SUPPORTING INFORMATION

Appendix I : Supporting tables and figures

Title: Connected macroalgal-sediment systems: blue carbon and foodwebs in the deep coastal ocean

Authors: Ana M Queirós, Nicholas Stephens, Stephen Widdicombe, Karen Tait, Sophie McCoy, Jeroen Ingels, Saskia Rühl, Ruth Airs, Amanda Beesley, Giorgia Carnovale, Pierre Cazenave, Sarah Dashfield, Er Hua, Mark Jones, Penelope Lindeque, Caroline L. McNeill, Joana Nunes, Helen Parry, Christine Pascoe, Claire Widdicombe, Tim Smyth, Angus Atkinson, Dorte Krause-Jensen and Paul J Somerfield

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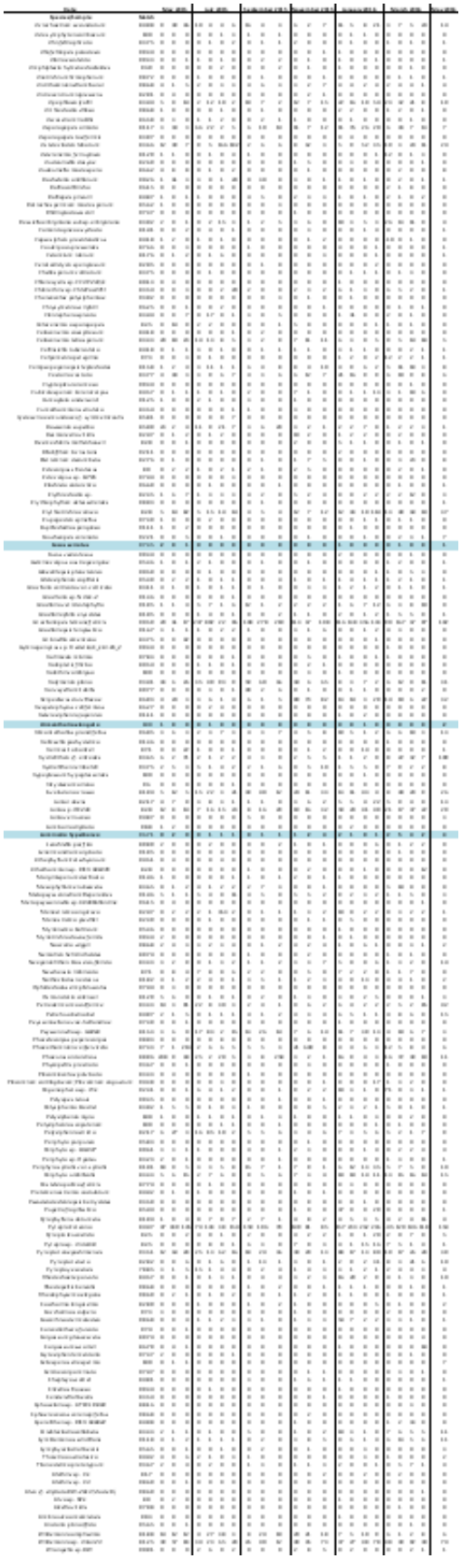
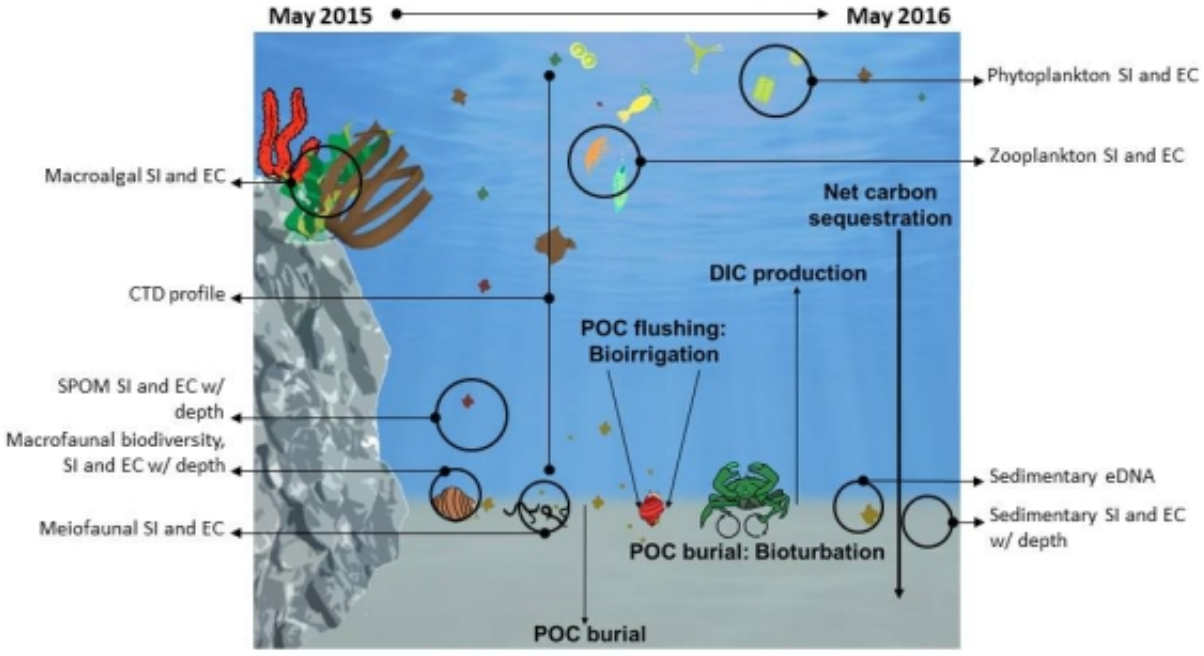
SI Table S1: Sequence counts for macroalagal taxa for which DNA was found within L4 sediments. Species we sampled on the shore are highlighted in bold. “Match”: minimum percent homology, that is, the minimum match between a sampled sequence and a (set of) record(s) in the NCBI BLAST.



SI Table S2: Macrofaunal community at Station L4's benthic site. Feeding modes extracted from the World Register of Marine Species. Depth refers to the sediment layer depth sampled in each multicorer core (10.5

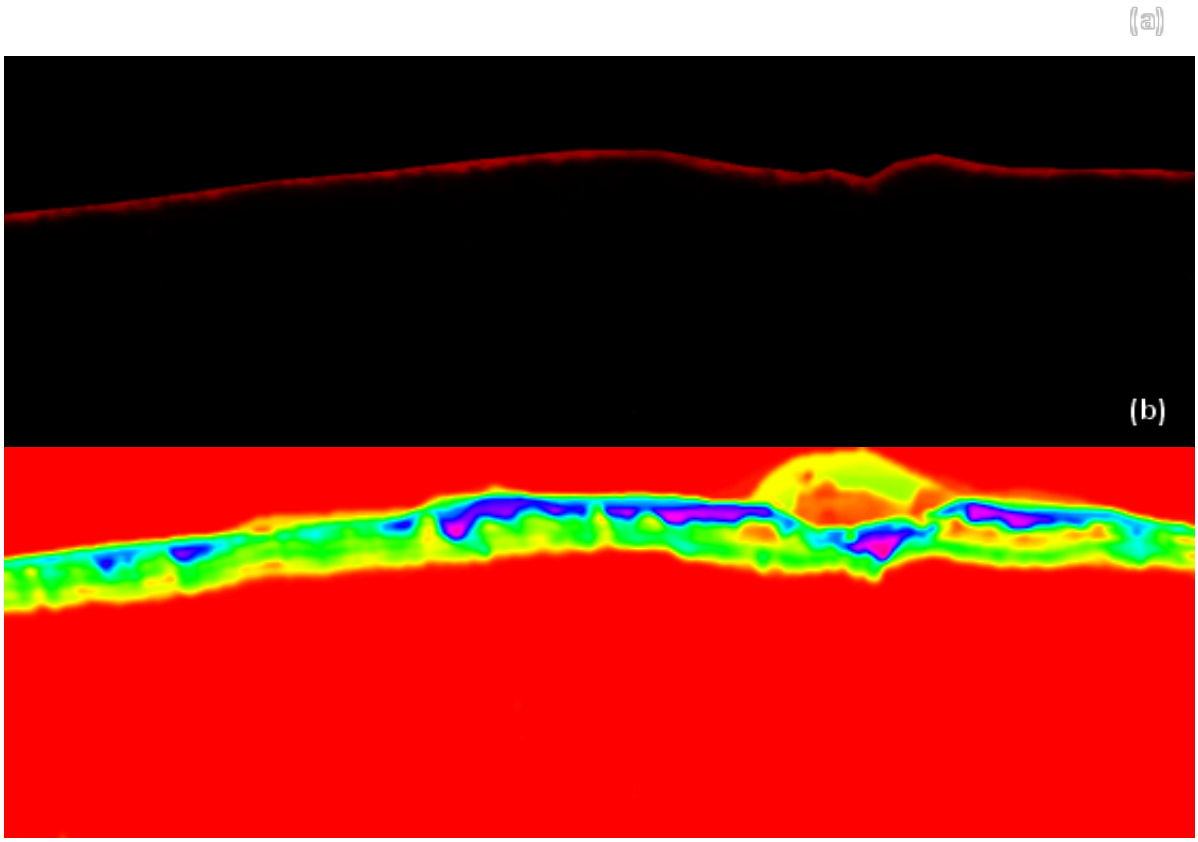
cm i.d.). Species numbers and biomass per feeding group (g) are the total over all replicates per sampling date and sediment depth. Feeding mode: “CDF” alternate carnivore-deposit feeder; “CS” carnivore, scavenger; “DF” deposit feeder; “DFSF” alternate deposit feeder -suspension feeder; “SF” suspension feeder.

Depth (cm)	Date	Nr Species	Feeding mode				
			CDF	CS	DF	DFSF	SF
0-2	May-15	33		0.27175	0.17928		0.07521
	Jul-15	43	0.00437	0.20997	0.11217	0.00362	0.170193
	Sep-15	39		0.72729	0.06352		0.16519
	Nov-15	6		0.26073	0.01237		0.00541
	Jan-16	20	0.00428	0.22529	0.01418		0.31553
	Mar-16	20	0.02457	0.23532	0.08884		0.01087
	May-16	31	0.02454	0.17967	0.01858	0.04358	0.16018
2-6	May-15	10		0.4573	0.362402		
	Jul-15	18		0.43245	2.02342		0.06562
	Sep-15	21		0.3761	0.01852		0.07847
	Nov-15	20		1.62983	2.30234		0.68928
	Jan-16	15		0.29813	0.037	0.00145	
	Mar-16	11		0.15301	0.08725		0.09851
	May-16	17	0.00091	0.20007	0.58421		0.00752
6-10	May-15	8		0.69043	0.00008		0.01094
	Jul-15	3		0.53614	0.36294		
	Sep-15	8		0.58652	2.11768		3.67501
	Nov-15	7		1.79206	0.04018		3.03283
	Jan-16	1			0.94274		
	Mar-16	1			0.05028		
	May-16	4			0.426539		

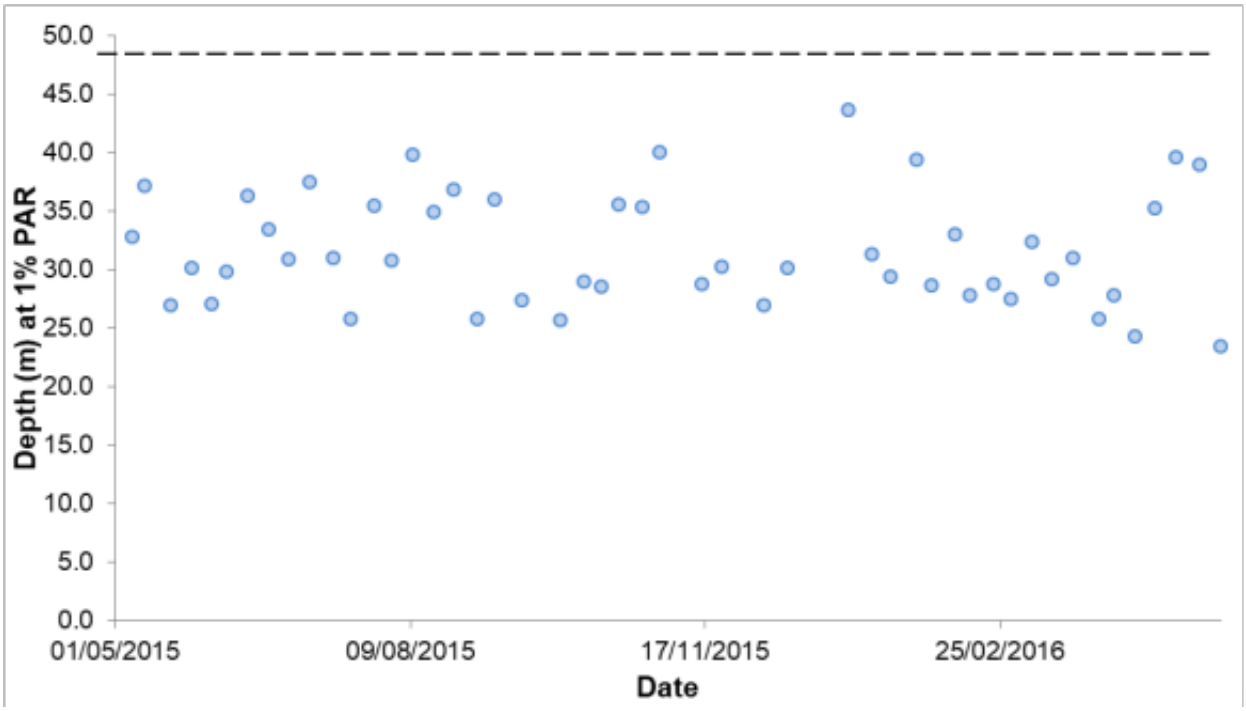


SI Figure S1. Schematic representation of the sampling programme, indicating fluxes, pools and processes measured at each of seven sampling occasions, during the seasonal cycle of May 2015-May 2016. DIC: dissolved inorganic carbon. EC: elemental composition (C and N). POC: particulate organic carbon. SI:

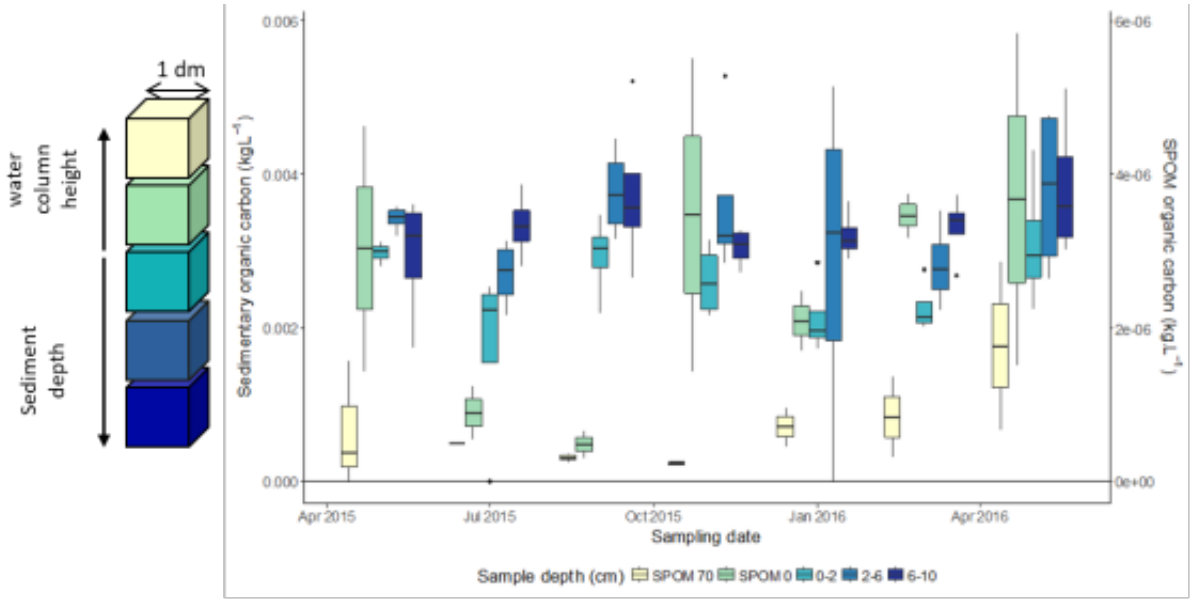
stable isotopes ($\delta^{13}C$, $\delta^{15}N$). SPOM: suspended particulate organic matter.



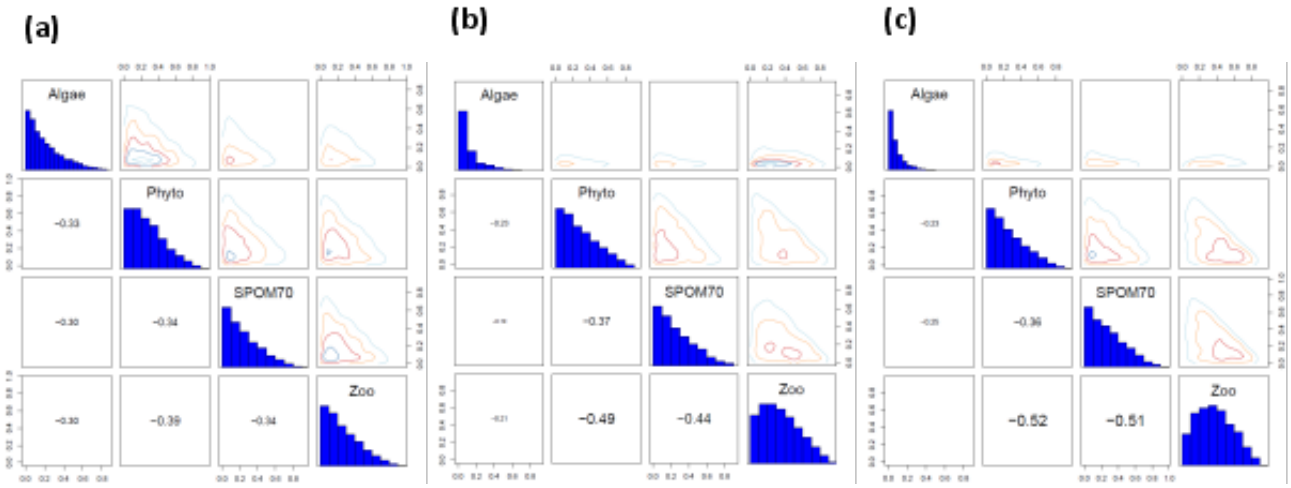
SI Figure S2. Processes measured during laboratory incubations of natural cores from L4: bioturbation. a) Cross section of sediment core at the beginning of a bioturbation incubation (10 cm wide), with fluorescent orange luminophore layer (bioturbation tracer) evident at the sediment surface. b) Composite of all 144 images captured on the same core over a 7 day period, with LUT spectral mask highlighting regions where luminophore pixels occur in the same position in all images (i.e. no mixing; blue to violet) and those where they do not (mixing occurs; orange to green), against the non-reflective background (red).



SI SI Figure S3. 1% light (photosynthetically active radiation - PAR) depth at station L4 in the Western Channel Observatory, during the samplign period. Publicy available dataset.



SI Figure S4. Seasonal cycle of organic carbon depth profiles ($kg.L^{-1}$), from 70 cm above the sediment water interface (SPOM70), to 10cm within sediments at L4. Data derived from either filter samples (SPOM0 and SPOM70) in which case they relate to seawater volume; or from sediment samples (0-2, 2-6 and 6-10 cm), in which case they relate to sediment volume.



SI Figure S5. Pairs plot of the posterior diet proportions of macrofauna communities at 0-2 cm (a), 2-6 cm (b), and 6-10 cm sediment depth (c).The upper-diagonal shows contour plots, the diagonal shows histograms, and the lower-diagonal shows the correlations between the different sources. Note the low correlation between SPOM70 and the other sources in each model.

Appendix II: Extended methods section

Title: Connected macroalgal-sediment systems: blue carbon and foodwebs in the deep coastal ocean

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Collection of macroalagal tissue samples during shore surveys

Samples were preferentially collected from fixed and homogenous areas at the centre of healthy looking

mature blades to avoid confounding by within organism variation in isotopic signatures (Stephenson et al. 1984). We avoided, as much as possible, decomposing tissue, active growing regions, grazing marks, gametes and epibionts. Exception was made between November 2015 and March 2016 for *H. elongata* and *F. serratus*, when only young individuals (year 1) were found after large biomass loss during the winter, in which case growing tissue was collected. For *H. elongata*, we also included reproductive fronds on some occasions. On all occasions, each tissue sample was rinsed upon collection with MilliQ water and placed in a pre-acid washed falcon tube which had been rinsed with MilliQ three times.

Sampling of CO_2 gas from water samples

Water samples for this analysis were acquired using glass syringes coupled *via* a luer lock to a filter head which lodged a GF/F grade pre-combusted filter (425ºC, Fisher) onto which a non-coring 22G needle had been mounted. This was rinsed with 10 mL of sample water three times before collection of each sample (Waldron et al. 2014), and pre-combusted filters replaced for the processing of each individual core. Insuring that no air bubbles were visible in the syringe, 2 mL of sample water were then immediately injected into a 10 mL Na- glass Exetainer®, through screw caps fitted with a chlorobutyl rubber septa. Prior to each sampling occasion, Exetainers® and septa had been prepared by acid-washing in 10% HCl and dried at 60ºC. When dry,150 µL of 85% Phosphoric acid were added to each Exetainer® which was sealed with a new, acid-washedrubber septa, and flushed with pure Helium gas using a double needle setup (Spötl 2005, Olack et al. 2018).

eDNA extraction, amplification and sequencing

eDNA was extracted from sediment samples using the Qiagen Dneasy PowerSoil © on 0.25 g wet sediment sub-samples, according to the manufacturers' instructions . Extraction quality was assessed in all cases using Nanodrop© spectrophotometry, as per manufacturer guidelines. DNA extracted from sediments was then amplified using polymerase chain reaction (PCR) using the 18S rRNA V9 region PCR primers Euk1391F (GTACACACCGCCCGTC) and EukBr (TGATCCTTCTGCAGGTTCACCTAC). The 50 µL reaction mixture contained 1 µL DNA, 5 µL PCR buffer (Qiagen, Manchester, UK), 2mM MgCL2, 0.2 mM dNTPs,

2.5 U of Taq polymerase (Qiagen, Manchester, UK) and 1mM of each primer. PCR products were amplified using the following conditions: 95 °C for 5 min, followed by 20 cycles of 95 °C for 1 min, 56 °C for 1 min and 72 °C for 1 min with a final extension time of 5 min at 72 °C.The success of the PCR was verified using 2μL of PCR product on 1% agarose gel electrophoresis. PCR products were then purified using commercially available kits (Qiagen) and sequenced by commercial contract (Mr DNA, Molecular Research LP, USA). Each sediment sample was amplified in triplicate, the triplicates pooled, cleaned using the QIAquick PCR purification kit (Qiagen, Manchester, UK) and sent for sequencing by commercial contract (www.mrdnalab.com, TX, USA). PCR products were then subjected to a further five PCR cycles using primer sets modified with multiplexing identifier (MID) adaptors for barcode tagging, thereby allowing for post-sequencing separation of the samples. Following PCR, all amplicon products from different samples were mixed in equal concentrations and purified using the Agencourt AMPure XP Purification System (Beckman Coulter, Bromley, UK). The pooled and purified PCR product was used to prepare DNA libraries by following the Illumina TruSeq DNA library preparation protocol. Sequencing was performed on a MiSeq following the manufacturer's guidelines. The resulting eDNA sequence data is publicly available at the National Center for Biotechnology Information (USA) *via* the SRA accession number SRP148635. Sequence data were processed using a proprietary analysis pipeline (www.mrdnalab.com, TX, USA) as follows: sequences were de-multiplexed, depleted of barcodes and primers, sequences <150 bp or with ambiguous base calls and with homopolymer runs exceeding 6 bp removed, denoised, operational taxonomic units (OTUs) generated (at 97% similarity) and chimeras removed.

Determination of $\delta^{13}C$ of DIC

Through the use of the Valco valve and a sample loop within the gas bench and the instrument Isodat NT software version 2.0, each Exetainer® was sampled nine times of which the last four values were averaged to give a single sample value. The stable carbon isotope ratio of DIC samples ($\delta^{13}C$ DIC) are expressed throughout as the per mil deviation from that of the commonly used standard material Vienna Pee-Dee Belemnite (VPDB, Coplen 1995) and are traceable to IAEA reference material NBS 19 TS-Limestone. Repeated analysis of a quality control standard gas indicated typical precision of the gas bench for analyzing $\delta^{13}C$ of CO_2 at a concentration of 450 ppm in Exetainers® was -35.03 ± 0.24 ‰ (mean ± SD, n = 65).

Preparation of samples for elemental scans and stable isotope determination

All phyto- and zooplankton, macrofauna, macroalgae, filters and BSed samples were fresh weighed. All samples were then oven dried at 45ºC for 48 hours in pre-weighted foil boats and glassware, which had been acid washed, combusted in foil wraps and weighed. Each whole sample was re-weighed to determine fresh weight to dry weight ratios ("FW/DW"), and then ground to a fine powder using acid-washed and pre-combusted agate pestle and mortars. Macrofauna, macroalgae and plankton samples were then divided into 2 portions of equal weight:1) one half was added to a USFDA approved 2 mL polypropylene, human touch-free centrifuge tube (ISO 13485, Isolab, GmbH) for combined TPC and TPN as well as $\delta^{15}N$ and $\delta^{13}C$ determination; 2) one half was acidified by adding drops of 10% HCl until all carbonates had been dissolved, and then oven dried, re-weighted, homogenised and added to the same type of centrifuge tube for the determination of $\delta^{13}C$ of POC, and POC content, as before. BSed samples were split into 2 equal weight portions: 1) one half was added to a USFDA approved 2 mL polypropylene, human touch-free centrifuge tube (Isolab, GmbH) for the determination of TPN content and $\delta^{15}N$ determination, as before; 2) one half was further split into two by weight for the determination of TPC and POC (*via* HCl acidification), and the determination $\delta^{13}C$, using the same methods. This is because low nitrogen values were identified in sediments in pilot elemental scans, necessitating a larger sample size. Early scans revealed that the nitrogen levels in SPOM were even lower. In this case, out of four filters per water column depth and sampling occasion, we used two whole filters for nitrogen analysis (TPN and $\delta^{15}N$), and two filters were divided into equal portions each: one half for TPC content and determination of its $\delta^{13}C$, and one half and for POC content and determination of its $\delta^{13}C$ (*via* HCl acidification, as before).

Elemental scanning and stable isotope determination of sediment, plankton, SPOM and macrofaunal samples

Samples and references were weight optimised for combined N and C isotope analysis, $\delta^{15}N$or $\delta^{13}C$analysis as appropriate to each sample type, except for filters and BSed, for which elemental and isotopic analysis for C and N were undertaken from different sub-samples (as described).IRMS calibration was scale normalised

using USGS-40 and USGS-41 scale anchors with QC sample checks. Percent nitrogen and carbon were determined from the IRMS total beam values. References and samples were weighed from bulk material to 6 decimal places using a Mettler UMX5 microbalance. Standard deviations on isotope reference materials were typically better than 0.2 for nitrogen and 0.15 for carbon. Standard deviation of percent elemental analysis values using total beam values is typically better than 0.5 for nitrogen and 0.8 for carbon.

The filter pre-combustion procedure (SPOM samples) generated a residue of total particulate carbon (TPC) = 0.02± 0.01 % (mean ± standard deviation), particulate organic carbon (POC) = 0.01± 0.01 %, and total particulate nitrogen (TPN) = 5.1 x 10^{-3}±1.6 x 10^{-3} %, which were considered appropriate, and did compromise the stability of the filters or their elemental and isotopic analysis.

Direct acidification of sediments is the best method to determine the concentration of organic carbon accurately in marine sediments (Ryba and Burgess 2002). We applied this method only in samples used to estimate POC (and not TPN, as described). In this study, in line with previous work (Ryba and Burgess 2002), dry weight of sediments post acidification with 10% HCl increased by 1.6±0.7%. Having used desiccators for sample storage throughout, we assign this increase in weight to H contamination and salt formation during acidification.

Washing of meiofaunal samples for elemental scanning and isotopic determination

Meiofaunal samples were thawed by rinsing the frozen sediment into sterile glass beakers using Milli-Q water and decantated. Decantation involved bringing the sample into resuspension through stirring a 1L diluted sample and waiting for 40 sec. for the heavier sediment particles to settle, before pouring the top half of the suspension on a 63 μm sieve; this process was repeated three times to extract the majority of the meiofauna. The residue on the sieve was then rinsed into a sterile glass beaker and placed on ice to avoid rapid degradation of the sample and continue processing in the lab. Each washed sample was placed in a Petri dish and using a stereoscope (10-50x magnification). 100-120 nematodes were picked out per sample and placed in a sterile cavity block containing Milli-Q water. From the cavity blocks the nematodes were picked out and placed in a new cavity block containing Milli-Q water in order to clean the nematodes from

adhering particles and other contaminants; the nematodes were washed three times this way.

Determination of bromide concentrations in seawater samples

Bromide concentrations were determined using reversed-phase high performance liquid chromatography (HPLC, Gazeau et al. 2014), which was executed using an Agilent 1100 system (Agilent Technologies, Cheadle, UK) comprising a micro vacuum degasser, quaternary pump, a thermostated autosampler and a photodiode array detector. The system was controlled using Agilent ChemStation software and elution was carried out using an IonPac AG14 RFIC (4 x 250 mm; particle size 2-50 µm; Thermo Scientific DIONEX,) protected with an IonPac AG14 RFIC guard (4 x 50 mm). Mobile phase comprised 1 % Sodium Carbonate (Na2CO3; Supplier) in MilliQ at a flow rate of 1.2 ml/minute. The column temperature was maintained at 21 °C. Bromide was detected at 210 nm. Prior to analysis, frozen water samples were thawed at 4^{o}C and 100 µl sample was diluted with 900 µl artificial sea water (35ppm Sodium Chloride in MilliQ) in a sample vial, capped, mixed using a vortex mixer and transferred to the HPLC autosampler. 10µl from each vial was injected and an average peak area was taken from each lot of six replicates per sample. Calibration was carried out regularly with the start of each new sample group by injecting freshly prepared solutions of 10 mM of sodium bromide (NaBr) at the following concentrations: 0.1 mM, 0.5 mM, 0.99 mM, 1.49 mM, 1.98 mM, 2.48 mM, 2.97 mM, 3.47 mM, 3.97 mM, 4.46 mM and 4.96 mM in artificial sea water. With each sample set of injections, six replicates of 10 molar NaBr solution in MilliQ were injected to check consistent detector response.