cause of increase in chl-*a* and organic matter as well as total lipids in the SPM can be

explained by a *Phaeocystis* bloom at the end of April, characterised by elevated concentrations in 18-PUFAs and 18:1ω9c (Braeckman et al., 2012; Dalsgaard et al., 2003; Hamm and Rousseau, 2003) and more enriched $\delta^{13}C$ values of the diatom, dinoflagellate and *Phaeocystis* markers (16:1ω7c, EPA, DHA and 18:1ω9c), reflecting potentially higher specific growth rates or use of ^{13}C enriched dissolved inorganic carbon pools during the bloom (Laws et al., 1995). During the post-bloom period, the signatures became more depleted in $d^{13}C$, which is related to sinking of senescent algae to the sea floor and replenishment with new communities. This temporal pattern in compound-specific stable isotope signatures of FA is in accordance with the bulk $\delta^{13}C$ signature of water column SPM in the same area (Kuerten et al., 2013b; van Oevelen et al., 2009).

The main phytodetritus pulse to the sediment in our study was detected at the end of June, 1–2 months after the peak bloom in the
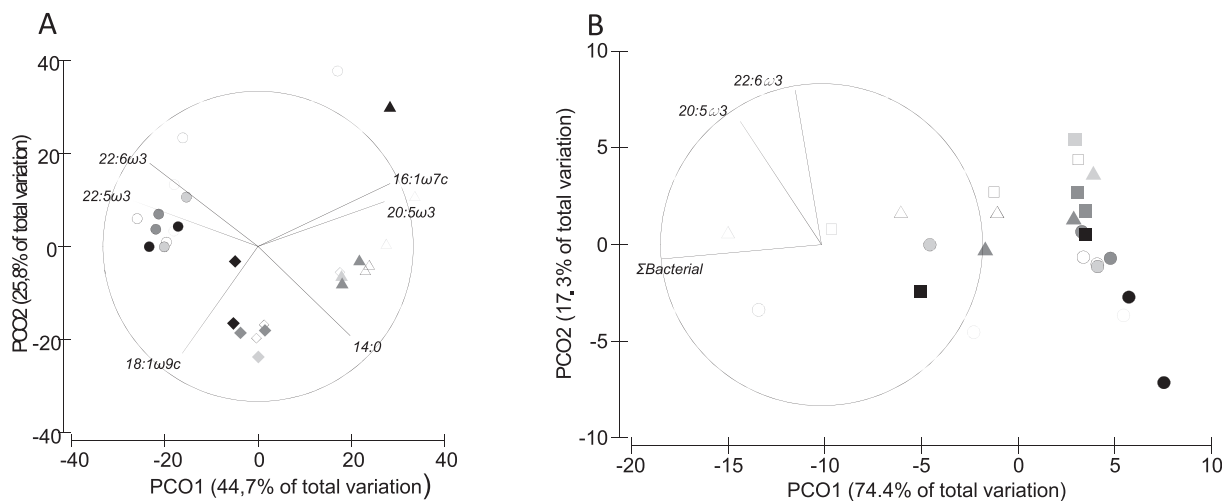

Fig. 7. PCO analysis of relative abundance (A) and $\delta^{13}C$ signature (B) of FA in Neutral Lipids in *Abra alba* (△), *Owenia fusiformis* (□) and *Sagartia* sp. (○) over the time course (colour codes: 18/2 □ 15/3 □ 14/4 □ 27/4 □ 22/6 ■ 20/9 ■). Vectors represent specific FA biomarkers correlating >80% with the first 2 PCO axes (A) or represent all selected FA biomarkers (B).

water column, by an increase in sediment chl-*a* and organic carbon content, as well as by elevated total fatty acid concentrations in the sediment samples. This timing is in accordance with the seasonal dynamics of oxygen consumption and benthic communities in this shallow coastal system (Braeckman et al., 2012; Franco et al., 2008b; Provoost et al., 2013; van Oevelen et al., 2009). However, the composition and δ^{13}C signatures of sedimentary FA did not change drastically. This is in contrast with earlier observations of clear peaks of diatom and dinoflagellate marker concentrations in the sediment shortly after the water column blooms (Braeckman et al., 2012). Still, we have to keep in mind that the bulk of the organic matter from primary production is mineralized in the water column (Rousseau et al., 2000) and only 25% of this matter arrives at the sea floor in our study area (Lancelot et al., 2005). The variances observed for variables measured on the sediment samples obtained during the phytoplankton bloom were high (see large error bars in chl-*a*, TOC and total PLFA fatty acid content as well as spread in PCO graphs on FA composition), probably indicating patchy distribution of the phytodetritus, which we may therefore not have representatively sampled given the small sampling area (surface of 10 mL syringe: <1 cm^2). An alternative explanation would be resuspension of the surface layer, as has been suggested for other sites in the Southern Bight of the North Sea (Boon and Duineveld, 1996; Boon et al., 1999; Wieking & Kröncke, 2005). However, our study site is located in a gully where organic matter is rather trapped than resuspended. Macrobenthic deposit-feeders are well adapted for fast consumption of uniformly spread, concentrated food and can spatially redistribute this organic matter within the sediment matrix (Van Nugteren et al., 2009). Through the activity of macrofauna, freshly deposited organic matter can be also strongly modified during efficient assimilation and gut passage, with essential PUFAs and even less essential bacterial FA being stripped from food sources as has been shown for sediment reworking animals like *Hediste diversicolor* and *Yoldia limatula* (Bradshaw et al., 1990; Sun et al., 1999; Woulds et al., 2014). An abundant and active macrobenthic community (van Oevelen et al., 2009) could thus partially explain why sediment organic matter FA do not mirror phytoplankton dynamics in the SPM of the water column. In addition, PUFAs also degrade quickly under microbial activity in the sediment (Carrie et al., 1998; Leduc et al., 2009).

4.2. Nematode diet composition and temporal patterns

Very little information exists on FA in marine free-living nematodes (Guilini et al., 2013; Leduc and Probert, 2009; Leduc, 2009; Leduc et al., 2015; Lins et al., 2015; Van Gaever et al., 2009). In line with these studies, we also found elevated PUFA levels in the investigated nematode groups, which confirms that nematodes can be considered a high-quality food source for higher trophic levels, although the total amount of lipids in the nematodes can be low (Guilini et al., 2013). *Sabatieria* had higher total FA concentrations per individual than 'other nematodes'. This is most probably related to the higher individual biomass of *Sabatieria* as compared to 'other nematodes' (U. Braeckman, pers. obs.). Relatively, *Sabatieria* contained more EPA and DHA than 'other nematodes', which makes them a better quality food source to higher trophic levels.

A DHA/EPA ratio >1 in combination with 16:1ω7c/16:0 ratio <1 suggest a flagellate−based diet (Dalsgaard et al., 2003). As for many FA biomarkers, care has to be taken when attributing them to specific food sources (Dalsgaard et al., 2003; Parrish et al., 2000). For nematodes, a diet mainly composed of flagellates by the entire nematode community throughout the year seems unlikely, based on the diversity in morphology of their buccal cavity (Wieser, 1953), but see 'Introduction' for criticisms on this study). In fact, EPA levels in the nematodes were similarly low as the EPA concentrations in

the sediment, whereas DHA levels in nematodes were 5-fold the DHA concentrations in the sediment. Biosynthesis is a possible explanation and has been shown for nematodes before, but DHA is seldom biosynthesised in nematodes (Honnens et al., 2014; Leduc and Probert, 2009; Schlechtriem et al., 2004; Watts and Browse, 2002). We cannot fully exclude this biosynthesis of DHA though, since intermediate products (22:5ω3; Kelly and Scheibling, 2012) in its biosynthesis were detected in small amounts (2.07 ± 0.45% on average ± SD; Table 2 Appendix), while the concentrations of 22:5ω3 in SPM and sediment were <0.7%. An alternative explanation is that nematodes selectively assimilated the PUFAs from the sediment and accumulated them, as has been shown for polychaetes (Woulds et al., 2014), echinoderms (Boon and Duineveld, 2012) and copepods (Graeve et al., 2005; Mayor et al., 2011) and tentatively suggested for deep-sea nematodes (Guilini et al., 2013; Lins et al., 2015).

Apart from these PUFAs, nematode FA composition seemed to be complemented with bacterial FA (<10% of total FA concentrations), indicating assimilation of bacterial derived organic matter. The 'other nematodes' seemed to rely more on sedimentary bacteria than *Sabatieria* (see similar δ^{13}C signatures of bacterial markers in 'other nematodes' and sediment signatures, Fig. 2 Appendix). Before and long after the bloom, *Sabatieria* δ^{13}C signatures of bacterial biomarkers and MUFAs were more enriched than those of 'other nematodes' and were situated between the δ^{13}C signatures of the bacterial markers and MUFAs in the sediment and SPM. During the bloom and its deposition (April, May, June), δ^{13}C signatures of both nematode groups converged to similar signatures probably reflecting a mixture between fresh input from the SPM in the water column and more degraded matter in the sediment. Interestingly, this diverges from Franco et al. (2008b) who did not find differences in bulk δ^{13}C between 'other nematodes' and *Sabatieria*. This shows the advantage of CSIA on more food source-specific biomarkers as opposed to bulk δ^{13}C analysis of the entire body.

Total absolute fatty acid concentrations in 'other nematodes' were on average 28% higher in surface nematodes than in deep-dwelling nematodes, but the actual FA composition remained similar. This indicates that 'other nematodes' at the surface that are supposed to have access to fresher organic matter actually accumulate higher fat reserves and/or grow bigger. The latter cannot be excluded since individual biomass data are not available for our dataset. The individual abundances of FA biomarkers in *Sabatieria*, however, were equal in both surface and deep-dwelling groups, which suggests access to the same quantities of food, supporting the hypothesis of vertical migration in the sediment (Franco et al., 2008b) or even genetic predetermination (Kuerten et al., 2013a). Nematode bulk δ^{13}C signature at the same station differed among nematodes inhabiting contrasting depth layers, especially for 'other nematodes' (Franco et al., 2008b). The authors attributed this difference to depth-segregated food webs. The 'other nematodes' in the present study did not show depth-related differences in δ^{13}C signatures of the main biomarkers, but there was a shorter distance between our depth layers (deepest layer in this study: 3−4 cm; 4−5 cm in Franco et al., 2008a,b). As deeper layers contain nematodes feeding on older material (Franco et al., 2008b), the difference between our surface layer and deep layer was expected to be smaller than that in Franco et al. (2008b).

Meiobenthic bulk δ^{13}C signature changed little over time and was decoupled from temporal patterns in SPM and sediment, which indicates selective feeding (Franco et al., 2008b). This was also the case for *Sabatieria* FA CSIA in this study, whereas 'other nematodes' seemed to experience some temporal fluctuations in their δ^{13}C signature, however, unrelated to the deposition of the phytoplankton bloom. Together with high PUFA levels, essential

energy-rich compounds to maintain their metabolism (Arts et al., 2001), this decoupling from temporal phytoplankton dynamics corroborates the results for deep-sea nematodes (Guilini et al., 2013; Lins et al., 2015; Veit-Köhler et al., 2013) where continuous feeding throughout the year on degraded matter was suggested. Only during phytoplankton bloom deposition, nematodes have access to and accumulate more FA. Interestingly, this suggests that irrespective of the region and extent in food supply, nematodes do not change their diet throughout the year.

4.3. Macrofauna diet composition and temporal patterns

Abra and *Owenia* seem to share the same food sources throughout the year, except during the phytoplankton bloom, when *Owenia* seemed to assimilate at least some of the abundant *Phaeocystis* in the SPM, while *Abra* consumed strictly diatoms. Since diatom abundances during the *Phaeocystis* bloom were roughly two orders of magnitude lower than *Phaeocystis* abundances, this means that *Abra* was selectively taking out the diatoms from the SPM. *Abra* spp. are known to select particles by size from the sediment (Hughes, 1975), but our data now also indicate that this bivalve can selectively pick up diatoms from deposited phytoplankton that consists mainly of *Phaeocystis* (Lancelot et al., 2005). It has been shown that the gelatinous *Phaeocystis* colonies have a low nutritional value and tend to have adverse effects, such as clogging of gills (Weisse et al., 1994). Selection of food particles is a common feature among suspension- and deposit-feeding invertebrates in relatively rich coastal environments (Boon and Duineveld, 2012; Ward and Shumway, 2004). For deposit-feeders, one would expect elevated bacterial FA concentrations. Indeed, *Abra* spp. can also distinguish between microbially coated particles and clean particles (Hughes, 1975). In the suspension-deposit feeding *Abra* and *Owenia*, however, bacterial contributions were low. Only during the deposition of the phytoplankton bloom on the sediment, both species accumulated >10% of bacterial biomarkers in their reserve lipids, probably attached to the algal detritus in the SPM. Altogether, this shows that *Abra* and *Owenia* are primary consumers selectively feeding on the fresh particulate organic matter of the water column.

Sagartia had a very different FA composition from *Abra* and was characterized by higher PUFA levels. The $\delta^{13}C$ signatures of the PUFAs in *Sagartia* were 3–4‰ depleted as compared to the sediment and SPM signatures. This could be evidence of biosynthesis from other FA (Ruess et al., 2005; Treignier et al., 2009); 22:5ω3 as an intermediate product in the synthesis of DHA was present in relatively high concentrations (6.7 ± 2.1% average ± SD). However, biosynthesis is thought to be a slower process than assimilation of FA in the neutral lipids (Graeve et al., 2005; Woulds et al., 2014). Taking into account an estimated decrease in $\delta^{13}C$ of PUFA of ~3.2‰ per trophic level increase (Bec et al., 2011; Gladyshev et al., 2014), this suggests rather predation. In addition, *Sagartia* is the only investigated species where 20:1ω9c, a biomarker for copepods (2.6–5.3%) was detected. This is in agreement with gut content observations of other burrowing anemones (Holohan et al., 1998). Together with elevated DHA levels, DHA/EPA ratio >1, this confirms the suggestion of van Oevelen et al. (2009) that *Sagartia* is a predatory anemone capturing flagellates and copepods.

Abra and *Sagartia* started to replenish their total FA reserves from the moment the phytoplankton bloom in the water column started. Especially *Abra* experienced strong variations in the reserve lipid concentrations, which declined strongly after the phytoplankton bloom. Similar to other invertebrates, this might be related to the period of spawning, since PUFAs are crucial for reproduction of bivalves (Hendriks et al., 2003; Wacker and Von Elert, 2003) and also for sea anemones (Pernet et al., 2002).

Unexpectedly, we observed very little influence of season on the FA *composition* of *Abra*, *Owenia* and *Sagartia*. Although the diet composition of bivalves and polychaetes for example, has been shown to depend on seasonal variations in resource availability (Braeckman et al., 2012; Ezgeta-Balić et al., 2012; Lefebvre et al., 2009; Richoux et al., 2014) other studies contradict this (Dubois et al., 2014; Kuerten et al., 2013a; Legeżyńska et al., 2014). Similar to the nematodes, biosynthesis can complicate the interpretation of CSIA, as PUFAs can be biosynthesized by cnidarians, bivalves and polychaetes (Monroig et al., 2013; Thurber, 2014). PUFAs can also be selectively retained by polychaetes and echinoids (Boon and Duineveld, 2012; Woulds et al., 2014), especially in periods of nutritional shortage (Boon and Duineveld, 2012; Freites et al., 2002), which would then obscure the higher uptake of PUFA during accumulation of fresh organic matter. Similarly, PUFA levels in marine bivalves are typically at levels >50% and remain relatively stable over time (Freites et al., 2002; Ventrella et al., 2008). On the other hand, the $\delta^{13}C$ signature of the bacterial and saturated FA in all investigated species became gradually more depleted towards the end of June, whereas EPA and DHA in macrofaunal reserve lipids were more enriched during this bloom deposition period. The latter is probably a mirror from the $\delta^{13}C$ signature of the SPM in the water column and constitutes this replenishment of lipid reserves (Lorrain et al., 2002; van Oevelen et al., 2009).

4.4. Benthic food web

In this study, we investigated the benthic food web from a fine–sandy depositional site in the Southern Bight of the North Sea. The seasonal input of fresh pelagically produced organic matter to the sediment is strongly mediated by suspension-deposit feeders through herbivory (e.g. by the bivalve *Abra alba* and polychaetes like *Owenia fusiformis* and *Lanice conchilega*) and predation (e.g. by the polychaete *Nephtys hombergii* and anemone *Sagartia* sp.) (van Oevelen et al., 2009; Braeckman et al., 2012; this study). Since the nematode FA biomarkers show a strong similarity to the benthic food sources in the sediment and experience little variation in time with respect to phytoplankton bloom deposition (Franco et al., 2008a,b; this study), we believe that the nematodes rely on different food sources than those preferred by the macrobenthic species mentioned above (Maria et al., 2012; van Oevelen et al., 2009). In the present study and a previous one (Braeckman et al., 2012), we chose to focus on the temporal variation in the diet of few selected species, in case of nematodes 'groups', at the expense of covering the diet of the entire community. As such, we miss detailed information on the diet composition and seasonal variation of true deposit feeders of the *Abra alba–Kurtiella bidentata* community (Van Hoey et al., 2004). Although *ex situ* experiments with suspension-deposit feeders and nematodes already excluded strong resource competition (Braeckman et al., 2011), additional information on the diet composition of macrobenthic deposit feeders would allow making stronger statements about trophic competition between the entire macrobenthic community and the nematode community mainly composed of non-selective deposit feeders present in depositional sites such as present in the Southern Bight of the North Sea. In such high energy systems at least, macrobenthos seems to be imposing stronger interference competition on meiobenthos than in systems with low food availability (Nascimento et al., 2011).

5. Conclusions

The phytoplankton dynamics in the Belgian Part of the North Sea were clearly mirrored in the FA biomarkers of the SPM in the water column, but less pronounced in the sediment, probably due

to patchy distribution, microbial decomposition processes or stripping of FA by macrofauna. The macrobenthic suspension-deposit feeders *Abra* and *Owenia* seemed to rapidly assimilate the fresh organic matter from the SPM, while *Sagartia* showed evidence of a predatory diet. Since these three consumers can make up 50% of the biomass of the entire macrobenthic community in the area, their rapid consumption could quickly deplete the easily degradable matter of the phytoplankton bloom once deposited on the sediment. Together with the sediment-resembling FA signatures of the nematodes, this is again evidence that macrobenthos and nematodes belong to two different food webs (Maria et al., 2012; van Oevelen et al., 2009) and at least the suspension-deposit feeding macrobenthos and non-selective deposit feeding nematodes do not compete for fresh organic matter from the phytoplankton bloom (Braeckman et al., 2011).

Acknowledgments

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.marenvres.2015.04.010.

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