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ORIGINAL ARTICLE

Resource quality affects carbon cycling in deep-sea sediments

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Deep-sea sediments cover ~70% of Earth's surface and represent the largest interface between the biological and geological cycles of carbon. Diatoms and zooplankton faecal pellets naturally transport organic material from the upper ocean down to the deep seabed, but how these qualitatively different substrates affect the fate of carbon in this permanently cold environment remains unknown. We added equal quantities of ^{13}C-labelled diatoms and faecal pellets to a cold water (−0.7 °C) sediment community retrieved from 1080 m in the Faroe-Shetland Channel, Northeast Atlantic, and quantified carbon mineralization and uptake by the resident bacteria and macrofauna over a 6-day period. High-quality, diatom-derived carbon was mineralized >300% faster than that from low-quality faecal pellets, demonstrating that qualitative differences in organic matter drive major changes in the residence time of carbon at the deep seabed. Benthic bacteria dominated biological carbon processing in our experiments, yet showed no evidence of resource quality-limited growth; they displayed lower growth efficiencies when respiring diatoms. These effects were consistent in contrasting months. We contend that respiration and growth in the resident sediment microbial communities were substrate and temperature limited, respectively. Our study has important implications for how future changes in the biochemical makeup of exported organic matter will affect the balance between mineralization and sequestration of organic carbon in the largest ecosystem on Earth.

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Introduction

A proportion of organic matter produced in the upper ocean via photosynthesis sinks down into the deep sea, carrying with it carbon from the atmosphere. Organisms inhabiting the deep seabed are the ultimate recipients of this particulate material. Any organic carbon that escapes mineralization in this environment is liable to be sequestered for millennia, ultimately representing the sequestration of atmospheric CO_2 (Lampitt *et al.*, 2008). Deep-sea sediments are estimated to bury up to 300 Tg C y^{-1} globally (Burdige, 2007) and hence play a major role in the global carbon cycle (Jahnke, 1996). Understanding the factors influencing the fate of carbon at the deep seabed is necessary for discerning the strength of marine sediments as a global carbon sink and thus predicting how their role in climate regulation will change in the future.

The quantities of benthic (seabed) organisms and sediment community oxygen consumption (SCOC) rates in the deep sea all correlate positively with the flux of particulate organic carbon (Smith *et al.*, 1997; Ruhl *et al.*, 2008), suggesting that biogeochemical processes in this environment are controlled by the quantity of available resources. Nevertheless, circumstantial evidence strongly suggests that the biochemical makeup of organic material, that is, 'resource quality', also affects the structure and functioning of deep-sea benthic communities (see, for example, Wolff *et al.*, 2011). Discrete pulses of diatoms and zooplankton faecal pellets can dominate the annual flux of organic matter to the deep seabed (Billett *et al.*, 1983; Bathmann *et al.*, 1987). These contrasting substrates can have similar bulk elemental stoichiometry, yet differ considerably at the biochemical level: the former are rich in labile compounds such as sugars, lipids and amino acids, whereas the latter are largely devoid of these,

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instead containing large quantities of insoluble carbohydrates (Cowie and Hedges, 1996; Mayor *et al*., 2011). An influx of diatom-derived organic matter to deep seabed communities initiates the rapid uptake and mineralization of carbon by the benthos (Moodley *et al*., 2005; Gontikaki *et al*., 2011b). A similar, rapid response has been proposed to occur in response to the arrival of zooplankton faecal material (Graf, 1989). Robust empirical comparisons of how the lability of organic material arriving at the deep seabed affects the fate of its constituent carbon are currently lacking.

We employed identical carbon-13 (^{13}C) tracer experiments during mid May and early October 2008 to investigate how resource quality affected carbon cycling in the bacteria-dominated sediment community found at 1080 m in the sub-zero waters of the Faroe Shetland Channel, Northeast Atlantic. Our aim was to examine how diatoms and copepod faecal pellets, naturally occurring substrates of contrasting qualities, influenced the rates and pathways of organic carbon cycling in this challenging environment.

Materials and methods

Production of ^{13}C-labelled substrates

The techniques for producing ^{13}C-labelled diatoms and faecal pellets are presented elsewhere (Mayor *et al*., 2011). In brief, sterile cultures of the marine diatom, *Chaetoceros radicans*, were grown in artificial sea water enriched with L1 medium and $NaH^{13}CO_3$ (99 atom % ^{13}C, Goss Scientific, Nantwich, UK). Concentrated diatoms were repeatedly washed with sterile filtered (0.2 μm) sea water to remove dissolved inorganic and organic ^{13}C ($DI^{13}C$ and $DO^{13}C$, respectively). Batches of ~1000 *Calanus* spp. copepods were fed the labelled diatoms. The resulting faecal pellets were separated from eggs, diatoms and other debris and repeatedly washed in sterile filtered sea water. The carbon/nitrogen (C/N) ratios of the diatoms and faecal pellets were 6.3 ± 0.2 and 8.2 ± 0.1 by atoms, respectively. The C/total fatty acid ratios in these substrates, a relative measure of increasing recalcitrance, were 0.30 ± 0.01 and 0.83 ± 0.05 μmol C/nmol fatty acid, respectively.

Experimental setup

Experiments were conducted on 11 May 2008 and 01 October 2008. On both occasions, a total of 18 undisturbed sediment cores (ID = 10 cm, $n = 6$ per treatment) were retrieved from 1080 m in the Faroe Shetland Channel, Northeast Atlantic (61°13′88″ N, 2°40′62″ W; Supplementary Figure S1). The sub-Arctic community at this location is well described (Gontikaki *et al*., 2011a, b) and dwells in fine- to medium-textured sediments that contain 0.3 wt % organic carbon. Core-warming artefacts were minimized because of the extremely cold water temperatures (<2 °C below 450 m) and rapid transferral of cores into the experimental incubator (~15 min for 8 cores). The effects of decompressing sediments and their resident biota on elemental cycling are considered to be negligible when retrieved from ⩽1000 m (Graf, 1989; Hensen *et al*., 2006), in part reflecting the barotolerant nature of the resident bacterial communities (Meyer-Reil and Köster, 1992). Recent ^{13}C pulse-chase experiments undertaken on communities from such depths have demonstrated that microbial and macrofaunal carbon uptake remains broadly consistent between studies conducted *in situ* and *ex situ* (Woulds *et al*., 2007). All cores were acclimated to the experimental conditions overnight. The equivalent of 16.7 mmol organic carbon m^{-2}, either in the form of diatoms or faecal pellets, was gently pipetted onto the surface of the experimental sediments ($n = 6$ in both cases). This represents ~10% of the annual carbon flux in this region (Schlüter *et al*., 2000). Treatments were randomized across corer deployments. All cores, including controls (without addition of organic matter, $n = 6$), were topped up with bottom water, sealed with custom-made lids to prevent gas exchange and incubated at the *in situ* temperature (−0.7 °C) for 6 days. Core lids sealed against the inner surface of the core via a nitrile rubber O-ring. Gas-tight water samples from each core were collected through lid ports immediately after the introduction of organic substrates ($t = 0$) and every 24 h thereafter for 6 days. This was achieved by applying gentle pressure to the core lid while simultaneously drawing on the sampling syringe, thereby allowing the removal of water without the introduction of a head space beneath the core lid. Samples for the analysis of dissolved inorganic carbon (DIC and $DI^{13}C$) were sterile filtered, fixed with 0.2% (vol) HgCl and stored in Exetainers (Labco, Ceredigion, UK) at 4 °C. Oxygen samples were transferred into 10 ml Winkler bottles, fixed for Winkler titration and stored in the dark until analysis. Oxygen and DIC concentration data were corrected for the proportional quantities removed from the overlying waters due to sampling. At the end of the experiment, three cores from each treatment were sectioned at the following horizons and stored frozen at −20 °C for analysis of bacterial and archaeal community composition using PCR-denaturing gradient gel electrophoresis (PCR-DGGE) analysis of 16S rRNA gene sequences, tracer carbon incorporation into sediment bacteria phospholipid fatty acids (PLFAs) and bulk organic carbon: 0–10, 10–20, 20–30, 30–50 and 50–100 mm. The remaining three replicate cores from each treatment were sectioned at 0–20, 20–50 and 50–100 mm depth horizons, gently sieved (250 μm mesh) and the residue preserved in 3.7% saline formaldehyde for subsequent determination of ^{13}C uptake by the constituent macrofauna. We focussed solely on the organisms inhabiting the upper 20 mm of sediment because tracer penetration was never apparent

below this depth (Supplementary Figure S2) and previous work at this location has demonstrated that 95% of tracer incorporation after 6 days of incubation occurs in the upper 2 cm (Gontikaki *et al.*, 2011a, b). Meiofauna (32–250 μm) were not examined owing to their negligible contribution to benthic carbon processing at our experimental location (<1% of total) (Gontikaki *et al.*, 2011a). Concentrations of labelled $DO^{13}C$ introduced at the outset of the experiment were not quantified, although these have previously been shown to be <5% of the total (Andersson *et al.*, 2008). A schematic of the experimental setup and sampling procedure is presented in Supplementary Figure S3.

Sample processing

Oxygen concentrations were determined using an automated Winkler titration system (785 DMP Titrino, Metrohm, Runcorn, UK). DIC samples were oxidized to carbon dioxide before the concentrations and carbon isotope ratios were determined using a Gas-bench II connected to a Delta^{Plus} Advantage isotope ratio mass spectrometer (IRMS; both from Thermo Finnigan, Bremen, Germany). The mean amplitude of five replicate sample peaks was used to calculate DIC concentration from a calibration curve derived from an appropriate range of sodium carbonate standard solutions. Purified PLFAs extracted from freeze-dried sediment samples (White *et al.*, 1979) were derivitized to yield fatty acid methyl esters. The concentrations and carbon isotope ratios of individual fatty acid methyl esters were measured using a GC Trace Ultra with combustion column attached via a GC Combustion III to a Delta V Advantage IRMS (all from Thermo Finnigan).

Individual PLFAs were quantified by combining the area of their mass peaks, m/z = 44, 45 and 46, after background subtraction, and comparison with a known internal standard (19:0) added to each sample (Thornton *et al.*, 2011). Bacterial carbon uptake was calculated from label incorporation into the bacterial biomarker PLFAs i15:0, ai15:0 and i16:0 (Moodley *et al.*, 2005), assuming these represent 10% of total bacterial PLFAs and 0.056 gC PLFA/gC biomass (Brinch-Iversen and King, 1990). Macrofauna were identified to the lowest taxonomic resolution possible (family or lower), dried, and their carbon concentrations and isotope ratios were determined using a Flash EA 1112 Series Elemental Analyser connected via a Conflo III to a Delta^{Plus} XP IRMS (Thermo Finnigan). The bulk sediment organic carbon content and isotopic signatures were determined on decalcified samples using a Costech (Cernusco sul Naviglio, Italy) ECS 4010 elemental analyser interfaced with a Delta^{Plus} XP IRMS (Thermo Electron, Bremen, Germany). All calculations relating to the uptake of ^{13}C were made using well-established equations (Moodley *et al.*, 2005) and are not repeated here for brevity. Data are expressed as the total uptake of added diatom- or faecal pellet-derived carbon ($^{12}C + ^{13}C$) to account for differences in the fractional abundance of ^{13}C between the two substrates (49.4 ± 0.3 and 31.9 ± 0.2 atom % ^{13}C, respectively).

Microbial community structures were profiled using PCR-DGGE analysis of 16S rRNA gene sequences using primers P3/P2 (Muyzer *et al.*, 1993) for the bacterial community and a nested approach using primers A109f/Ar9r followed by rSAf/PARCH519r (Nicol *et al.*, 2007) for the total archaeal community. Details of reagent concentrations are as described previously (Nicol *et al.*, 2007) and thermal cycling conditions were 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min, followed by 1 cycle of 72 °C for 10 min for all assays, except rSAF/PARCH5195r where the annealing temperature was 63 °C. DGGE analysis was performed using a Bio-Rad DCode Universal Mutation Detection System (Bio-Rad, Hertfordshire, UK) with gels containing 8% (w/v) polyacrylamide and a linear gradient of 35–75% denaturant. Gels were electrophoresed in 7 l of 1 × TAE buffer at a constant temperature of 60 °C for 900 min at 100 V and silver stained before scanning using an Epson GT9600 scanner with transparency unit (Epson Ltd, Hemel Hempstead, UK) as described previously (Nicol *et al.*, 2007). To compare community profiles, relative (within lane) band intensities were quantified by densitometry analysis of normalized DGGE profiles using Phoretix 1-D gel analysis software (Phoretix International, Newcastle upon Tyne, UK) as previously described (Nicol *et al.*, 2007).

Estimating bacterial growth efficiency

The contribution of meiofauna to carbon processing in the Faroe-Shetland Channel is negligible (<1% of total) (Gontikaki *et al.*, 2011a). Archaea typically represent <<5% of the total prokaryotic communities found in the surficial sediments of Arctic and deep-sea environments (Sahm and Berninger, 1998; Vetriani *et al.*, 1999; Lipp *et al.*, 2008). We therefore assume that their metabolic response to our substrate addition experiments was also negligible, making it possible to partition the observed carbon mineralization between macrofauna and bacteria: $R_B = R_T - [I_M/NPE_M \times (1 - NPE_M)]$, where R_B and R_T are the quantities of carbon mineralized over the duration of the experiment by bacteria and in total, respectively, I_M is the quantity of added carbon incorporated into macrofaunal tissues over the duration of the experiment and NPE_M is the net production efficiency of the macrofauna. We assume that $NPE_M = 0.5$, although the small values of I_M (Table 1) necessitate that estimates of bacterial growth efficiency (BGE) are insensitive to this parameter. BGE can then be estimated: $BGE = I_B/(I_B + R_B)$, where I_B is the quantity of added carbon incorporated in the bacterial biomass over the duration of the experiment.

Table 1 Benthic carbon budgets for the 6-day experimental duration (mmol C m^{-2} exp^{-1} ± s.e.m.) in May and October

	Treatment	
	Faecal pellet	*Diatom*
May		
Mineralization	0.29 ± 0.10 (1.71)	0.94 ± 0.40 (5.64)
Bacterial uptake[a]	0.47 ± 0.25 (2.79)	0.70 ± 0.13 (4.20)
Macrofaunal uptake[a]	<0.01 ± <0.01 (0.03)	0.03 ± 0.02 (0.18)
Total	0.76 ± 0.27 (4.53)	1.67 ± 0.42 (10.01)
BGE	0.62	0.43
October		
Mineralization	0.29 ± 0.10 (1.71)	1.08 ± 0.40 (6.46)
Bacterial uptake[a]	0.68 ± 0.01 (4.10)	0.40 ± 0.01 (2.40)
Macrofaunal uptake[a]	0.04 ± 0.01 (0.23)	0.03 ± 0.01 (0.16)
Total	1.01 ± 0.10 (6.04)	1.50 ± 0.40 (9.02)
BGE	0.73	0.28

Abbreviation: BGE, bacterial growth efficiency.
[a]Uptake values are net and expressed for the 6-day experimental duration to avoid the assumption that rates are linear.
Values in parentheses represent the percentage of added carbon.

Statistical analyses
Initial data exploration was undertaken to identify outliers and to determine the most appropriate statistical analysis (Zuur *et al.*, 2010). Repeated sampling of sea water from each core gave an *a priori* reason to analyse the oxygen drawdown and carbon mineralization data sets using linear mixed-effect models (Zuur *et al.*, 2009). This approach allowed a correlation structure (the compound symmetrical correlation) to be imposed on all observations within each core by incorporating core identity as a random effect. The protocol for model selection in linear mixed-effect modelling is described elsewhere (Zuur *et al.*, 2009). In brief, the optimal random structure of the model was identified before the optimal fixed structure was determined. Full models with and without core identity as a random effect (random intercept) were compared using a likelihood ratio (L. ratio) test using restricted maximum likelihood estimation. The resulting *P*-values were corrected for boundary effects (Zuur *et al.*, 2009). Variance covariate terms were also incorporated where data exploration revealed instances of unequal variance. This technique allows the residual spread to vary between individual levels of a particular explanatory variable, or combinations thereof. The significance of the random effect was reassessed after the incorporation of variance covariate terms using the L. ratio test, corrected for testing on the boundary (Zuur *et al.*, 2009). The fixed structures of the optimal models, which initially incorporated time, treatment and month and all possible interaction terms, were determined using backwards selection based on the L. ratio test using maximum likelihood estimation. Model parameters were generated using restricted maximum likelihood estimation.

Bacterial and macrofaunal biomass-specific carbon uptake data were Box–Cox transformed before analysis to attain homogeneity of variance. Preliminary linear regression analysis of transformed data to examine the interaction between treatment × month × group ID revealed strong heterogeneity in the residual plots. Data were therefore split by month and analysed separately using linear regression. Backwards selection using the F-statistic was used to determine the optimal models.

The relative proportions of sediment PLFAs reflect the microbial community composition, and the incorporation of tracer carbon into specific PLFAs provides information on the active component of the microbial community (Boschker and Middelburg, 2002). Differences in the incorporation of added carbon into individual PLFAs between treatments and months were therefore assessed with permutational multivariate analysis of variance (perMANOVA). This test can be considered as a nonparametric analogue to multidimensional ANOVA, permitting the simultaneous response of numerous variables to be examined in an ANOVA experimental design. The tests statistics are derived from a distance (dissimilarity) matrix using permutation tests. Distance between samples was assessed using Gower's coefficient. The perMANOVA randomizations were fixed by month to account for the hierarchical sampling design. The PLFAs 14:0, 16:1w7, 16:1w5, 16:0 and all C18 s were excluded from the perMANOVA analysis because of the prevalence of these fatty acids in the diatoms and faecal pellets (Mayor *et al.*, 2011). Additional perMANOVA analyses were used to examine month-, treatment- and month × treatment effects on the normalized DGGE profiles determined at the end of our experiments.

All statistical models were validated to check that the underlying assumptions were met. In the univariate analyses, normality of residuals was examined by plotting theoretical quantiles versus standardized residuals (Q-Q plots); homogeneity of variance was assessed by plotting residual versus fitted values; independence was verified by plotting residuals versus each covariate (Zuur *et al.*, 2009, 2010). Homogeneity of multivariate dispersion was verified by comparing distances with spatial medians using the ANOVA F-statistic. All statistical analyses were conducted in the 'R' programming environment (R Development Core Team, 2010).

Results

Benthic respiration
Mineralization rates of the introduced substrates (Figure 1) were significantly affected by resource quality (time × treatment interaction; L. ratio = 57.79, d.f.$_1$, $P < 0.001$; Supplementary Table S1), with diatom-derived carbon being respired ∼300% faster than that derived from faecal pellets (Table 1).

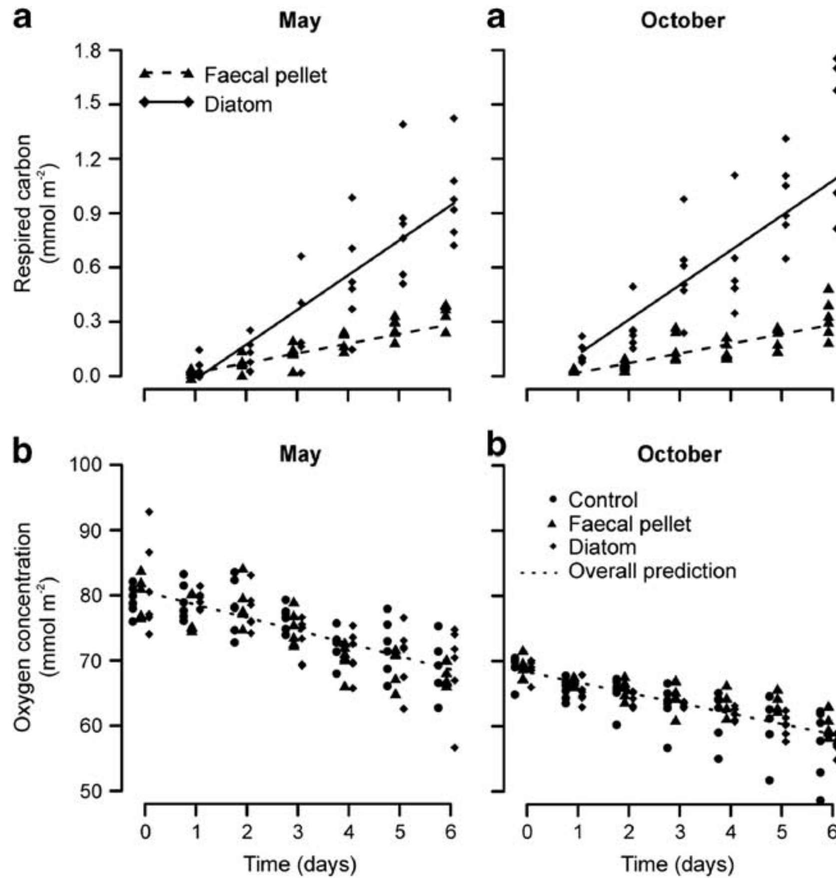

Figure 1 Food-quality effects on the rates of (**a**) carbon mineralization and (**b**) sediment community oxygen consumption in May and October.

Mineralization rates in the different treatments did not differ between months (time × treatment × month interaction; L. ratio = 2.99, d.f.$_1$, $P = 0.084$). The time lag between substrate addition and the first appearance of DI^{13}C in the experimental water differed by treatment and month (treatment × month interaction; L. ratio = 10.19, d.f.$_1$, $P = 0.001$), largely reflecting a delayed response in the May diatom treatment: the intercept of this treatment was lower than those of the diatom treatment in October ($t = 3.52$, d.f.$_{20}$, $P = 0.002$) and the faecal pellet treatment in May ($t = -4.14$, d.f.$_{20}$, $P < 0.001$). The SCOC rates in May and October differed significantly (time × month interaction; L. ratio = 5.98, d.f.$_1$, $P = 0.015$; Figure 1 and Supplementary Table S2), with rates of 1.98 and 1.59 mmol m^{-2} day^{-1} respectively. They were not affected by resource quality (time × treatment interaction; L. ratio = 3.75, d.f.$_2$, $P = 0.153$).

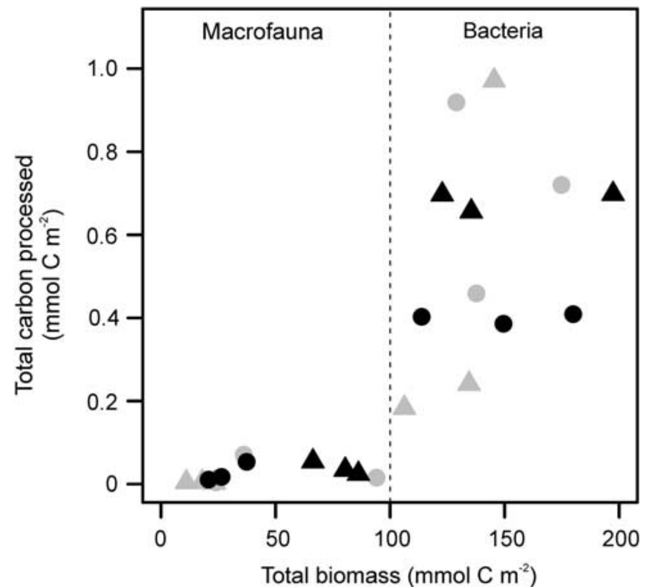

Figure 2 Benthic biomass and carbon uptake. Triangles and circles represent data from May and October, respectively; grey and black fills denote the faecal pellet and diatom treatments, respectively.

Organismal response

Benthic bacteria dominated the macrofauna in terms of biomass and carbon uptake in all of our experiments (Figure 2 and Table 1). The majority of substrate-derived carbon incorporated into PLFAs occurred in the generic bacterial biomarkers i15:0, a15:0 and i16:0. Much lower and relatively uniform uptake of tracer carbon was also apparent in the PLFAs a17:0, 12 Me16:0, 17:1ω8c, 17:0cy, 17:0,

19:1ω8 and 19:0cy (Supplementary Table S3). Carbon-specific uptake rates by the two groups (bacteria and macrofauna) in May were significantly different (group ID; $F=26.83$, $d.f._{1,10}$, $P<0.001$; Figure 2) but treatment effects were not apparent (treatment; $F = 1.13$, $d.f._{1,9}$, $P=0.315$). A marginal treatment × group ID interaction was present in October ($F = 6.78$, $d.f._{1,8}$, $P=0.031$), although subsequent examination did not reveal any significant intragroup treatment effects (Tukey's honest significant different test, $P_{adj}>0.1$ in both cases). Continued model selection demonstrated that carbon-specific uptake rates of the two groups in October were different (group ID; $F = 45.80$, $d.f._{1,10}$, $P<0.001$) but not affected by treatment ($F=0.44$, $d.f._{1,9}$, $P=0.523$). The distribution of added ^{13}C incorporated into the individual PLFAs was affected by a marginally significant treatment × month interaction (perMANOVA: $F=4.60$, $P=0.061$; Supplementary Table S4). In contrast, the relative compositions of the bacterial and archaeal communities at the end of our experiments (Supplementary Figure S4) were not affected by treatment, month or the interaction between these factors (perMANOVA, $F\leqslant 5.78$, $P\geqslant 0.482$ in all cases; Supplementary Table S4); they remained constant across all treatment–month combinations. Estimated values of BGE in May and October are presented in Table 1.

Discussion

Deep-sea benthic organisms, from bacteria to large metazoans, are well known to respond rapidly to pulses of organic matter (Gooday and Turley, 1990). Numerous studies have used ^{13}C-labelled diatoms to quantify the rates and pathways of carbon cycling at the deep seabed (see, for example, Witte *et al*., 2003; Moodley *et al*., 2005; Woulds *et al*., 2007; Gontikaki *et al*., 2011a, b), and there is a growing appreciation that the biochemical composition of organic matter may further influence the benthic response (Wolff *et al*., 2011). Our study quantitatively demonstrates that resource quality exerts a profound and largely unexplored effect on the rate at which the constituent organic carbon is mineralized in permanently cold, deep-sea sediments: diatom-derived carbon was respired >300% faster than that derived from faecal pellets. This observation is consistent with differences in carbon quality between these treatments. On the basis of C/total fatty acid ratios, diatoms contain ~300% more labile carbon. Our data demonstrate that bulk organic carbon flux is a poor predictor of mineralization rates in this environment. Phenomenological relationships between observed bulk carbon flux and deep-sea benthic function do not, therefore, necessitate cause and effect.

Mineralization rates in the different treatments did not differ between months, illustrating that the benthic community at our experimental location is capable of rapidly capitalizing on sporadic food inputs whenever they occur, irrespective of background levels of biological activity: the SCOC rate in May was significantly greater than that in October, likely reflecting seasonal activity patterns that relate to the productivity regime and hence food supply from the overlying waters (Ruhl *et al*., 2008). Resource-quality effects on SCOC were not discernable, which agrees well with previous studies in which relatively small quantities of organic carbon tracers have been introduced to deep-sea benthic communities (Moodley *et al*., 2005; Gontikaki *et al*., 2011b). Indeed, the observed SCOC rates were an order of magnitude greater than would be expected from the ^{13}C mineralization data alone, illustrating that the majority of oxygen consumption was fuelled by substrates other than those introduced experimentally.

Biomass and carbon uptake in all of our experiments were dominated by bacteria, in accordance with previous ^{13}C-labelled diatom addition experiments conducted on deep-sea benthic communities (see, for example, Moodley *et al*., 2005; Woulds *et al*., 2007; Gontikaki *et al*., 2011b). A notable exception to this pattern is the rapid macrofaunal response and retarded bacterial uptake of ^{13}C-tracer observed at 4850 m on the Porcupine Abyssal Plane, Northeast Atlantic, using an automated lander system (Witte *et al*., 2003). Rather than reflecting a unique response at the Porcupine Abyssal Plane, we suggest that this apparent discrepancy reflects the relatively large quantity of diatoms (83 mmol organic $C\,m^{-2}$) administered in that study; macrofauna outcompete bacteria when accessing concentrated patches of organic resources and vice versa (van Nugteren *et al*., 2009). Contrary to our expectations, we found no evidence to suggest that substrate uptake into bacterial or macrofaunal biomass was affected by resource quality. Indeed, the observed patterns of mineralization and growth resulted in BGE being consistently lower in the diatom treatments. It is possible that treatment effects on macrofaunal growth may not have been discernable over the relatively short duration of our experiments as our methods cannot distinguish between ingested and assimilated substrates. However, the same does not hold for bacteria. Tracer incorporation into bacterial PLFAs is a highly sensitive and targeted technique for determining their rates of biosynthesis (Boschker and Middelburg, 2002); deep-sea benthic bacteria have previously been shown to incorporate diatom-derived carbon into their PLFAs within 24 h (Moodley *et al*., 2005). Treatment effects on bacterial growth, if apparent, should therefore have been readily discernable in our experiments after 6 days of incubation. A previous, 6-day study at the same experimental location documented a temporal shift from high to low BGE following the addition of diatoms. This was attributed to a switch from labile to refractory respiratory substrates as the former

became exhausted (Gontikaki *et al*., 2011b). However, this interpretation is not consistent with the relatively high BGE observed in our faecal pellet treatment. Differences in BGE in our experiments could plausibly reflect the development of metabolically distinct microbial communities in the different treatments and months. Some support for this hypothesis comes from the data on ^{13}C uptake into individual PLFAs, which were affected by a marginally significant ($P = 0.061$) treatment × month interaction. However, this interpretation is not supported by our PCR-DGGE analyses (Supplementary Figure S4) and is further refuted by the statistically consistent mineralization rates in May and October. It is possible that treatment effects on carbon mineralization and hence BGE were attributable to a differential archaeal response, although their negligible contributions ($<<5\%$) to the prokaryotic communities of surficial sediments (Sahm and Berninger, 1998; Vetriani *et al*., 1999; Lipp *et al*., 2008) indicates that this is unlikely. Furthermore, we found no evidence to suggest that the archaeal community composition changed in response to our different experimental treatments (Supplementary Figure S4). We therefore suggest a more parsimonious explanation is that differences in carbon quality between the treatments caused an uncoupling of bacterial catabolic and anabolic processes. Bacteria solubilize and catabolize available substrates at maximal rates, independent of their specific requirements (del Giorgio and Cole, 1998; Vetter *et al*., 1998). This results in reduced BGE when growth is constrained by factors such as temperature and nutrient supply, rather than organic carbon (energy) (del Giorgio and Cole, 1998). Previous studies have demonstrated that the growth of barophilic bacteria isolated from depths $\geqslant 3584$ m is affected by temperature, pressure and substrate supply (Yayanos, 1986; Wirsen and Molyneaux, 1999; Eloe *et al*., 2011), with the production of new biomass declining rapidly as temperatures drop below 0 °C (Yayanos, 1986). Experiments conducted on barotolerant microbial communities from much shallower ($\leqslant 2003$ m), Arctic sediments have demonstrated that the effects of labile substrate supply on bacterial catabolic activities predominate over those of temperature, salinity and pressure (Meyer-Reil and Köster, 1992; Arnosti *et al*., 1998). We therefore contend that the counterintuitive effect of resource quality on BGE demonstrates that bacterial community respiration in our sub-zero experiments was substrate limited, whereas their PLFA biosynthesis (growth) was not. This interpretation may also explain why deep-sea benthic BGE apparently decreases when offered increased quantities of organic substrates (Bühring *et al*., 2006).

We propose that bacterial biosynthetic pathways in our near-freezing experiments were temperature limited, a mechanism frequently invoked to explain the production of bacterioplankton communities outside of seasonal temperature maxima (Shiah and Ducklow, 1994; Apple *et al*., 2006). The notion that respiration and growth were substrate and temperature limited, respectively, is consistent with the understanding that these factors influence the growth efficiencies and production of heterotrophic bacteria to differing extents (Arnosti *et al*., 1998; Pomeroy and Wiebe, 2001; Apple *et al*., 2006). This, in turn, reflects that respiration and growth are the summation of numerous, discrete enzymatic pathways, all of which respond differently to temperature (Clarke, 1991). Similar, carbon-quality effects at a low temperature have previously been demonstrated in a study examining microbial activity in the accreted ice of Lake Vostok: melt water samples enriched with qualitatively different carbon substrates and incubated at 1 °C revealed clear substrate-identity effects on mineralization but not growth; substrate effects on growth were, however, apparent when incubated at 23 °C (Karl *et al*., 1999). The recent observation that the effects of food stoichiometry on growth rate diminish as an organism approaches its lower thermal limit (Persson *et al*., 2011) lends further support to our data interpretation. Nevertheless, considering the similar bulk elemental composition of the diatoms and faecal pellets (C/N ratio $= 6.3 \pm 0.2$ and 8.2 ± 0.1 by atoms, respectively), we cannot conclusively exclude the possibility of nitrogen-limited bacterial growth (Boetius and Lochte, 1996) in both treatments and stoichiometric regulation of the excess, labile organic carbon in the diatom treatment via futile metabolic cycles (del Giorgio and Cole, 1998). It is unlikely that that enhanced bacterial growth was apparent but not discernable owing to rapid protistan grazing (Boetius and Lochte, 1996), although we cannot dismiss losses due to viral lysis (Danovaro *et al*., 2008).

We have shown that the residence time of organic carbon at the deep seabed is profoundly affected by the lability of the vector within which it is delivered, and assert that the respiration and growth of the resident microbial communities are limited by the supply of labile substrates and temperature, respectively. It follows that future climate-driven changes in the composition of marine phytoplankton communities (Edwards and Richardson, 2004), and hence the biochemical makeup of exported organic matter, have the potential to impact upon the balance between carbon mineralization and burial in the largest habitat on Earth. A more complete understanding of the physiology and ecology of deep-sea organisms is required before the processes regulating their growth, and ultimately the fate of elements in this environment, can be fully appreciated.

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Author contributions

DJM designed and performed the research; DJM and BT analysed the samples; DJM and AFZ analysed the data; DJM wrote the paper and all co-authors commented; SH, DJM and UFMW co-authored the original grant.

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