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Key role of bacteria in the short-term cycling of carbon at the abyssal seafloor in a low particulate organic carbon flux region of the eastern Pacific Ocean

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Abstract

The cycling of carbon (C) by benthic organisms is a key ecosystem function in the deep sea. Pulse-chase experiments are designed to quantify this process, yet few studies have been carried out using abyssal (3500–6000 m) sediments and only a handful of studies have been undertaken in situ. We undertook eight in situ pulse-chase experiments in three abyssal strata (4050–4200 m water depth) separated by tens to hundreds of kilometers in the eastern Clarion-Clipperton Fracture Zone (CCFZ). These experiments demonstrated that benthic bacteria dominated the consumption of phytodetritus over short (~ 1.5 d) time scales, with metazoan macrofauna playing a minor role. These results contrast with the only other comparable in situ abyssal study, where macrofauna dominated phytodetritus assimilation over short (2.5 d) time scales in the eutrophic NE Atlantic. We also demonstrated that benthic bacteria were capable of converting dissolved inorganic C into biomass and showed that this process can occur at rates that are as high as the bacterial assimilation of algal-derived organic C. This demonstrates the potential importance of inorganic C uptake to abyssal ecosystems in this region. It also alludes to the possibility that some of the C incorporation by bacteria in our algal-addition studies may have resulted from the incorporation of labeled dissolved inorganic carbon initially respired by other unstudied organisms. Our findings reveal the key importance of benthic bacteria in the short-term cycling of C in abyssal habitats in the eastern CCFZ and provide important information on benthic ecosystem functioning in an area targeted for commercial-scale, deep-sea mining activities.

The abyssal seafloor (3500–6000 m) covers 54% of the Earth's surface (Smith et al. 2008*a*), making it the largest seafloor habitat on the planet. With the exception of abyssal reducing ecosystems (Pedersen et al. 2010), metabolic processes occurring in the vast majority of the abyssal seafloor are fueled by the supply of photosynthetically derived organic matter from the euphotic zone (Smith et al. 2008*a*, 2009). The flux of particulate organic carbon (POC) to the abyss can be episodic (Billett et al. 1983; Lampitt and Antia 1997; Lutz et al. 2007), with bloom-derived pulses of phytodetritus providing the dominant source of C and energy to abyssal seafloor communities (Graf 1989; Pfannkuche 1993; Smith and Kaufmann 1999). Since the late 1970s, a variety of studies have shown a tight coupling between the flux of POC and a number of benthic faunal characteristics and functions,

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including sediment community respiration (Smith and Kaufmann 1999; Smith et al. 2001, 2009), macrofauna (hereafter referred to as organisms > 300 μm) and microbial abundance, biomass and biodiversity (Smith et al. 2008*a*; Ruhl et al. 2008; Wei et al. 2010; Wooley et al. 2016), and bioturbation depth and intensity (Smith and Rabouille 2002; Smith et al. 2008*a*).

Benthic POC cycling is an important ecosystem function and service in the deep sea as it plays a key role in influencing the amount of C that is ultimately sequestered in seafloor sediments (Dunne et al. 2007; Thurber et al. 2014). To date, the role of POC in deep-sea food webs has largely been explored through gut pigment (Drazen et al. 1998; Wigham et al. 2003; Wigham et al. 2008), fatty acid (Hudson et al. 2004), and natural-abundance stable-isotope signature analyses (Iken et al. 2001; Drazen et al. 2008). However, these methods do not allow the quantification of the rates of phytodetritus C cycling through the benthos to be determined. Other studies have used food web modeling (Rowe et al. 2008; van Oevelen et al. 2012) or introduced stable or radioactive isotope labeled substrates to quantify assimilation of organic matter by different sediment-dwelling organisms (Rowe and Deming 1985; Cahet and Sibuet 1986; Middelburg 2014, 2018). These experiments, referred to as pulse-chase studies, allow the most important metazoan and microbial groups involved in C cycling to be identified and rates of C turnover to be assessed (Boschker and Middelburg 1997; Boschker et al. 2014; Middelburg 2014, 2018). Microbial community structure is known to fluctuate with energy availability (Kanzog et al. 2009; Bienhold et al. 2012; Hoffmann et al. 2017), and can change in cold (0°C) deep-sea environments within periods as short as 1 week when exposed to fresh organic material (Kanzog et al. 2009). Hence, pulse-chase experiments can be supplemented with methodologies designed to detect changes in microbial community composition (e.g., 16S rRNA analysis) resulting from the addition of organic matter. To date, only five in situ pulse-chase experiments have been carried out at the abyssal seafloor, and all have been conducted under relatively eutrophic waters masses, including the NE Pacific (Sweetman and Witte 2008; Enge et al. 2011; Jeffreys et al. 2013), SE Pacific (Stratmann et al. 2018), and NE Atlantic (Witte et al. 2003*a*). However, only Witte et al. (2003*a*) quantified the collective response of bacteria and macrofauna to a fresh phytodetritus input event in an nondisturbed abyssal setting. Moreover, in contrast to shipboard studies that have shown that bacteria are important regulators of short-term degradation of organic material in the deep sea (Rowe and Deming 1985; Lochte and Turley 1988; Boetius and Lochte 1996; Kanzog et al. 2009; Hoffmann et al. 2017), the study by Witte et al. (2003*a*) identified macrofauna as the dominant group of organisms responsible for the initial stages of organic matter remineralisation. Such conflicting results highlight our limited understanding of the role of different organism size classes in the degradation of POC at the abyssal seafloor.

Direct measurements of POC supply and consumption have revealed that the input of POC is, at times, not sufficient to fulfill the C demands of abyssal ecosystems (Smith and Kaufmann 1999; Smith et al. 2002, 2013, 2014). While episodic food falls (e.g., gelatinous zooplankton carcasses) and aggregates can explain much of the discrepancy between POC supply and demand in areas close to the continental margin (NE Pacific; Smith et al. 2013, 2014), it is possible that organic C produced in situ through inorganic C fixation may provide another C source to deep-sea communities (Middelburg 2011; Molari et al. 2013). Christensen and Rowe (1984) and Middelburg (2011) both suggested that inorganic C fixation processes (e.g., nitrification) may produce organic material for benthic communities, but the rates of inorganic C fixation in deep-sea sediments were estimated to be relatively minor compared to incoming POC fluxes (Christensen and Rowe 1984) and shallower environments (Middelburg 2011). Brunnegard et al. (2004), however, showed that nitrification was the most important N-cycling process in sediments of the abyssal NE Atlantic accounting for 65% of the incoming N flux. More recently, a study in the abyssal NE Atlantic and Mediterranean Sea showed that deep-sea benthic prokaryotic communities could incorporate inorganic C into their biomass at very high rates (equivalent to approximately 20% of the heterotrophic C-production) through chemoautotrophic or mixotrophic processes (Molari et al. 2013). However, this study did not assess the transfer of fixed microbial C to metazoan consumers, and was undertaken on abyssal samples incubated at atmospheric pressure, which may have led to pressure-related artifacts (e.g., an increase in the activity of nonpiezophilic microbes). We know of no in situ study that has quantified inorganic C fixation at the abyssal seafloor or assessed C transfer through the metazoan community. Thus, the importance of this process in abyssal ecosystems is still unclear.

The abyssal seafloor underlying the eastern equatorial Pacific receives an annual POC flux equivalent to ~ 20% of the flux at the equator (e.g., 0.4 g C m^{-2} yr^{-1} at 10°N vs. 2.2 g C m^{-2} yr^{-1} at the equator; Smith et al. 1997; Smith and Demopoulos 2003). The low POC flux to the seabed here allows the precipitation of manganese and iron oxides from the water column and sediment pore waters, forming polymetallic nodules at the seafloor that are rich in nickel and copper (e.g., exceeding 1.6% by weight; International Seabed Authority 2010*a*), as well as other commercially important metals (e.g., cobalt, molybdenum, and lithium) (International Seabed Authority 2010*a*). In the Clarion-Clipperton Fracture Zone (CCFZ) of the equatorial Pacific Ocean, nodules are found over an area of ~ 5 × 10^6 km^2 at weights ranging from < 1 to > 35 kg m^{-2} (International Seabed Authority 2010*a*). Because of the sheer volume of nodules in the CCFZ and the increasing difficulty of extracting the important metals they contain from land-based mines, the CCFZ has become a prime area of interest for future seabed mining. Currently, 16 contractors have claimed exploration rights in this region, with

contractors conducting surveys to gather baseline data on biodiversity and genetic connectivity across mine claim areas. If mining proceeds in the CCFZ, nodule extraction will significantly disturb the seafloor environment (e.g., 600–800 km^2 of seabed per mining operation per year affected by direct nodule removal and sediment plumes; Smith et al. 2008*b*; Levin et al. 2016). Thus, this represents a serious environmental issue, heightened by the lack of present knowledge about abyssal seafloor biology and ecology in this region.

Because C cycling and eventual C sequestration are key ecosystem functions and services in the deep sea (Thurber et al. 2014), the fate of POC should be included in baseline surveys, impact assessments, and later monitoring surveys at mine claim sites, so mining-related changes in this important ecosystem function can be detected. We undertook in situ pulse-chase studies in the eastern CCFZ to quantify C flow through benthic bacteria and macrofauna, determining how the abyssal benthos processes fresh POC beneath mesotrophic waters and whether seafloor processes in the CCFZ differ relative to other deep-sea environments. We also assessed the amount of inorganic C fixation that occurs in the seafloor of the CCFZ, and possible short-term changes in microbial community composition following the addition of organic and inorganic C. Our study focused on two different locations in the UK Seabed Resources UK1 claim area, and a location in the Ocean Minerals of Singapore claim area, in the eastern end of the CCFZ (Fig. 1). The most northerly and southerly sites were separated by over 200 km, which allowed us to address regional patterns in benthic processes. Specifically, we tested the following hypotheses. (1) Macrofauna dominate the short-term assimilation of phytodetritus at the abyssal seafloor of the eastern CCFZ. (2) Abyssal benthic bacteria actively assimilate inorganic C into their biomass in the eastern CCFZ. (3) Microbial community composition changes significantly over short-term time scales following the addition of organic and inorganic C.

Materials and methods

Study sites

In situ experiments were carried out in the eastern CCFZ (Fig. 1) in two separate 30 x 30 km blocks (Strata A and B) of the UK1 claim area, as well as s 30 × 30 km block at the southerly end of the Ocean Minerals of Singapore (OMS) claim area 1 (Fig. 1). The UK1 strata (ca. 4100–4200 m water depth, bottom temperature approximately 1.5°C) were located at the northern and southern end of the UK1 contract area. The more northerly UK1 Stratum A was visited in October 2013 during the AB01 cruise aboard the R/V *Melville*, while the southern UK1 Stratum B and OMS stratum (ca. 4040 m water depth, bottom temperature approximately 1.5°C) were visited in February/March 2015 during the AB02 cruise aboard the R/V *Thomas G. Thompson*. The seafloor in the UK1 and OMS areas is characterized by abundant polymetallic nodules and silty clay sediments (radiolarian oozes).

Labeled algae preparation for phytodetritus-addition experiments

An axenic clone of the diatom *Phaeodactylum* sp. was used as an isotopically labeled food source in our phytodetritus-addition experiments. This species was chosen as a suitable food source because it belongs to a widely distributed diatom genus that occurs throughout the Pacific Ocean (De Martino et al. 2007) and possibly sinks to the seafloor in phytodetrital aggregates, although this requires confirmation. The phytoplankton culture was grown in F/2 algal medium in artificial seawater (Guillard 1975; Grasshoff et al. 1999) and isotopically labeled by replacing 50% of ^{12}C bicarbonate in the culture

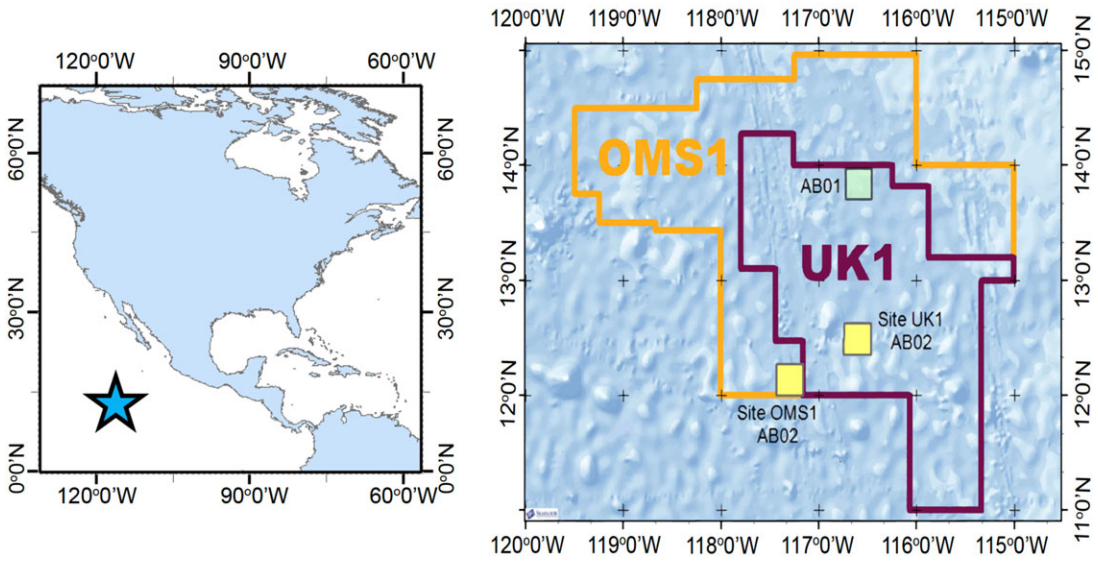

Fig. 1. Location of the UK1 and OMS study areas in the polymetallic nodule province of the eastern CCFZ.

medium with $NaH^{13}CO_3$. To harvest the algae, the cultures were filtered onto a 0.45-μm filter (cellulose acetate) and then rinsed with unlabeled F/2 medium to remove excess ^{13}C-labeled bicarbonate. The algal material was then centrifuged (404 g x 5 min) and washed with unlabeled F/2 medium five times, then frozen and freeze-dried. Isotope ratio mass spectrometry (IRMS) analyses on the algal biomass revealed that the diatom cells had a mass C content of 18.6% and a ^{13}C content of 37.8 atom %. This algal material was fresh and was likely significantly less degraded than the phytoplankton detritus that is typically deposited at the abyssal seafloor. As a result, the overall benthic responses observed maybe different from responses to naturally occurring phytodetritus at the seafloor (Aspetsberger et al. 2007). However, short-term (1.5–4 d) labeling studies testing C cycling processes using identically grown and harvested algal sources have been carried out in the deep sea (Witte et al. 2003*a*; Sweetman and Witte 2008; Jeffreys et al. 2013; Stratmann et al. 2018), allowing direct comparisons to be made with our data. Moreover, fresh algal cells, including diatoms, have been found at abyssal depths where they are likely available to benthic organisms (Lochte and Turley 1988; Agusti et al. 2015).

Benthic lander deployments

Phytodetritus-addition experiments

In situ experiments were undertaken with a deep-sea benthic chamber lander (KUM GmbH). Four lander experiments were carried out in the UK1 Stratum A (2013), three in the UK1 Stratum B (2015), and two in the OMS Stratum (2015) (Table 1). One additional lander deployment (without labeled algae, and hereafter referred to as a background deployment) was carried out in 2013 to collect sediment samples for background isotope measurements. A background deployment was also undertaken in 2015 to measure background sediment community oxygen consumption (SCOC) rates.

The lander carried three independent, autonomous, square benthic chambers (484 cm^2) separated by approximately 0.5 m. Immediately before each algal-addition lander deployment, approximately 47 mg of labile *Phaeodactylum* sp. culture (equivalent to approximately 180 mg C m^{-2} or 18% of the annual POC flux; Amon et al. 2016) were hydrated with cold (4°C), filtered (0.2-μm) seawater and placed in an algal injector mounted in the lid of each benthic chamber. The lander was then deployed and descended to the seafloor in approximately 2 h. After landing at the seafloor, the benthic chambers were slowly driven into the sediment by chamber motors that were commanded by an on board computer. Two hours after its arrival at the seafloor, the ^{13}C labeled algal biomass was injected into each chamber and homogeneously distributed by stirrers mounted on the chamber lids. Approximately 1 min after algal injection, the chamber stirrers were switched off for 1.5 h to allow the labeled algae to sink to the sediment surface (sinking rates were determined from previous laboratory experiments). Stirrers were then turned on, and the chamber waters gently mixed (60 rpm) for the duration of the 36 h (AB01) and 34 h (AB02) in situ incubations. Contros Hydroflash® oxygen optodes were mounted on the lid of each chamber to measure SCOC rates at the seafloor during the AB02 expedition. Unfortunately, SCOC could only be measured in a single algal-addition and single background experiment due to two malfunctioning optodes. At the end of the experiments, the chambers were sealed and the lander was acoustically recalled from the seabed, arriving at the sea surface approximately 1 h later. Once the lander was back aboard the ship, the depth of the overlying water in each chamber was measured to calculate SCOC rates, the top water was siphoned off and filtered through a 63-μm mesh, and the sediment and nodules enclosed in each chamber were recovered from 0 to 2 cm and 2 to 5 cm deep sediment horizons. If nodules were found to cross the 0–2 cm or 2–5 cm sediment boundary within a chamber, they were classified as coming from the sediment layer where more than 50% of the nodule was present. Sediments from each vertical stratum were homogenized by mixing thoroughly with a spackle knife. From this homogenate, approximately 40 mL of sediment was removed and placed in 50 mL plastic vials for bacterial

Table 1. Description of sampling sites and experiments undertaken during the AB01 and AB02 cruises.

Date	Cruise	Study site	Depth (m)	Lander deployment	Substrate added	Number of labeled algae experiments and chamber (Ch.) number(s)	Number of labeled DIC experiments and chamber (Ch.) number
18 Oct. 2013	AB01	UK1 (stratum A)	4120	RL1	*Phaeodactylum* sp.	1 (Ch.2)	0
21 Oct. 2013	AB01	UK1 (stratum A)	4102	RL2	*Phaeodactylum* sp.	3 (Ch. 1–3)	0
20 Feb. 2015	AB02	UK1 (stratum B)	4149	RL3	*Phaeodactylum* sp.	1 (Ch. 1)	0
26 Feb. 2015	AB02	OMS	4044	RL4	*Phaeodactylum* sp.	2 (Ch. 1,3)	0
06 Mar. 2015	AB02	UK1 (stratum B)	4146	RL5	*Phaeodactylum* sp. or bicarbonate	1 (Ch. 1)	1 (Ch. 2)
13 Mar. 2015	AB02	OMS	4037	RL6	Bicarbonate	0	1 (Ch. 2)
Total						8	2

phospholipid-derived fatty acid (PLFA) analysis and frozen at −20°C. Samples (5 mL) for determining microbial community structure were collected from pooled 0–5 cm sediment samples using a sterile 20 mL syringe, and frozen at −80°C. The remaining sediment was sieved for macrofauna using a 300-μm sieve and 4°C, 0.2-μm-filtered seawater. Polymetallic nodules from each sediment layer were washed with a 300-μm sieve to remove any attached fauna (e.g., hydrozoans/nodule crevice-dwelling animals, such as nematodes), and the nodule volume was later calculated by water displacement. Sieved macrofauna from the polymetallic nodule residues (including any nodule biota) were pooled with the macrofaunal sample from the same sediment layer, and preserved in 4% buffered formaldehyde seawater solution. Macrofauna and bacteria from the background lander deployment from the AB01 expedition were sampled using the same procedures as above.

Dissolved inorganic carbon assimilation experiments

To quantify the assimilation of dissolved inorganic carbon (DIC) by benthic bacteria, and its subsequent transfer to the metazoan macrofaunal community, seafloor sediments were incubated in situ in two benthic experiments with 46 mL of 20 mM DI^{13}C-labeled seawater (1.7 g L^{-1} of 99 atom % NaH$^{13}CO_3$) at one UK1 Stratum B and one OMS stratum site during the AB02 cruise (Table 1). The DI^{13}C solution was injected into each chamber approximately 2 h after the lander reached the seafloor from a 60-mL plastic syringe mounted in a syringe injection system located on the outside of the benthic chambers. The sediments were then incubated for 34 h before the chambers and enclosed sediments were retrieved by the lander, and the lander was recalled to the surface. All sediments were sampled in the same way as described above.

Sample processing and analysis of microbial community structure and diversity

Short-term changes in microbial community structure and diversity following the addition of the labeled phytodetritus or DIC were assessed by comparing microbial community structure in the experimental chambers to measurements made in background sediments (0–5 cm) collected using an OSIL Bowers and Connolly mega-corer (with 12 x 10-cm diameter tubes) in the same study area. DNA was extracted from all sediments using the FastDNA Spin Kit for Soil (MP Biomedicals) as previously described (Shulse et al. 2017). For the samples collected during the AB01 cruise, the V4 region of the 16S rRNA gene was polymerase chain reaction (PCR)-amplified using the oligonucleotide primer pair F515/R806, which included the Illumina flowcell adapter sequences (Caporaso et al. 2011). For experiments performed during the AB02 cruise, amplicon processing was performed as described in Lindh et al. (2015) using primers 341F and 805R targeting the V3-V4 region of the 16S rRNA gene (Herlemann et al. 2011). Sequencing of the PCR products from all experiments was performed on an Illumina MiSeq at the Hawaii Institute of Marine Biology Genetics Core Facility. Illumina MiSeq sequences obtained from the AB01 cruise experiment were processed as described in Caporaso et al. (2011), while data obtained from the AB02 experiments were processed as described in Lindh et al. (2015). Operational Taxonomic Units (OTUs) from all experiments were delineated at 97% 16S rRNA gene identity. DNA sequences have been deposited in the National Center for Biotechnology Information Sequence Read Archive under accession numbers SRP057408 and SRP078396.

Isotopic sample preparation and analysis

In a shore-based laboratory, frozen sediment samples for PLFA analysis were freeze dried and subsequently ground with a mortar and pestle. Lipids were then extracted from approximately 3 g of dried sediment using a Bligh and Dyer extraction procedure in which lipids were sequentially isolated by rinsing on a silicic acid column with chloroform, acetone, and methanol (Sweetman et al. 2010, 2014, 2016). The lipid extract was then derivatized to volatile fatty-acid methyl esters and measured by gas chromatography IRMS (GC-IRMS) for PLFA concentration and delta ^{13}C (δ^{13}C) signatures. The C-isotope ratios were corrected for the single methyl group inserted during derivatization. Bacterial biomass in the algal-addition and DIC-addition experiments was calculated from the weighted-average PLFA (iC$_{15:0}$, aiC$_{15:0}$) concentration (μmol) mL^{-1} sediment/(a x b), where a is the average PLFA concentration in bacteria (for this study, we assumed 0.056 g C PLFA g^{-1} biomass; Brinch-Iversen and King 1990) and b is the average fraction-specific bacterial PLFA encountered in sediment dominated by bacteria (0.07; calculated after Rajendran et al. 1993, 1994). The prefixes "*i*" and "*ai*" refer to "*iso*" and "*antiso*," respectively. Areal estimates of bacterial biomass (mg C m^{-2}) were then corrected for the fraction of nodule-free sediment.

Metazoan macrofauna were removed from formaldehyde solution by gently sieving on a 300-μm mesh in cool, filtered seawater in the laboratory. The sieve residue was then transferred to a petri-dish containing cold, filtered seawater, and macrofauna were picked out and identified under a dissecting microscope. Macrofauna from the AB01 expedition were grouped into polychaetes, crustaceans, and molluscs. In the AB02 samples, polychaetes were identified to family, while crustaceans and other taxa were identified to order or major taxon. Separate sorting utensils were used for background and enriched-isotope samples to avoid contamination with enriched ^{13}C. Due to insufficient biomass being available for measuring isotope signatures of specific animals, macrofauna were pooled into total macrofauna for the AB01 samples, and polychaete family, crustacean order, and major taxa for the AB02 samples. Organisms were washed to remove attached organic debris in cooled, filtered seawater before being pooled in silver cups, frozen, and freeze dried. All freeze-dried cups were then acidified using the methods of Sweetman et al. (2010, 2014, 2016) and dried at 45°C for 7 d prior to isotopic analysis.

The isotopic ratios (^{13}C : ^{12}C) and biomass of macrofaunal organisms from the algae- and DIC-addition experiments were measured using a Thermo Flash EA 1112 elemental analyzer (EA; Thermo Fisher Scientific) coupled to a DELTA V Advantage IRMS (Thermo Fisher Scientific). Total incorporation of ^{13}C by macrofauna (mg ^{13}C m^{-2}) from the algal-addition experiments was calculated following the methods of Sweetman et al. (2010, 2014, 2016). Total bacterial assimilation (mg ^{13}C m^{-2}) of ^{13}C from the algal-addition experiments was calculated from label incorporation into the bacterial fatty acids ($iC_{15:0}$, $aiC_{15:0}$) following the methods of Sweetman et al. (2010, 2014, 2016) using an average fraction-specific bacterial PLFA encountered in sediment dominated by bacteria (0.07; calculated after Rajendran et al. 1993, 1994). The ^{13}C-assimilation values (mg ^{13}C m^{-2}) for macrofauna and bacteria were then converted to daily C-assimilation rates (mg C m^{-2} d^{-1}) by accounting for the fractional abundance of ^{13}C in the added algae as follows: C assimilation = ^{13}C incorporated (mg ^{13}C m^{-2})/fractional abundance of ^{13}C in algae, and dividing by 1.5 (AB01 experiments) or 1.42 (AB02 experiments) depending on the length of the experiment. Macrofaunal biomass (mg C m^{-2}) and daily C assimilation rates (mg C m^{-2} d^{-1}) for bacteria and macrofauna for each depth interval were then corrected for the fraction of nodule-free sediment.

The uptake of $DI^{13}C$ into macrofauna biomass and bacterial PLFA was calculated by measuring specific uptake ($\Delta\delta^{13}$) of macrofauna and the bacterial PLFAs $iC_{15:0}$, $aiC_{15:0}$, $C_{16:1\omega7c}$, $iC_{17:1\omega7c}$, 10-Methyl $C_{16:0}$, $C_{17:1\omega8c}$, $C17:1_{\omega6c}$, and Cy-$C_{19:0}$. Specific uptake of bacterial PLFA was calculated as excess (above background): $\Delta\delta^{13}C = \delta^{13}C_{PLFA\ sample} - \delta^{13}C_{PLFA\ background}$. $\Delta\delta^{13}$-values of macrofauna were calculated as $\Delta\delta^{13}C = \delta^{13}C_{macrofauna\ sample} - \delta^{13}C_{macrofauna\ background}$. Positive $\Delta\delta^{13}C$ values indicated the incorporation of ^{13}C label into a specific bacterial PLFA and into macrofauna. Assimilation rates of $DI^{13}C$ by bacteria (mg ^{13}C m^{-2}) were calculated from label incorporation into the bacterial fatty acids $iC_{15:0}$, $aiC_{15:0}$, $C_{16:1\omega7c}$, $iC_{17:1\omega7c}$, 10-Methyl $C_{16:0}$, $C_{17:1\omega8c}$, $C17:1_{\omega6c}$, and Cy-$C_{19:0}$ following the methods of Sweetman et al. (2010, 2014, 2016), using an average fraction-specific bacterial PLFA encountered in sediment dominated by bacteria (0.43; calculated after Rajendran et al. 1993, 1994). To estimate total daily assimilation of DIC into bacterial biomass (mg C m^{-2} d^{-1}), we accounted for the fractional abundance of ^{13}C in the water phase overlying the sediment in each chamber as follows: C assimilation = ^{13}C incorporated (mg ^{13}C m^{-2})/fractional abundance of ^{13}C in water phase and dividing by 1.42. The fractional abundance of ^{13}C in the water phase ranged between 0.06 and 0.1, and was dependent on the volume of water within each chamber. It was calculated from the concentration (20 mM) and amount of ^{13}C label (99%) in the injected bicarbonate medium, and an average background DIC concentration at the seafloor of 2.4 mM, which was derived from four sites in the same locality as our AB02 study sites using the World Ocean Database and Ocean Data View (Version 4).

Data analysis

All data analysis was carried out in R. Prior to statistical analysis, data were checked for normality and heteroscedasticity. Data were transformed, when necessary, to meet parametric assumptions. For datasets meeting parametric assumptions, differences in biomass and daily C-assimilation rates were tested using a two-way ANOVA with organism group (macrofauna and bacteria) and cruise as factors. Significant differences were explored further using Tukey HSD post hoc tests. If datasets failed to meet parametric assumptions after transformation, nonparametric Kruskal–Wallis tests were used, and significance tests were explored via nonparametric multiple comparison procedures. An alpha level of 0.05 was used as a criterion for statistical significance throughout. For microbial community structure and diversity analyses, all data were analyzed using the R-package "vegan." Prior to community structure analyses, the 16S rRNA gene dataset was normalized by dividing the observed number of sequence reads for each individual OTU by the total number of sequence reads in the entire sample to calculate relative abundances (i.e., percentages of total sequences) of OTUs. To compare community structure between samples, we calculated Bray-Curtis distances using the function "vegdist" in the R-package "vegan" and performed permuational ANOVA using the function "adonis" in "vegan." Graphical outputs for microbial data were made using the R package "ggplot2."

Results

Benthic community structure

Mean bacterial biomass measured in benthic chambers that were incubated with algae or DIC during the AB01 and AB02 campaigns were 530 ± 104 mg C m^{-2} (n = 4, standard error of the mean [SEM]) and 277 ± 52 mg C m^{-2} (n = 6, SEM), respectively (Fig. 2). Mean macrofaunal biomass was less in

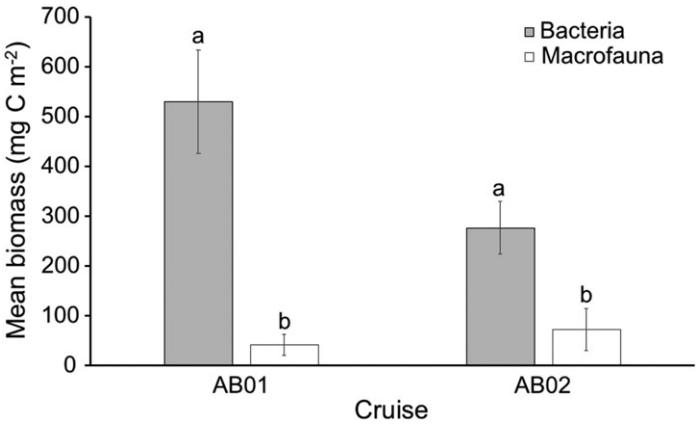

Fig. 2. Mean bacterial and metazoan macrofaunal biomass (mg C m^{-2}) in the 0–5 cm sediment layer of sediments collected during the AB01 and AB02 cruises. Different letters denote significant differences ($p < 0.05$) between means. Error bars denote ± 1 SEM (n = 4 for AB01 and n = 6 for AB02).

the AB01 (42.3 ± 20.7 mg C m^{-2}, n = 4, SEM) compared to the AB02 (72.3 ± 41.6 mg C m^{-2}, n = 6, SEM) samples. No identifiable xenophyophores were found in any of the macrofaunal samples from the AB01 and AB02 experiments, but fragments of potential nodule-dwelling fauna (hydrozoans) were recovered, and contributed 0.7 ± 0.6 mg C m^{-2} (n = 6, SEM) to mean macrofauna biomass in the AB02 samples. There was a statistically significant difference between mean macrofaunal and bacterial biomass in both sample sets (two-way ANOVA, $p < 0.001$; Fig. 2), with bacterial biomass being significantly greater than macrofaunal biomass. No difference was detected in the mean bacterial biomasses between the AB01 and AB02 studies. This was also true for mean macrofaunal biomass, despite the mean biomass of macrofauna in the AB02 study being heavily influenced by a single, large unidentified vermiform animal (233 mg C m^{-2}). Mean macrofaunal abundance in the AB01 and AB02 samples was 546 ± 51 (n = 4, SEM) and 313 ± 64 individuals (ind.) m^{-2} (n = 6, SEM), respectively, with the metazoan assemblage in the AB02 samples largely composed of crustaceans (46%) and polychaetes (26%). Of the crustacean fauna, tanaids (34%), harpacticoids (21%), and isopods (24%) contributed most to crustacean abundance, while the Nereidae (15%), Spionidae (14%), and unknown polychaetes (19%) contributed most to the polychaete fauna.

Phytodetritus carbon assimilation by the benthic community

Mean total (summed bacteria and macrofauna) daily C-assimilation rates were not significantly different (Welch two-sample t test, p = 0.14) between the AB01 (0.97 ± 0.18 mg C m^{-2} d^{-1}, n = 4, SEM) and AB02 experiments (0.60 ± 0.10 mg C m^{-2} d^{-1}, n = 4, SEM), although the statistical power of the test was low (due to between chamber variability being large). The pooled background $\delta^{13}C$ signature for abyssal macrofauna sampled during the AB01 cruise was −18.6‰. Druffel et al. (1998) showed that POC settling at a depth of 3450 m in the water column in the NE Pacific possessed a $\delta^{13}C$ signature of ~ −21‰. Assuming a $\delta^{13}C$ enrichment of 1‰ per trophic step (Fry and Sherr 1984), it seems plausible that phytodetritus was the main source of C to macrofauna in the AB01 samples, and possibly the AB02 samples as well given the relatively close proximity of the AB01 and AB02 study sites (Fig. 1). Mean $\delta^{13}C$ values for macrofauna from the algal-addition chambers for AB01 and AB02 were −10.0 ± 3.9‰ and −6.1 ± 10.1‰, respectively (n = 4, SEM), suggesting minimal uptake of ^{13}C-labeled phytodetritus. This was also reflected in mean daily C assimilation rates of macrofauna in the AB01 and AB02 experiments, namely 0.9 ± 0.4 x 10^{-3} mg C m^{-2} d^{-1} (n = 4, SEM) and 5.0 ± 4.0 x 10^{-3} mg C m^{-2} d^{-1} (n = 4, SEM), respectively (Table 2; Fig. 3). The single large vermiform animal had a $\delta^{13}C$ signature of −18.9‰ indicating no uptake of labeled phytodetritus. In contrast, mean daily C-assimilation rates for bacteria were several orders of magnitude higher at 0.97 ± 0.18 mg C m^{-2} d^{-1} (n = 4, SEM) and 0.59 ± 0.10 mg C m^{-2} d^{-1} (n = 4, SEM) for the AB01 and AB02 studies, respectively (Fig. 3). Mean daily C-assimilation rates by bacteria during both cruises were significantly greater (two-way ANOVA, $p < 0.001$) than daily macrofauna C-assimilation rates (Fig. 3) from the AB01 and AB02 cruises, but no significant cruise effect was identified, although the power of the statistical test was low. The dominant macrofaunal taxa involved in C assimilation in the AB02 experiments were nereid polychaetes, followed by ostracods, gastropods and tanaids (Table 2; Fig. 4). Subsurface and surface deposit-feeding polychaetes (e.g., orbinid and spionid polychaetes, respectively), as well as carnivores (e.g., pilargids and pisionids) and other omnivorous polychaetes (e.g., lumbrinerids) consumed little or no algal C (Table 2; Fig. 4). SCOC rates that were measured in a single background and algal-addition experiment during the AB02 expedition were 2.70 mg C m^{-2} d^{-1} (0.22 mmol O_2 m^{-2} d^{-1}) and 4.77 mg C m^{-2} d^{-1} (0.40 mmol O_2 m^{-2} d^{-1}), respectively (assuming a DIC: O_2 respiratory quotient of 1).

Evidence for chemoautotrophy at the abyssal seafloor

Positive $\Delta\delta^{13}$ values indicative of assimilation of $DI^{13}C$ were identified in six out of eight bacterial-specific PLFAs studied (Fig. 5). The highest $\Delta\delta^{13}$ values were detected in the bacterial PLFA $iC_{17:1\omega7c}$, 10-Methyl $C_{16:0}$, and $C_{17:1\omega8c}$ (Fig. 5). No ^{13}C uptake was detected in the PLFA $C_{17:1\omega6c}$ or Cy-$C_{19:0}$. $\Delta\delta^{13}$ values of bacteria-specific PLFA were generally higher in the 0–2 cm sediment layer compared to 2–5 cm sediment depth (Fig. 5). The mean $\Delta\delta^{13}$ values of macrofauna from the DIC-incubation experiments was −3.2 ± 0.2‰ (n = 2), indicating no transfer of labeled C from bacterial biomass into the metazoan macrofauna after 34 h. Mean daily incorporation of inorganic C into benthic bacteria was 1.20 ± 0.35 mg C m^{-2} d^{-1} (n = 2), which was 1.2 and 2.0 times greater than the mean daily bacterial assimilation rates of labeled algal C from the AB01 and AB02 cruises, respectively. A statistically significant difference was detected (Kruskal–Wallis, p = 0.0015) between median daily algal C-incorporation rates by fauna and bacteria (AB01 and AB02 data pooled) and daily inorganic C-incorporation rates by bacteria. Median daily inorganic C fixation by bacteria was significantly greater than median daily algal C incorporation by metazoan macrofauna, and median daily algal C incorporation by bacteria was significantly greater than median daily algal C incorporation by metazoan macrofauna. No significant difference was detected between median daily algal C or inorganic C assimilation into bacterial biomass.

Microbial community structure and diversity

Alpha-diversity was very stable for both the AB01 and AB02 experiments with an average Shannon–Weiner diversity index of 6.25 ± 0.11 (n = 5, standard deviation [SD]) and 6.65 ± 0.08 (n = 14, SD), respectively. Analysis of microbial community composition by Permutational ANOVA

Table 2. Delta ^{13}C (‰) values and daily C-uptake rates ($\times 10^{-3}$ mg C m^{-2} d^{-1}) of macrofauna from the AB01 and AB02 experiments.

	Taxa	Tracer added	$\delta^{13}C$ (‰)	C-uptake ($\times 10^{-3}$ mg C m^{-2} d^{-1})
AB01 experiment				
Background (natural abundance) sample	Pooled macrofauna	—	−18.58	—
RL4 Ch. 2 (0–2cm)	Pooled macrofauna	^{13}C *Phaeodactylum* sp.	−3.19	0.329
RL4 Ch. 2 (2–5cm)	Pooled macrofauna	^{13}C *Phaeodactylum* sp.	−18.63	0.000
RL5 Ch. 1 (0–2cm)	Pooled macrofauna	^{13}C *Phaeodactylum* sp.	−18.88	0.000
RL5 Ch. 1 (2–5cm)	Pooled macrofauna	^{13}C *Phaeodactylum* sp.	−18.33	0.119
RL5 Ch. 2 (0–2cm)	Pooled macrofauna	^{13}C *Phaeodactylum* sp.	−4.24	0.210
RL5 Ch. 2 (2–5cm)	Pooled macrofauna	^{13}C *Phaeodactylum* sp.	−17.13	1.783
RL5 Ch. 3 (0–2cm)	Pooled macrofauna	^{13}C *Phaeodactylum* sp.	19.60	1.139
RL5 Ch. 3 (2–5cm)	Pooled macrofauna	^{13}C *Phaeodactylum* sp.	−19.24	0.000
AB02 experiment				
RL1 Ch. 1 (2–5cm)	Dorvelleidae	^{13}C *Phaeodactylum* sp.	−17.53	0.053
RL1 Ch. 1 (0–2cm)	Lumbrineridae	^{13}C *Phaeodactylum* sp.	−15.75	0.023
RL1 Ch. 1 (2–5cm)	Orbiniidae	^{13}C *Phaeodactylum* sp.	−18.80	0.000
RL1 Ch. 1 (2–5cm)	Pilargiidae	^{13}C *Phaeodactylum* sp.	−17.95	0.005
RL1 Ch. 1 (2–5cm)	Spionidae	^{13}C *Phaeodactylum* sp.	−17.82	0.008
RL1 Ch. 1 (0–2cm)	Undetermined polychaetes	^{13}C *Phaeodactylum* sp.	−17.37	0.015
RL1 Ch. 1 (2–5cm)	Undetermined polychaetes	^{13}C *Phaeodactylum* sp.	−17.72	0.013
RL1 Ch. 1 (2–5cm)	Tanaidacea	^{13}C *Phaeodactylum* sp.	−16.37	0.858
RL1 Ch. 1 (0–2cm)	Pooled crustaceans	^{13}C *Phaeodactylum* sp.	−18.72	0.000
RL1 Ch. 1 (0–2cm)	Hydrozoa	^{13}C *Phaeodactylum* sp.	−25.96	0.000
RL1 Ch. 1 (0–2cm)	Nematodes	^{13}C *Phaeodactylum* sp.	−21.51	0.000
RL1 Ch. 1 (0–2cm)	Undetermined	^{13}C *Phaeodactylum* sp.	−16.36	0.551
RL1 Ch. 1 (2–5cm)	Undetermined	^{13}C *Phaeodactylum* sp.	−18.92	0.000
RL1 Ch. 1 (2–5cm)	Undetermined	^{13}C *Phaeodactylum* sp.	−19.12	0.000
RL3 Ch. 1 (0–2cm)	Nereidae	^{13}C *Phaeodactylum* sp.	8.49	12.591
RL3 Ch. 1 (0–2cm)	Nereidae	^{13}C *Phaeodactylum* sp.	10.38	0.667
RL3 Ch. 1 (0–2cm)	Undetermined polychaete	^{13}C *Phaeodactylum* sp.	−11.34	0.536
RL3 Ch. 1 (0–2cm)	Ostracoda	^{13}C *Phaeodactylum* sp.	266.92	1.950
RL3 Ch. 1 (2–5cm)	Tanaidacea	^{13}C *Phaeodactylum* sp.	−8.73	1.287
RL3 Ch. 1 (0–2cm)	Bivalvia	^{13}C *Phaeodactylum* sp.	−17.10	0.026
RL3 Ch. 1 (0–2cm)	Gastropoda	^{13}C *Phaeodactylum* sp.	−14.73	1.305
RL3 Ch. 1 (0–2cm)	Crinoidea	^{13}C *Phaeodactylum* sp.	−2.76	0.126
RL3 Ch. 1 (2–5cm)	Undetermined	^{13}C *Phaeodactylum* sp.	−19.52	0.000
RL3 Ch. 3 (0–2cm)	Undetermined polychaetes	^{13}C *Phaeodactylum* sp.	−10.00	0.090
RL3 Ch. 3 (2–5cm)	Isopoda	^{13}C *Phaeodactylum* sp.	−21.60	0.000
RL3 Ch. 3 (0–2cm)	Undetermined	^{13}C *Phaeodactylum* sp.	−24.04	0.000
RL5 Ch. 1 (0–2cm)	Pisionidae	^{13}C *Phaeodactylum* sp.	−11.39	0.236
RL5 Ch. 1 (0–2cm)	Cumacea	^{13}C *Phaeodactylum* sp.	−13.68	0.099
RL5 Ch. 1 (0–2cm)	Isopoda	^{13}C *Phaeodactylum* sp.	−13.83	0.050
RL5 Ch. 1 (0–2cm)	Hydrozoa	^{13}C *Phaeodactylum* sp.	−10.00	0.620
RL5 Ch. 1 (0–2cm)	Oligochaeta	^{13}C *Phaeodactylum* sp.	−4.17	0.086
RL5 Ch. 2 (0–2cm)	Lumbrineridae	^{13}C bicarbonate	−19.70	0.000
RL5 Ch. 2 (2–5cm)	Serpulidae	^{13}C bicarbonate	−23.49	0.000
RL5 Ch. 2 (0–2cm)	Spionidae	^{13}C bicarbonate	−22.87	0.000
RL5 Ch. 2 (2–5cm)	Trichobranchidae	^{13}C bicarbonate	−20.56	0.000
RL5 Ch. 2 (0–2cm)	Undetermined polychaetes	^{13}C bicarbonate	−20.47	0.000
RL5 Ch. 2 (2–5cm)	Undetermined polychaetes	^{13}C bicarbonate	−22.62	0.000
RL5 Ch. 2 (0–2cm)	Amphipoda	^{13}C bicarbonate	−22.27	0.000

(Continues)

Table 2. Continued

	Taxa	Tracer added	$\delta^{13}C$ (‰)	C-uptake ($\times 10^{-3}$ mg C m^{-2} d^{-1})
RL5 Ch. 2 (2–5cm)	Amphipoda	^{13}C bicarbonate	−21.20	0.000
RL5 Ch. 2 (0–2cm)	Undetermined	^{13}C bicarbonate	−25.08	0.000
RL6 Ch. 2 (2–5cm)	Lacydoniidae	^{13}C bicarbonate	−19.53	0.000
RL6 Ch. 2 (0–2cm)	Spionidae	^{13}C bicarbonate	−19.48	0.000
RL6 Ch. 2 (0–2cm)	Undetermined polychaetes	^{13}C bicarbonate	−21.04	0.000
RL6 Ch. 2 (2–5cm)	Undetermined polychaetes	^{13}C bicarbonate	−20.29	0.000
RL6 Ch. 2 (0–2cm)	Isopoda	^{13}C bicarbonate	−20.59	0.000
RL6 Ch. 2 (2–5cm)	Isopoda	^{13}C bicarbonate	−21.06	0.000
RL6 Ch. 2 (0–2cm)	Ostracoda	^{13}C bicarbonate	−25.78	0.000
RL6 Ch. 2 (0–2cm)	Tanaidacea	^{13}C bicarbonate	−20.27	0.000
RL6 Ch. 2 (2–5cm)	Tanaidacea	^{13}C bicarbonate	−21.67	0.000
RL6 Ch. 2 (0–2cm)	Bivalvia	^{13}C bicarbonate	−23.48	0.000
RL6 Ch. 2 (2–5cm)	Bivalvia	^{13}C bicarbonate	−24.64	0.000
RL6 Ch. 2 (0–2cm)	Undetermined	^{13}C bicarbonate	−20.86	0.000

(PERMANOVA) using Bray–Curtis distances confirmed that there were no statistically significant differences in microbial taxonomic composition when sediments were incubated with fresh phytodetritus compared to background sediments (PERMANOVA, $p > 0.05$, n = 9–15; Fig. 6A,B).

Analysis of the taxonomic composition among OTUs binned at phylum/class level in background and algal-addition treatments revealed highly similar relative abundances (% of total sequences) of the major microbial groups (Fig. 7A,B). This pattern was consistent for both the AB01 and AB02 experiments (Fig. 7A,B). At the OTU level, the top 10 most abundant OTUs detected in the AB01 experiments included the Acidobacteria AT-s2-57 (OTU 4425583), which had the highest observed relative abundance (~ 5%) in treatments amended with algal biomass (Table 3). Most of the top 10 OTUs, such as OTU 619389 affiliated with *Flavobacteriales*, had either similar or lower relative abundances in the algal-amended compared to the background samples from AB01 (Table 3). Forty percent of the top 10 most abundant OTUs-found in the sediments from the AB02 experiments had similar relative abundances in the algae treatment relative to background samples (Table 3). Exceptions included OTU

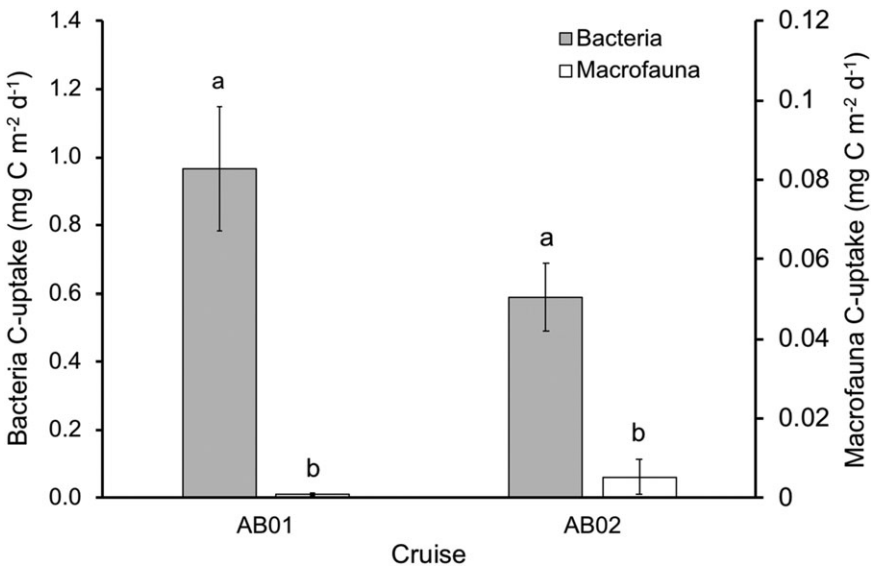

Fig. 3. Mean daily bacterial and macrofaunal algal C-assimilation rates (mg C m^{-2} d^{-1}) in the 0–5 cm sediment layer of benthic chamber sediment samples collected during the AB01 and AB02 cruises. Different letters denote significant differences ($p < 0.05$) between medians. Error bars denote ± 1 SEM (n = 4). Note: Different y-axes for bacteria and metazoan macrofauna algal C-assimilation rates.

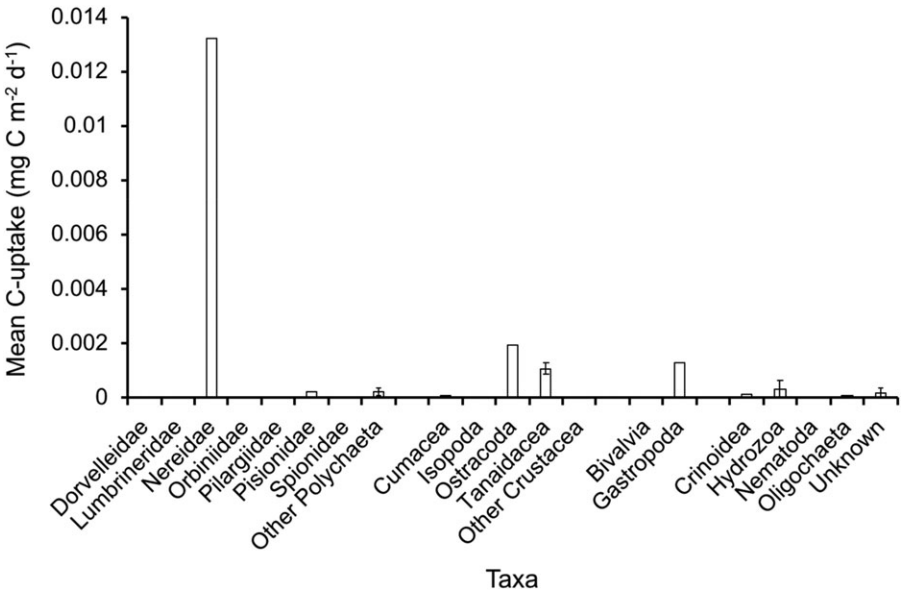

Fig. 4. Mean daily phytodetritus C-assimilation rates (mg C m^{-2} d^{-1}) of different metazoan macrofauna taxa in the 0–5 cm sediment layer of benthic chamber sediment samples collected during the AB02 cruise. Error bars denote ± range for all taxa, except other polychaetes (n = 3) and unknown taxa (n = 3), where the error bar denotes ± 1 SEM.

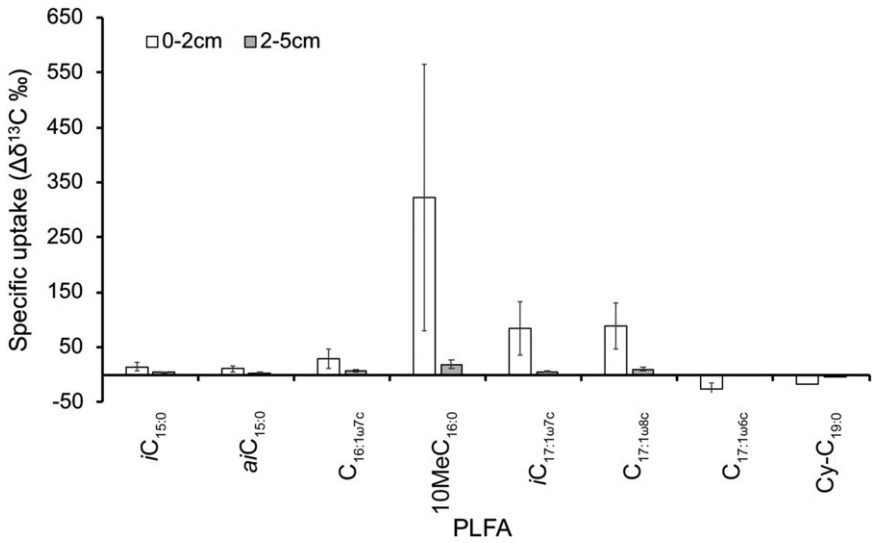

Fig. 5. Mean specific uptake of ^{13}C ($\Delta\delta^{13}C$) from labeled DIC into bacterial PLFA in the 0–2 and 2–5 cm sediment layers of benthic chamber sediment samples. Error bars denote range (n = 2).

50 (Planctomycetes), OTU 15 (Rhodospirillaceae), OTU 6 (Nitrospirales), OTU 12 (Chloroflexi), the gammaproteobacterial OTUs affiliated with JTB255 marine benthic group (2 + 19), which had slightly higher relative abundances in algal-amended compared to background samples.

Microbial community structure was similar in sediments amended with labeled DIC and in background samples (PERMANOVA, $p > 0.05$, n = 5; Fig. 6C). The top 10 most abundant OTUs observed in the DIC-addition experiment were the same as for the AB02 algal-addition experiments (Table 3). Nevertheless, OTUs 2 and 19 affiliated with JTB255 marine benthic group bacteria and OTU 50 and 15 affiliated with Planctomycetes and Rhodospirillaceae, respectively, had slightly higher relative abundances in the DIC-incubation treatments compared to background samples (Table 3).

Discussion

Bacteria dominated the short-term assimilation of fresh phytodetritus in both the AB01 and AB02 experiments, consuming 99.9% ± 0.0% (n = 4, SEM) and 98.8% ± 1.1% (n = 4, SEM) of the 0.97 ± 0.18 mg C m^{-2} (n = 4, SEM) and

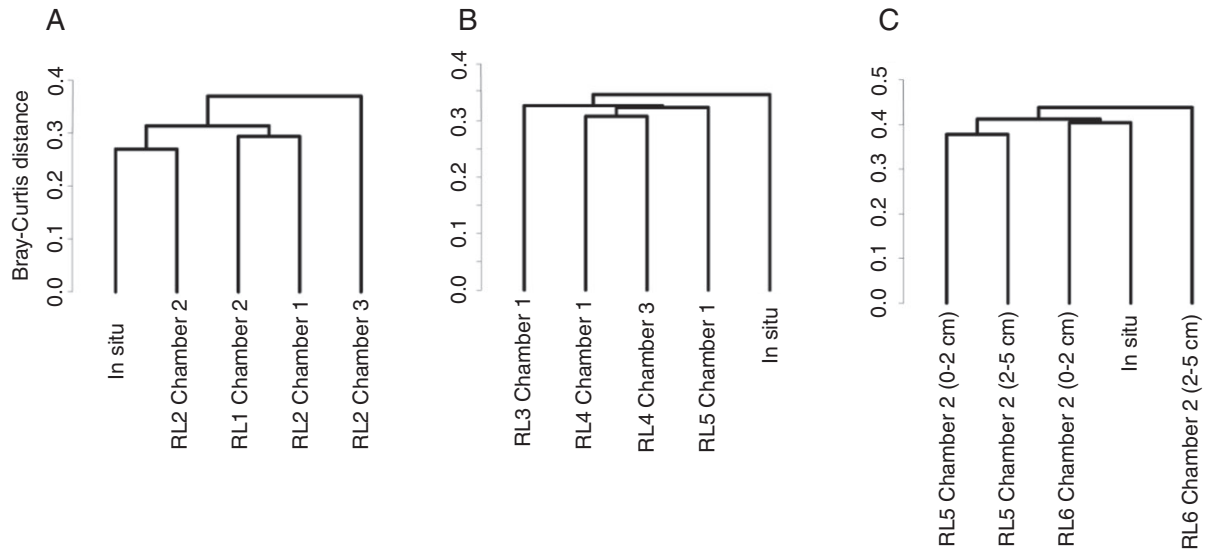

Fig. 6. Bray Curtis similarities in microbial community structure in background sediments (in situ) compared to sediments from the algal-addition experiments from **(A)** AB01 and **(B)** AB02 and **(C)** the DIC-addition experiments.

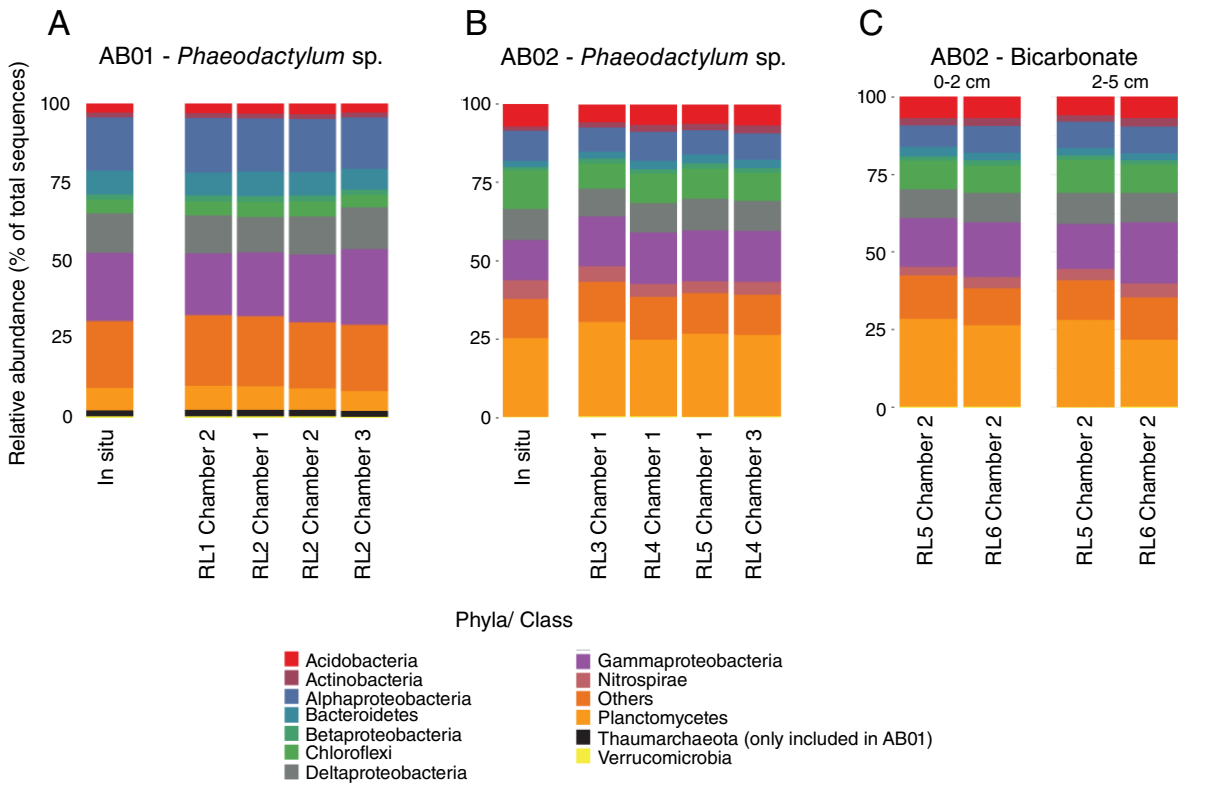

Fig. 7. Microbial taxonomic composition in background sediments (in situ) compared to sediments from the algal-addition experiments from **(A)** AB01 and **(B)** AB02 and **(C)** the DIC-addition experiments. Bar charts denote the relative abundance (% of total 16S rRNA sequences) of OTUs classified to phylum/class level obtained from high-throughput sequence data.

0.60 ± 0.10 mg C m^{-2} ($n = 4$, SEM), respectively, that was traced into benthic (macrofauna and bacteria) biomass over 34–36 h. Thus, our first hypothesis that macrofauna dominate the short-term assimilation of phytodetritus in this region of the Pacific can be rejected. We found similar responses by the abyssal benthic community across all three strata that were

Table 3. The top 10 most abundant OTUs detected during the AB01 and AB02 cruises in background sediments (in situ) and benthic chamber lander sediments that were treated with either labeled algae or DIC, with the average relative abundance (% of total sequences) indicated for each microbial group.

					Average relative abundance (% of total sequences)	
Cruise	Treatment	OTU	Phylum/class	Taxa	Treatment	In situ
AB01	*Phaeodactylum* sp.	4425583	Acidobacteria	AT-s2-57	4.95	3.82
		104620	Actinobacteria	Acidimicrobiales	1.58	1.94
		619389	Bacteroidetes	Flavobacteriales	1.54	1.50
		182872	Chloroflexi	S085	2.22	2.93
		742886	Chloroflexi	mle1-48	1.85	1.78
		730215	Deltaproteobacteria	Nitrospina	1.83	1.98
		547475	Gammaproteobacteria	Oceanospirillales	2.24	3.53
		4475363	Proteobacteria	NB1-j	1.24	1.29
		737234	TA18	PHOS-HD29	2.66	2.98
		271272	TM6	S1198	1.85	1.72
AB02	*Phaeodactylum* sp.	13	Alphaproteobacteria	Rhodospirillaceae	1.13	1.23
		11	Alphaproteobacteria	Defluviicoccus	1.01	0.79
		3	Alphaproteobacteria	Rhodospirillaceae	0.81	0.87
		12	Chloroflexi	S085	1.13	0.70
		2	Gammaproteobacteria	JTB255_marine_benthic_group	1.79	1.00
		19	Gammaproteobacteria	JTB255_marine_benthic_group	1.42	0.80
		6	Nitrospirae	Nitrospirales	2.60	2.22
		50	Planctomycetes	Urania-1B-19_marine_sediment_group	1.85	0.81
		38	Planctomycetes	Urania-1B-19_marine_sediment_group	0.60	0.74
		15	Proteobacteria	Rhodospirillaceae	1.47	0.50
AB02	Bicarbonate	13	Alphaproteobacteria	Rhodospirillaceae	0.96	1.23
		11	Alphaproteobacteria	Defluviicoccus	0.96	0.79
		3	Alphaproteobacteria	Rhodospirillaceae	0.59	0.87
		12	Chloroflexi	S085	0.96	0.70
		2	Gammaproteobacteria	JTB255_marine_benthic_group	1.76	1.00
		19	Gammaproteobacteria	JTB255_marine_benthic_group	1.69	0.80
		6	Nitrospirae	Nitrospirales	1.75	2.22
		50	Planctomycetes	Urania-1B-19_marine_sediment_group	1.85	0.81
		38	Planctomycetes	Urania-1B-19_marine_sediment_group	0.59	0.74
		15	Proteobacteria	Rhodospirillaceae	1.15	0.50

separated by tens to hundreds of kilometers, so our results may be generalizable over large spatial scales in the eastern CCFZ. We did not consider the role played by metazoan meiofauna (32–300 μm), meiofaunal protists, nanobiota, fungi, or Archaea in C cycling during our experimental studies. Previous investigations in the abyssal central Pacific have shown that the metazoan meiofaunal taxa (32 to 300-μm), protists, and nanobiota constitute a large fraction of the infaunal abundance and biomass (Snider et al. 1984). While the exclusion of metazoan meiofauna could have altered our results somewhat, this likely had less effect on our results than the exclusion of the Foraminfera. Previous investigations have shown metazoan meiofauna do not respond as strongly to phytodetritus C input as macrofauna or Foraminifera (Gooday et al. 1996; Moodley et al. 2000, 2002; Nomaki et al. 2005; Guilini et al. 2010; Pozzato et al. 2013; Mevenkamp et al. 2017; Stratmann et al. 2018). Among the Foraminifera, monothalmids dominate assemblages in the eastern CCFZ, including the UK1 and OMS areas (Nozawa et al. 2006; Goineau and Gooday 2017). There is some evidence that, as a group, these "primitive," single-chambered Foraminifera are less responsive to pulsed inputs of labile organic matter than multichambered taxa, particularly some rotaliid species (Bertram and Cowan 1999; Enge et al., 2011). However, the high dominance of monothalamid Foraminifera at our study sites and the rapid response by bacteria to phytodetritus input may lead to monothalamids playing a much larger role in C cycling in the eastern CCFZ than other areas (e.g., NE Pacific; Enge et al. 2011) as exclusive feeding on bacterial biofilms by allogromiid species has been previously documented (e.g., Bernhard and

Bowser 1992). Therefore, the role of the meiofaunal Foraminifera community, in particular the monothalamid forams, should be investigated in follow-up studies. The contribution of macrofauna-sized Foraminifera (> 300 μm), which in the CCZ mainly comprise monothalamids, such as komokiaceans and tubular forms, to short-term C-uptake was assessed during the AB01 experiments. The amount of C-uptake by these forams was insignificant (3.3 ± 1.7 x 10^{-3} mg C m^{-2} d^{-1}) compared to that of bacteria as also found by Sweetman et al. (2009). As a result, we did not include these large Foraminifera in the AB02 experiments. Conversely, some small rotaliids (notably *Alabaminella weddellensis* and *Epistominella exigua)* that are known to feed on fresh phytodetritus in the North Atlantic Ocean (Gooday 1988) are a minor component of sediment assemblages in samples from the UK1 and OMS areas. There is evidence that pulsed inputs of phytodetritus occur occasionally in the eastern CCZ (Radziejewska 2002). If such an event occurred at one of our study sites, it is likely that species such as *A. weddellensis* and *E. exigua* would consume the phytodetritus, rapidly increase their population density and undertake an active role in processing this labile material.

Community responses to phytodetritus

Rapid responses by bacteria to the input of phytodetritus/fresh organic material have been shown at a variety of deep-sea sites (Rowe and Deming 1985; Lochte and Turley 1988; Boetius and Lochte 1996; Kanzog et al. 2009; Hoffmann et al. 2017). For example, Rowe and Deming (1985) found rapid (< 5 d) utilization of ^{14}C-labeled glutamic acid by benthic microbes that had been collected from abyssal depths in the Bay of Biscay and on the Demerara Abyssal Plain (DAP). Lochte and Turley (1988) also documented significant bacterial growth after 2 d on freshly deposited detrital material recovered from 4500 m depth and incubated at in situ pressure. More recently, Kanzog et al. (2009) and Hoffmann et al. (2017) found significant positive responses by microbial communities from the bathyal Arctic Ocean to the addition of chitin and phytodetritus in 7 d in situ and 23 d ex situ experiments, respectively. Finally, unpressurized shipboard studies using ^{13}C-labeled detritus revealed that abyssal bacteria can process much more phytodetritus C than macrofauna (Moodley et al. 2005). Despite our in situ abyssal data from the CCFZ being consistent with these findings, pressure-related artifacts associated with sediment retrieval from depth (e.g., decompression of organisms, macrofaunal death, and an increase in the activity of nonpiezophilic microbes) may significantly alter benthic activities and resource partitioning among organism groups even if sediments are repressurized following seabed sampling. Care should therefore be taken when comparing results from ex situ (shipboard) to in situ (seabed) experiments.

To our knowledge, only the study of Witte et al. (2003*a*) on the Porcupine Abyssal Plain (PAP, 4800 m) in the eutrophic NE Atlantic has assessed the in situ short-term response of both benthic bacteria and macrofauna to the addition of identically grown and harvested, fresh phytodetritus in an undisturbed abyssal setting. Interestingly, as in our study, bacterial biomass in the NE Atlantic was much greater (2.5 x 10^3 mg C m^{-2}) than macrofaunal biomass (120 mg C m^{-2}) (Witte et al. 2003*a*). Yet, in the NE Atlantic, macrofauna consumed more than 95% of the total amount of C that was assimilated by the benthos over 2.5 d, with an almost undetectable contribution by bacteria (Witte et al. 2003*a*). Moreover, mean total daily C-assimilation rates by the benthic bacterial and macrofaunal community in the CCFZ were 39–64% of those recorded from the abyssal PAP by Witte et al. (2003*a*) and 47–79% of the total modeled heterotrophic organic C-utilization rates recorded from the DAP by Rowe and Deming (1985) (summed bacteria and macrofauna C-assimilation rates: 0.6 to 1.0 mg C m^{-2} d^{-1} for the CCFZ vs. 1.52 mg C m^{-2} d^{-1} for the PAP after 2.5 d [Witte et al. 2003*a*] and 1.23 to 1.29 mg C m^{-2} d^{-1} for the DAP [Rowe and Deming 1985]). It is unlikely that the lower response by macrofauna we detected (relative to the PAP and DAP studies) was an artifact of our experimental design, as very little organic material would need to be consumed by the macrofauna to detect positive C-uptake into the macrofaunal biomass (e.g., 0.6 μg C would need to be consumed by 1 mg C of macrofaunal biomass to detect a positive shift in δ^{13} of 20 ‰ assuming the algae were labeled with 37.8 atom % ^{13}C). Although the lower total C-assimilation rates measured in our study were partly due to daily bacterial and macrofaunal C-assimilation rates being normalized to account for the presence of nodules, a number of other factors could possibly account for the differences in the benthic response we observed. First, the shorter duration of our experiments compared to those of Witte et al. (2003*a*) may have been a factor. Short-term in situ tracer studies are logistically simpler to run as they require less ship-time, are not as prone to de-oxygenation artifacts, and useful for identifying uptake by fast-growing organisms at the bottom of the food web (e.g., microbes) (Middelburg 2014). However, they do not always allow adequate detection of transfer from basal resources via intermediates to eventual consumers (Middelburg 2014). As a result, they can show completely different responses by the benthic community compared to longer-term in situ investigations. In a 1.5 d in situ study at 1250 m in Sognefjorden (Norway), Witte et al. (2003*b*) showed more C incorporation by macrofauna relative to bacteria, and lower amounts of C assimilation, with the relative roles being reversed and more C being processed over a 3 d period. This response was also seen in the abyssal NE Atlantic (Witte et al. 2003*a*). Thus, the shorter duration of our experiments may have been one cause for the different organism responses we observed compared to those seen by Witte et al. (2003*a*). Longer studies (weeks to months) may thus reveal differences in the dominant C-processing organisms. Second, the smaller amounts of phytodetritus added to sediments in our study may have also played a role. Our algal C-addition was equivalent to ~ 18% of the annual POC flux to the seafloor estimated for the CCFZ study

area (1 g C m^{-2} yr^{-1}; Amon et al. 2016). The amount added in the NE Atlantic study was much larger in absolute (1 g C m^{-2}) and relative (33–50% of the annual POC flux at their study site) terms (Lutz et al. 2007). Modeling studies simulating benthic C cycling at abyssal depths have revealed that the bacterial contribution to total benthic C cycling increases under low POC flux relative to high POC flux conditions (Dunlop et al. 2016), which can be explained by competition for substrates between macrofauna and bacteria. In a shallow-water experiment, macrofauna exploited labile C-sources to a greater extent than bacteria when food was more concentrated in seafloor sediments but were outcompeted by bacteria when food was less concentrated (Van Nugteren et al. 2009), though this is not always evident (e.g., Sweetman et al. 2016). Therefore, lower amounts of C added may explain the more significant and consistent bacterial response at our study sites. If our results reflect abyssal community responses to smaller phytodetritus pulses that are typical for many mesotrophic and oligotrophic abyssal sites, they suggest that bacteria may play a key role in the short-term cycling of phytodetritus at the abyssal seafloor of the CCFZ. Finally, differences in benthic community structure between study sites could also explain the lower amount of C processed, and lower macrobenthic response to phytodetritus in our study compared to at the PAP. At our study sites, total benthic biomass was lower (mean summed biomass: ~ 0.44 g C m^{-2}) and known surface deposit-feeding fauna (e.g., spionids) contributed little to macrofaunal abundance (14% of polychaetes) and a negligible amount to C assimilation (Table 2; Fig. 4). In contrast, benthic biomass at the PAP was much greater (summed bacterial and macrofaunal biomass: ~ 2.6 g C m^{-2}) and surface-feeding cirratulid and spionid polychaetes were very abundant (50% of all polychaetes), and they contributed heavily to macrobenthic biomass (57% of polychaete biomass) and C assimilation (Aberle and Witte 2003; Witte et al. 2003*a*).

Assuming that our results can be generalized to the greater CCFZ, our findings have implications for the extraction of polymetallic nodules in this region (Smith et al. 2008*b*; Levin et al. 2016). Previous abyssal investigations assessing the response of the abyssal benthos to low-intensity, small-scale disturbance (e.g., at the DISCOL, Peru Basin and Inter-Ocean Metals, CCFZ study sites where < 11 km^2 of seafloor was disturbed) have shown that deep-sea benthic prokaryotic abundances and activities remain depressed relative to control areas for decades following disturbance (Weaver et al. 2016; Stratmann et al. 2018). Full-scale mining will be significantly greater in intensity and scale (600–800 km^2 of seafloor will be disturbed per year per mining operation, Smith et al. 2008*b*; Levin et al. 2016) compared to the disturbance created at the DISCOL and IOM study sites (Jones et al. 2017). Consequently, the disturbance effects on benthic communities are likely to be greater and persist for much longer (Miljutin et al. 2011; Levin et al. 2016; Jones et al. 2017). Our findings, which confirm the key role of deep-sea benthic bacteria in the initial stages of organic matter mineralization as shown by others (Rowe and Deming 1985; Lochte and Turley 1988; Boetius and Lochte 1996; Moodley et al. 2002), together with findings showing limited microbial recovery from small-scale disturbance in the abyss (Weaver et al. 2016; Stratmann et al. 2018), imply that key benthic ecosystem functions (e.g., bacterial C remineralization and C-burial efficiency) could be significantly impacted for decades, and perhaps even longer if polymetallic nodule mining commences. Benthic biodiversity and community structure assessments are a requirement for baseline surveys in mine claim areas in the CCFZ (International Seabed Authority 2010*b*). However, studies assessing C flow through the benthic community are not specified in the ISA regulations for the assessment of possible environmental impacts arising from polymetallic nodule extraction in the Area (International Seabed Authority 2010*b*). A power analysis based on our data reveals that only a small number of experiments (n = 10) would be required at both impacted and control sites to detect a 50% change in microbial C-cycling rates (one-sided test, power = 0.8). We therefore strongly recommend that studies assessing organic C processing by the benthic community be undertaken during baseline and monitoring surveys in mining claim zones, as they may provide a powerful tool for detecting changes in benthic ecosystem functioning resulting from mining.

Uptake of dissolved inorganic carbon by bacteria

Although there was considerable variability in the $\Delta\delta^{13}$ values among the extracted bacterial lipids, the positive $\Delta\delta^{13}$ values for six bacteria-specific PLFA in the chambers where $DI^{13}C$ was injected indicated active assimilation of inorganic C into bacterial biomass. This is consistent with our second hypothesis that "abyssal benthic bacteria actively assimilate inorganic C into their biomass in the eastern CCFZ." Estimated daily $DI^{13}C$-incorporation rates were statistically indistinguishable to the bacterial algal-C assimilation rates we measured, which suggests the possibility that some of the algal-C incorporation we detected in the bacteria could have resulted from the incorporation of labeled DIC produced during the remineralisation of algal material by other unstudied organism groups, such as meiofauna or protists. Our ^{13}C-incorporation rates from the labeled DIC study were 24 to 44 times greater than the estimated chemoautotrophy rates reported for open ocean sediments (0.04 mg C m^{-2} d^{-1}) by Middelburg (2011), but were similar to daily inorganic C-fixation rates measured using sediment cores that were recovered from abyssal depths and incubated at atmospheric pressure by Molari et al. (2013) (e.g., ~ 0.24–0.96 mg C m^{-2} d^{-1}, recalculated from fig. 4A in Molari et al. 2013, assuming a sediment density of 4 mL g dry $sediment^{-1}$ and a sediment depth of 2 cm). The rate of inorganic carbon assimilation by bacteria was equivalent to 31% to 57% of the incoming POC flux

estimated for our study area (2.7 mg C m^{-2} d^{-1}; Amon et al. 2016), suggesting inorganic C assimilation by deep-sea bacteria, and the in situ production of organic C may be avery important process in abyssal benthic food webs. Although the lack of positive $\Delta\delta^{13}$ values in the macrofauna indicated zero transfer of microbial-assimilated C into the metazoan macrofaunal food web over the short duration of our experiments, it is possible that this does occur but over longer durations than studied here. Clearly, further fieldwork using longer incubation times are required to confirm the generality of these findings, as well as to quantify C transfer from DIC via microbial intermediates to benthic metazoan communities.

It is unclear what energy source was utilized by bacteria to incorporate inorganic C into their biomass. Christensen and Rowe (1984) proposed that nitrification could play a role in supplying organic C to the deep-sea benthos, and Brunnegard et al. (2004) showed that nitrification can be a key N-cycling mechanism in abyssal sediments. However, it is unlikely that nitrification was the main inorganic C fixation process in our experiments. Assuming a molar stoichiometry of CO_2 fixed : NH_3 oxidized of 0.05–0.1 (based on Christensen and Rowe [1984], Middelburg [2011], and Könneke et al. [2014]), a NH_3 oxidized : O_2 utilized stoichiometry of 0.5 and 35% of oxygen consumption is due to nitrification (Christensen and Rowe 1984) would mean that 0.88–1.75 mol of CO_2 would be fixed per 100 mol of O_2 consumed. Our background SCOC rates were 0.22 mmol O_2 m^{-2} d^{-1}, implying the amount of CO_2 fixed by nitrification would be equivalent to 1.9 x 10^{-3} to 3.9 x 10^{-3} mmol C m^{-2} d^{-1} or 2.3 x 10^{-2} to 4.6 x 10^{-2} mg C m^{-2} d^{-1}. As our rates were much larger (1.20 $\pm$ 0.35 mg C m^{-2} d^{-1}) this suggests only a minor role for nitrification in the bacterial incorporation of inorganic C that we detected. The limited role of nitrification in inorganic C fixation is also apparent if we assume that all of the particulate organic nitrogen (PON) transported to the seafloor is oxidized by nitrification. For example, if the C flux to the seafloor is 1000 mg C m^{-2} yr^{-1} (Amon et al. 2016) or 2.74 mg C m^{-2} d^{-1} and the C:N ratio of the particulate organic material reaching the seafloor is 12:1 (at 4000 m depth at Station Aloha; Karl et al. 2012), the daily PON flux to the seafloor would be 0.23 mg N m^{-2} d^{-1} or 1.6 x 10^{-2} mmol N m^{-2} d^{-1}. If all of the PON was then nitrified at the seafloor and 0.05–0.1 mol of CO_2 were fixed per mole of NH_3 oxidized, then the CO_2 fixation rate would be 0.8 x 10^{-3} to 1.6 x 10^{-3} mmol C m^{-2} d^{-1} or 1.0 x 10^{-2} to 2.0 x 10^{-2} mg C m^{-2} d^{-1}, which is 1–2 orders of magnitude lower than our mean inorganic C fixation rate. Providing the C incorporation we detected was due to only chemoautotrophic C-fixation processes, sulphide oxidation could also serve as an energy source. However, again it is unclear where the source of sulphide would come from at our study sites since POC fluxes here are low (Lutz et al. 2007; Amon et al. 2016). Nitrite oxidation (Pachiadaki et al. 2017), hydrogen oxidation resulting from the radiolysis of water (D'Hondt et al. 2009), and oxidation of reduced iron or manganese particles that have been deposited from nearby hydrothermal vent sites (Resing et al. 2015; Tully and Heidelberg 2016) could offer alternative energy sources for at least some of the inorganic C fixation. Alternatively, it could be the result of heterotrophic bacteria using anaplerotic C-fixation mechanisms. However, estimates of the contribution of anaplerotic C fixation to biomass production suggest this pathway supplies only 5% to 10% to total biomass production (Boschker et al. 2014). Thus, if this process was the main cause for the inorganic C-fixation we measured, mean bacterial biomass production would range from 12 to 24 mg C m^{-2} d^{-1}, which is 4.4 to 8.8 times greater than the incoming POC flux, and 1.5 to 3.1 times greater than published deep-sea bacterial biomass production estimates (e.g., 7.76 mg C m^{-2} d^{-1} based on a global bacterial biomass production of 0.88 Pg C yr^{-1} in Danovaro et al. [2015] and an open-ocean area of 31.07 x 10^{13} m^2 in Middelburg [2011]). Clearly inorganic C fixation by abyssal microbial communities requires further study, including an assessment of the dominant mechanisms involved, and identification of the major energy sources being used.

Microbial diversity and assemblage composition responses

Deep-sea benthic microbial community structure and diversity can rapidly change following exposure to organic matter. Hoffmann et al. (2017) documented a significant decrease in microbial diversity (number of OTUs) over a 23 d period following the addition of phytodetritus. Similarly, Kanzog et al. (2009) showed that the addition of chitin led to significant shifts in microbial community structure in a 7 d in situ experiment. Despite bacteria dominating the assimilation of phytodetritus and incorporating DIC into their biomass, we did not observe a significant shift in microbial community structure or diversity (relative to background sediments) in any of the lander experiments over a period of 34–36 h. Thus, our third hypothesis that "microbial community composition changes significantly over short-term time scales following the addition of organic and inorganic C" can be rejected. Previous microbial community structure studies at Station M in the more eutrophic NE Pacific (POC flux ~ 3 g C m^{-2} yr^{-1}) have shown that abyssal benthic bacterial community structure remains relatively stable over long periods of time (weeks-years) despite fluctuations (4–5X) in POC flux (Moeseneder et al. 2012). We suspect the lack of a significant overall community response in our experiments was due to the short incubation times (34–36 h).

Although no significant shift in microbial community structure was seen during the AB02 experiments, we did detect small shifts in the relative abundance of some OTUs between treatments with and without added algae. The OTUs belonging to the Gammaproteobacteria JTB255 Marine Benthic Group (e.g., OTU 2 and 19), Nitrospirales (OTU 6), Chloroflexi (OTU 12), Planctomycetes (OTU 50), and Rhodospirillaceae (OTU 15) increased slightly in relative abundance when

exposed to fresh phytodetritus (Table 3). Hoffmann et al. (2017) showed an increase in the relative abundance of 12 bacterial families from Arctic deep-sea sediments following exposure to phytodetritus at cold temperatures (0°C) over a period of 23 d. There were also slight OTU changes following the addition of labeled DIC. For example, OTUs affiliated with JTB255 marine benthic group bacteria (OTUs 2 + 19), Planctomycetes (OTU 50), and Rhodospirillaceae (OTU 15) had approximately or greater than twice as high relative abundances in the DIC experiments compared to background conditions after ~ 1.5 d. As the added algae were axenic, it is unlikely that the slight positive responses we observed in the algae-addition experiments were due to the addition of algae-associated microbes. Therefore, the slight increase in the relative abundance of some OTUs could have been caused by specific microbial groups responding positively to the new conditions created by our additions. However, Deming (1985) showed bacteria doubling times of weeks to months when sediment samples from abyssal depths were incubated under in situ conditions. Moreover, Alongi (1990) showed slow bacterial growth rates (0.001–0.12 d^{-1}) in surface sediment samples collected from 10 bathyal and abyssal (695–4350 m) sites in the Solomon and Coral Sea. It is therefore more probable that the cold temperatures at the seafloor in our study region (~ 1.5°C) limited changes in in situ microbial community composition, and the slight differences we observed in the relative abundance of specific OTUs were simply due to natural variability in the sediments. Our deep-sea study area in the eastern CCFZ is also replete with DIC (bottom water concentration: ~ 2.4 mM) so our small DIC addition (~ 5–10% of total DIC concentration) should not have led to significant changes in DIC concentration at the seafloor and significant shifts in microbial community composition. We recommend that more long-term investigations, as well as the use of other methods (e.g., dual stable isotope probing; Wegener et al. 2012) should be undertaken to identify the nutritional pathways and feeding responses of specific microbes in abyssal sediments.

Conclusions

Our results reveal a key role for bacteria in the initial degradation of fresh phytodetritus at the abyssal seafloor of the eastern CCFZ, which is consistent with findings from earlier investigations from other deep-sea regions. The consistent nature of our results suggest that these characteristics may be generalizable over large spatial scales (distances of tens to hundreds of kilometers). The results presented highlight the differences that may occur in short-term C-cycling processes in abyssal environments characterized by different POC fluxes (i.e., mesotrophic vs. eutrophic systems). Our in situ DIC-addition experiments indicated that fixation of inorganic C by benthic bacteria does occur at the abyssal seafloor in this region. The daily DIC assimilation rates were statistically indistinguishable to bacterial assimilation rates of phytodetritus POC, which further highlights the importance of benthic bacteria in abyssal sediments, and points to the possibility that DIC fixation by bacteria may provide a different source of C to abyssal benthic communities in this region. Given the large changes that are predicted to occur in the eastern Pacific over the next century due to climate change (e.g., declining POC flux and benthic biomass; Sweetman et al. 2017) and mineral extraction (Jones et al. 2017), more short- and long-term studies are now needed in other abyssal areas to determine the generality of our findings, and assess the ecosystem consequences that could arise from significant changes to benthic microbial structure and ecosystem functioning in the abyss.

References

Aberle, N., and U. Witte. 2003. Deep-sea macrofauna exposed to a stimulated sedimentation event in the abyssal NE Atlantic: In situ pulse-chase experiments using ^{13}C-labeled phytodetritus. Mar. Ecol. Prog. Ser. **251**: 37–47. doi:10.3354/meps251037

Agusti, S., and others. 2015. Ubiquitous healthy diatoms in the deep sea confirm deep carbon injection by the biological pump. Nat. Commun. **6**: 7608. doi:10.1038/ncomms8608

Alongi, D. M. 1990. Bacterial growth rates, production and estimates of detrital carbon utilization in deep-sea sediments of the Solomon and Coral seas. Deep-Sea Res. Part A **37**: 731–746.

Amon, D. J., A. F. Ziegler, T. G. Dahlgren, A. G. Glover, A. Goineau, A. J. Gooday, H. Wiklund, and C. R. Smith. 2016. Insights into the abundance and diversity of abyssal megafauna in a polymetallic-nodule region in the eastern clarion-Clipperton zone. Sci. Rep. **6**: 30492.

Aspetsberger, F., M. Zabel, T. Ferdelman, U. Struck, A. Mackensen, A. Ahke, and U. Witte. 2007. Instantaneous benthic response to different organic matter quality: In situ experiments in the Benguela upwelling system. Mar. Biol. Res. **3**: 342–356. doi:10.1080/17451000701632885

Bernhard, J. M., and S. S. Bowser. 1992. Bacterial biofilms as a trophic resource for certain benthic foraminifera. Mar. Ecol. Prog. Ser. **83**: 263–272. doi:10.3354/meps083263

Bertram, M. A., and J. P. Cowan. 1999. Temporal variations in the deep-water colonization rates of small benthic foraminifera: The results of an experiment on cross seamount. Deep-Sea Res. Part I **46**: 1021–1049.

Bienhold, C., A. Boetius, and A. Ramette. 2012. The energy–diversity relationship of complex bacterial communities in Arctic deep-sea sediments. ISME **6**: 724–732. doi:10.1038/ismej.2011.140

Billett, D. S. M., R. S. Lampitt, A. L. Rice, and R. F. C. Mantoura. 1983. Seasonal sedimentation of phytoplankton

to the deep-sea benthos. Nature **302**: 520–522. doi:10.1038/302520a0

Boetius, A., and K. Lochte. 1996. Effect of organic enrichments on hydrolytic potentials and growth of bacteria in deep-sea sediments. Mar. Ecol. Prog. Ser. **140**: 239–250. doi:10.3354/meps140239

Boschker, H. T. S., and J. J. Middelburg. 2002. Stable isotopes and biomarkers in microbial ecology. FEMS Microbiol. Ecol. **40**: 85–95. doi:10.1111/j.1574-6941.2002.tb00940.x

Boschker, H. T. S., D. Vasquez-Cardenas, H. Bolhuis, T. W. C. Moerdijk-Poortvliet, and L. Moodley. 2014. Chemoautotrophic carbon fixation rates and active bacterial communitites in intertidal marine sediments. PLoS One **9**: e101443. doi:10.1371/journal.pone.0101443

Brinch-Iversen, J., and G. M. King. 1990. Effects of substrate concentration, growth state, and oxygen availability on relationships among bacterial carbon, nitrogen and phospholipids phosphorous content. FEMS Microbiol. Ecol. **74**: 345–356.

Brunnegard, J., S. Grandel, H. Stahl, A. Tengberg, and P. O. J. Hall. 2004. Nitrogen cycling in deep-sea sediments of the porcupine abyssal plain. NE Atlantic Prog. Oceanogr. **63**: 159–181.

Cahet, G., and M. Sibuet. 1986. Activité biologique en domaine profond: Transformations biochimiques *in situ* de composés organiqués marquis à carbone-14 h l'interface eau-sédiment par 2000 m de profondeur dans le golfe de Gascogne. Mar. Biol. **90**: 307–315. doi:10.1007/BF00569143

Caporaso, J. G., and others. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc. Natl. Acad. Sci. USA **108**: 4516–4522.

Christensen, J. P., and G. T. Rowe. 1984. Nitrification and oxygen consumption in Northwest Atlantic deep-sea sediments. Mar. Res. **42**: 1099–1111. doi:10.1357/002224084788520828

Danovaro, R., C. Corinaldesi, E. Rastelli, and A. Dell'Anno. 2015. Towards a better quantitative assessment of the relevance of deep-sea viruses, *Bacteria* and *Archaea* in the functioning of the ocean seafloor. Aquat. Microb. Ecol. **75**: 81–90. doi:10.3354/ame01747

De Martino, A., and others. 2007. Genetic and phenotypic characterization of Phaeodactylum tricornutum (Bacillariophyceae) accessions. J. Phycol. **43**: 992–1009. doi:10.1111/j.1529-8817.2007.00384.x

Deming, J. W. 1985. Bacterial growth in deep-sea sediment trap and boxcore samples. Mar. Ecol. Prog. Ser. **25**: 305–312. doi:10.3354/meps025305

D'Hondt, S., and others. 2009. Subseafloor sedimentary life in the South Pacific gyre. Proc. Natl. Acad. Sci. USA **106**: 11651–11656.

Drazen, J. C., R. J. Baldwin, K. L. Smith, and K. L. Jr. 1998. Sediment community response to a temporally varying food supply at an abyssal station in the NE Pacific. Deep-Sea Res. Part II **45**: 893–913.

Drazen, J. C., B. N. Popp, C. A. Choy, T. Clemente, L. De Forest, and K. L. Smith Jr. 2008. Bypassing the abyssal benthic food web: Macrourid diet in the eastern North Pacific inferred from stomach content and stable isotopes analyses. Limnol. Oceanogr. **53**: 2644–2654. doi:10.4319/lo.2008.53.6.2644

Druffel, E. R. M., S. Griffin, J. E. Bauer, D. M. Wolgast, and X. C. Wang. 1998. Distribution and Particulate Organic Carbon and radiocarbon in the water column from the upper slope to the abyssal NE Pacific Ocean. Deep-Sea Res. Part II **45**: 667–687.

Dunlop, K. M., D. van Oevelen, H. A. Ruhl, C. L. Huffard, L. A. Kuhnz, and K. L. S. Jr. 2016. Carbon cycling in the deep eastern North Pacific benthic food web: Investigating the effect of organic carbon input. Limnol. Oceanogr. **61**: 1956–1968. doi:10.1002/lno.10345

Dunne, J. P., J. L. Sarmiento, and A. Gnanadesikan. 2007. A synthesis of global particle export from the surface ocean and cycling through the ocean interior and on the seafloor, global Biogeochem. Cycles **21**: GB4006. doi:10.1029/2006GB002907

Enge, A. J., and others. 2011. Response of the foraminiferal community to a simulated phytodetritus pulse in the abyssal North Pacific. Mar. Ecol. Prog. Ser. **438**: 129–142. doi:10.3354/meps09298

Fry, B., and E. B. Sherr. 1984. $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. Contrib. Mar. Sci. **27**: 13–47.

Goineau, A., and A. J. Gooday. 2017. Novel benthic foraminifera are abundant and diverse in an area of the abyssal equatorial Pacific licensed for polymetallic nodule exploration. Sci. Rep. **7**: 45288. doi:10.1038/srep45288

Gooday, A. J. 1988. A response by benthic foraminifera to the deposition of phytodetritus in the deep sea. Nature **332**: 70–73. doi:10.1038/332070a0

Gooday, A. J., O. Pfannkuche, and P. J. D. Lambshead. 1996. An apparent lack of response by metazoan meiofauna to phytodetritus deposition in the bathyal north-eastern Atlantic. J. Mar. Biol. Assoc. UK **76**: 297–310.

Graf, G. 1989. Benthic-pelagic coupling in a deep-sea benthic community. Nature **341**: 437–439. doi:10.1038/341437a0

Grasshoff, K., K. Kremling, and M. Ehrhardt. 1999. Methods of seawater analysis. New York: Wiley.

Guillard, R. R. L. 1975. Culture of marine invertebrate animals, p. 26–60. *In* W. L. Smith and M. H. Chanley [eds.], Culture of phytoplankton for feeding marine invertebrates. Plenum Press.

Guilini, K., D. Van, K. S. Oevelen, J. J. Middelburg, and A. Vanreusel. 2010. Nutritional importance of benthic bacteria for deep-sea nematodes from the Arctic ice margin: Results of an isotope tracer experiment. Limnol. Oceanogr. **55**: 1977–1989. doi:10.4319/lo.2010.55.5.1977

Herlemann, M. Labrenz, K. Jürgens, S. Bertilsson, J. J. Waniek, and A. F. Andersson. 2011. Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. ISME J. **5**: 1571–1579. doi:10.1038/ismej.2011.41

Hoffmann, K., C. Hassenruck, V. Salman-Carvalho, M. Holtappels, and C. Bienhold. 2017. Response of bacterial communities to different detritus compositions in Arctic deep-sea sediments. Front. Microbiol. **8**: 266. doi:10.3389/fmicb.2017.00266

Hudson, I. R., and others. 2004. Temporal variations in fatty acid composition of deep-sea holothurians: Evidence of bentho-pelagic coupling. Mar. Ecol. Prog. Ser. **201**: 109–120.

Iken, K., and others. 2001. Food-web structure of the benthic community at the porcupine abyssal plain (NE Atlantic): A stable isotope analysis. Prog. Oceanography **50**: 383–405.

International Seabed Authority. 2010*a*. Development of geological models for the Clarion-Clipperton Zone polymetallic nodule deposits. ISA Technical Study 6. Kingston, Jamaica.

International Seabed Authority. 2010*b*. Recommendations for the guidance of contractors for the assessment of the possible environmental impacts arising from exploration for polymetallic nodules in the Area. Issued by the Legal and Technical Commission (ISBA/16/LTC/7).

Jeffreys, R. M., and others. 2013. Feeding preferences of abyssal macrofauna inferred from in situ pulse chase experiments. PLoS One **8**: e80510. doi:10.1371/journal.pone.0080510

Jones, D. O. B., and others. 2017. Biological responses to disturbance from simulated deep-sea polymetallic nodule mining. PLoS One **12**: e017750.

Kanzog, C., A. Ramette, N. V. Quéric, and M. Klages. 2009. Response of benthic microbial communities to chitin enrichment: An in situ study in the deep Arctic Ocean. Polar Biol. **32**: 105–112. doi:10.1007/s00300-008-0510-4

Karl, D. M., M. J. Church, J. E. Dore, R. M. Letelier, and C. Mahaffey. 2012. Predictable and efficient carbon sequestration in the North Pacific Ocean supported by symbiotic nitrogen fixation. Proc. Nat. Acad. Sci. USA **109**: 1842–1849.

Könneke, M., and others. 2014. Ammonia-oxidizing archaea use the most energy efficient aerobic pathway for CO_2 fixation. Proc. Natl. Acad. Sci. USA **111**: 8239–8244.

Lampitt, R. S., and A. N. Antia. 1997. Particle flux in deep seas: Regional characteristics and temporal variability. Deep-Sea Res. Part II **44**: 1377–1403.

Levin, L. A., and others. 2016. Defining "serious-harm" to the marine environment in the context of deep-seabed mining. Mar. Foreign Policy **74**: 245–259.

Lindh, M. V., and others. 2015. Transplant experiments uncover Baltic Sea basin-specific responses in bacterioplankton community composition and metabolic activities. Front. Microbiol. **6**: 223.

Lochte, K., and C. M. Turley. 1988. Bacteria and cyanobacteria associated with phytodetritus in the deep sea. Nature **333**: 67–69. doi:10.1038/333067a0

Lutz, M. J., K. Caldeira, R. B. Dunbar, and M. J. Behrenfeld. 2007. Seasonal rhythms of net primary production and particulate organic carbon flux to depth describe the efficiency of biological pump in the global ocean. J. Geophys. Res. **112**: C10011.

Mevenkamp, L., T. Stratmann, K. Guilini, L. Moodley, D. van Oevelen, A. Vanreusel, S. Westerlund, and A. K. Sweetman. 2017. Impaired short-term functioning of a benthic community from a deep Norwegian fjord following deposition of mine tailings and sediments. Front. Mar. Sci. **4**: 169. doi:10.3389/fmars.2017.00169

Middelburg, J. J. 2011. Chemoautotrophy in the ocean. Geophys. Res. Lett. **38**: L24604.

Middelburg, J. J. 2014. Stable isotopes dissect aquatic food webs from the top to the bottom. Biogeosciences **11**: 2357–2371. doi:10.5194/bg-11-2357-2014

Middelburg, J. J. 2018. Reviews and syntheses: To the bottom of carbon processing at the seafloor. Biogeosciences **15**: 413–427. doi:10.5194/bg-15-413-2018

Miljutin, D. M., M. A. Milijutin, P. M. Arbizu, and J. Galéron. 2011. Deep-sea nematode assemblage has not recovered 26 years after experimental mining of polymetallic nodules (Clarion-Clipperton fracture zone, tropical eastern Pacific). Deep-Sea Res. Part I **58**: 885–897.

Moeseneder, M.M., K.L. Smith Jr, H.A. Ruhl, D.O.B. Jones, U. Witte, and J.I. Prosser. 2012. Temporal and depth-related differences in prokaryotic communities in abyssal sediments associated with particulate organic carbon flux. Deep-Sea Res. Part I **70**: 26–35.

Molari, M., E. Manini, and A. Dell'Anno. 2013. Dark inorganic carbon fixation sustains the functioning of benthic deep-sea ecosystems. Global Biogeochem. Cycles **27**: 212–221. doi:10.1002/gbc.20030

Moodley, L., H. T. S. Boschker, J. J. Middleburg, R. Pel, P. M. J. Herman, E. DeDeckere, and C. H. R. Heip. 2000. Ecological significance of benthic foraminifera: 13C-labelling experiments. Mar. Ecol. Prog. Ser. **202**: 289–295. doi:10.3354/meps202289

Moodley, L., J. J. Middelburg, H. T. S. Boschker, G. Duineveld, R. Pel, and P. M. J. Herman. 2002. Bacteria and foraminifera: Key players in a short-term deep-sea benthic response to phytodetritus. Mar. Ecol. Prog. Ser. **236**: 23–29. doi:10.3354/meps236023

Moodley, L., J. J. Middelburg, K. Soetaert, H. T. S. Boschker, P. M. J. Herman, and C. H. R. Heip. 2005. Similar rapid responses to phytodetritus deposition in shallow and deep-sea sediments. J. Mar. Res. **63**: 457–469. doi:10.1357/0022240053693662

Nomaki, H., P. Heinz, T. Kakatsuka, M. Shimanaga, and H. Kitazato. 2005. Species-specific ingestion of organic carbon

by deep-sea benthic foraminifera and meiobenthos: In-situ tracer experiments: Limnol. Oceanography **50**: 134–146.

Nozawa, F., H. Kitazato, M. Tsuchiya, and A. J. Gooday. 2006. Live' benthic foraminifera at an abyssal site in the equatorial Pacific nodule province: Abundance, diversity and taxonomic composition. Deep-Sea Res. Part I **51**: 1406–1422.

Pachiadaki, M. G., and others. 2017. Major role of nitrite-oxidizing bacteria in dark ocean carbon fixation. Science **358**: 1046–1051. doi:10.1126/science.aan8260

Pedersen, R. B., and others. 2010. Discovery of a black smoker field and a novel vent fauna at the ultraslow spreading Arctic Mid-Ocean ridges. Nat. Commun. **1**: 126. doi:10.1038/ncomms1124

Pfannkuche, O. 1993. Benthic response to the sedimentation of particulate organic matter at the BIOTRANS station, 47°N, 20°W. Deep-Sea Res. Part II **40**: 135–149.

Pozzato, L., D. Van Oevelen, L. Moodley, K. Soetaert, and J. J. Middelburg. 2013. Sink or link? The bacterial role in benthic carbon cycling in the Arabian Sea's oxygen minimum zone. Biogeosciences **10**: 6879–6891. doi:10.5194/bg-10-6879-2013

Radziejewska, T. 2002. Responses of deep-sea meiobenthic communities to sediment disturbance simulating effects of polymetallic nodule mining. Int. Rev. Hydrobiol. **87**: 457–477. doi:10.1002/1522-2632(200207)87:4<457::AID-IROH457>3.0.CO;2-3

Rajendran, N., Y. Suwa, and Y. Urushigawa. 1993. Distribution of phospholipid ester-linked fatty acid biomarkers for bacteria in the sediments of Ise Bay, Japan. Mar. Chem. **42**: 39–56. doi:10.1016/0304-4203(93)90248-M

Rajendran, N., O. Matsuda, Y. Urushigawa, and U. Simidu. 1994. Characterization of microbial community structure in the surface sediment of Osaka Bay, Japan, by phospholipid fatty acid analysis. Appl. Environ. Microbiol. **60**: 248–257.

Resing, J. A., and others. 2015. Basin-scale transport of hydrothermal dissolved metals across South Pacific Ocean. Nature **523**: 200–203. doi:10.1038/nature14577

Rowe, G. T., and J. W. Deming. 1985. The role of bacteria in the turnover of organic carbon in deep-sea sediments. J. Mar. Res. **43**: 925–950. doi:10.1357/002224085788453877

Rowe, G. T., and others. 2008. Comparative biomass structure and estimated carbon flow in food webs in the deep Gulf of Mexico. Deep-Sea Res. **II**: 2699–2711.

Ruhl, H. A., J. A. Ellena, and K. L. Smith Jr. 2008. Connections between climate, food limitation, and carbon cycling in abyssal sediment communities. Proc. Natl. Acad. Sci. USA **105**: 17006–17011.

Shulse, C. N., B. Maillot, C. R. Smith, and M. J. Church. 2017. Polymetallic nodules, sediments, and deep waters in the equatorial North Pacific exhibit highly diverse and distinct bacterial, archaeal, and microeukaryotic communities. Microbiol. Open **6**: e00428. doi:10.1002/mbo3.428

Smith, C. R., and C. Rabouille. 2002. What controls the mixed-layer depth in deep-sea sediments: The importance of POC flux. Limnol. Oceanogr. **47**: 418–426. doi:10.4319/lo.2002.47.2.0418

Smith, C. R., and A. W. J. Demopoulos. 2003. Ecology of the deep Pacific Ocean floor, p. 179–218. *In* P. A. Tyler [ed.], Ecosystems of the world, volume 28: Ecosystems of the deep ocean. Elsevier.

Smith, C. R., F. C. De Leo, A. F. Bernardino, A. K. Sweetman, and P. M. Arbizu. 2008*a*. Abyssal food limitation, ecosystem structure and climate change. Trends Ecol. Evol. **23**: 518–528.

Smith, C. R., L. A. Levin, J. A. Koslow, P. A. Tyler, and A. G. Glover. 2008*b*. The near future of deep seafloor ecosystems, p. 334–351. *In* N. Polunin [ed.], Aquatic ecosystems: Trends and global prospects. Cambridge Univ. Press.

Smith, C. R., and others. 1997. Latitudinal variations in benthic processes in the abyssal equatorial Pacific: Controlled by biogenic particle flux. Deep-Sea Res. Part II. **44** (9–10): 2295–2317.

Smith, K. L., Jr., and R. S. Kaufmann. 1999. Long-term discrepancy between food supply and demand in the deep eastern North Pacific. Science **284**: 1174–1177. doi:10.1126/science.284.5417.1174

Smith, K. L., Jr., R. S. Kaufmann, R. J. Baldwin, and A. F. Carlucci. 2001. Pelagic-benthic coupling in the abyssal eastern North Pacific: An 8-year time-series study of food supply and demand. Limnol. Oceanogr. **51**: 166–176.

Smith, K. L., Jr., H. A. Ruhl, M. Kahru, C. L. Huffard, and A. D. Sherman. 2013. Deep ocean communities impacted by changing climate over 24 y in the abyssal Northeast Pacific. Proc. Nat. Acad. Sci. USA **110**: 19838–19841.

Smith, K. L., Jr., and others. 2009. Climate, carbon cycling and deep-ocean ecosystems. Proc. Natl. Acad. Sci. USA **106**: 19211–19218.

Smith, K. L., Jr., and others. 2014. Large salp bloom export from the upper ocean and benthic community response in the abyssal Northeast Pacific: Day to week resolution. Limnol. Oceanography **59**: 745–757.

Snider, L. J., B. R. Burnett, and R. R. Hessler. 1984. The composition and distribution of meiofauna and nanobiota in a central North Pacific deep-sea area. Deep-Sea Res. Part. A **31**: 1225–1249.

Stratmann, T., L. Mevenkamp, A. K. Sweetman, A. Vanreusel, and D. van Oevelen. 2018. Has phytodetritus processing by an abyssal soft-sediment community recovered 26 years after an experimental disturbance? Front. Mar. Sci. **5**: 59.

Sweetman, A. K., S. Sommer, O. Pfannkuche, and U. Witte. 2009. Retarded response by macrofauna-size foraminifera to phytodetritus in a deep Norwegian fjord. J. Foramin. Res. **39**: 15–22.

Sweetman, A. K., and U. Witte. 2008. Response of an abyssal macrofaunal community to a phytodetrital pulse. Mar. Ecol. Prog. Ser. **355**: 73–84. doi:10.3354/meps07240

Sweetman, A. K., K. Norling, C. Gunderstad, B. Haugland, and T. Dale. 2014. Benthic ecosystem functioning beneath fish farms in different hydrodynamic environments. Limnol. Oceanogr. **59**: 1139–1151. doi:10.4319/lo.2014.59.4.1139

Sweetman, A. K., and others. 2010. Impacts of exotic mangrove forests and mangrove deforestation on carbon remineralization and ecosystem functioning in marine sediments. Biogeosciences **7**: 2129–2145. doi:10.5194/bg-7-2129-2010

Sweetman, A. K., and others. 2016. Jellyfish decomposition at the seafloor rapidly alter biogeochemical cycling and carbon flow through benthic food-webs. Limnol. Oceanogr. **61**: 1449–1461.

Sweetman, A. K., and others. 2017. Major impacts of climate change on deep-sea benthic ecosystems. Elem. Sci. Anthropol. **5**: 4.

Thurber, A. R., A. K. Sweetman, B. E. Narayanaswamy, D. O. B. Jones, J. Ingels, and R. L. Hansmann. 2014. Ecosystem functions and services in the deep sea. Biogeosciences **11**: 3941–3963.

Tully, B. J., and J. F. Heidelberg. 2016. Potential mechanisms for microbial energy acquisition in oxic deep-sea sediments. Appl. Environ. Microbiol. **82**: 4232–4243. doi:10.1128/AEM.01023-16

Van Nugteren, P., P. M. J. Herman, L. Moodley, J. J. Middelburg, M. Vos, and C. H. R. Heip. 2009. Spatial distribution of detrital resources determines the outcome of competition between bacteria and a facultative detritivorous worm. Limnol. Oceanogr. **54**: 1413–1419. doi:10.4319/lo.2009.54.5.1413

van Oevelen, D., K. Soetaert, and C. H. R. Heip. 2012. Carbon flows in the benthic food web of the porcupine abyssal plain: The (un)importance of labile detritus in supporting microbial and faunal carbon demands. Limnol. Oceanogr. **57**: 645–664, DOI: 10.4319/lo.2012.57.2.0645

Weaver, P., and others. 2016. Research highlights. MIDAS: Managing impacts of deep-sea resource exploitation. Available from http://www.eu-midas.net/sites/default/files/downloads/MIDAS_research_highlights_low_res.pdf.

Wegener, G., M. Bausch, T. Holler, N. M. Thang, X. P. Mollar, M. Y. Kellermann, K.-U. Hinrichs, and A. Boetius. 2012. Assessing sub-seafloor microbial activity by combined stable isotope probing with deuterated water and ^{13}C-bicarbonate. Environ. Microbiol. **14**: 1517–1527.

Wei, C. L., and others. 2010. Global patterns and predictions of seafloor biomass using random forests. PLoS One **5**: e15323. doi:10.1371/journal.pone.0015323

Wigham, B. D., I. R. Hudson, D. S. M. Billett, and G. A. Wolff. 2003. Is long-term change in the abyssal Northeast Atlantic driven by qualitative changes in export flux? Evidence from selective feeding in deep-sea holothurians. Prog. Oceanogr. **59**: 409–441. doi:10.1016/j.pocean.2003.11.003

Wigham, B. D., E. A. Galley, C. R. Smith, and P. A. Tyler. 2008. Interannual variability and potential for selectivity in the diets of deep-water Antarctic echinoderms. Deep-Sea Res. Part II **55**: 2478–2490.

Witte, U., and others. 2003*a*. In situ experimental evidence of the fate of a phytodetritus pulse at the abyssal sea floor. Nature **424**: 763–765. doi:10.1038/nature01799

Witte, U., N. Aberle, W. Sand, and F. Wenzhofer. 2003*b*. Rapid response of a deep-sea benthic community to POM enrichment: An in situ experimental study. Mar. Ecol. Prog. Ser. **251**: 27–36. doi:10.3354/meps251027

Wooley, S. N. C., and others. 2016. Deep-sea diversity patterns are shaped by energy availability. Nature **533**: 393–396. doi:10.1038/nature17937

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Conflict of interest

There are no competing financial interests.

Author contributions

A.K.S. designed the experiments, and together with C.R.S., A.J.G., and M.J.C., they generated the funding for the study. A.K.S. carried out the experiments with K.S.M. Analysis on the AB01 and AB02 samples was conducted by A.K.S., C.N.S., B.M., T.S., M.L., and D.v.O. Interpretation of the data was performed by A.K.S., C.R.S., C.N.S., M.L., M.J.C., D.v.O., and A.J.G. A.K.S. wrote the manuscript with input from all coauthors. All authors edited various versions of the article.

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