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# Benthic microbial and whole-community responses to different amounts of $^{13}C$-enriched algae: In situ experiments in the deep Cretan Sea (Eastern Mediterranean)

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*Abstract*

The deep eastern Mediterranean Sea is one of the most oligotrophic regions of the world's oceans and is therefore highly suitable for investigating the response of a resource-limited deep-sea benthic community to food pulses. Additionally, the benthic response to organic matter (OM) deposition can differ according to the amount of OM entering the sediment. Here, we report on 36-h pulse–chase experiments with $^{13}C$-labeled diatoms as a tracer of labile OM. Two loads of OM, equivalent to 25 mg C $m^{-2}$ (low-carbon [L] experiment) and 250 mg C $m^{-2}$ (high-carbon [H] experiment), were injected into chambers of a benthic lander. The fate of the added carbon was followed in total community respiration and bacterial assimilation (through $^{13}C$ enrichment in bacteria-specific fatty acids). A benthic response was clearly evident in both experiments, but carbon turnover rates were higher with a higher OM addition. However, although a larger amount of carbon was assimilated by bacteria in the H experiment, bacterial assimilation accounted for a relatively lower proportion of total carbon processing compared with the L experiment. This result could indicate a modified bacterial response, a larger role of other biotic components in the high-load experiment, or both. This study demonstrates that an impoverished deep-sea benthic community rapidly responds to an input of fresh OM. Furthermore, the carbon flow pathways can differ according to the amount of OM entering the sediment. Consequently, comparison of benthic responses across systems can only be made in cases in which similar quantities and qualities of OM have been used.

With the discovery of life in the deep ocean and the first profound studies of deep-sea fauna (e.g., with the HMS *Porcupine* in 1868–1869 and the HMS *Challenger* in 1872–1876), the question arose of how these organisms could survive without obvious food resources. In the early 20th century, the concept that all life in the ocean ultimately depended on primary production in the euphotic zone was developed, and it was not until the late 1970s that the belief of a constant, fine "rain" of small particles to the deep-sea floor was challenged by the hypothesis of the aggregation and subsequent rapid sinking of marine snow (Trent et al. 1978). In the early 1980s, time-lapse cameras moored in the deep sea provided photographic evidence for seasonally occurring fluffy layers of fresh phytodetritus covering the deep-sea floor (Billett et al. 1983). Great effort was put into investigation of both sedimentation events (e.g., Honjo and Manganini 1993) as well as the benthic response to such an event. Although data are available for different functional

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groups (e.g., Boetius and Lochte 1996), the unpredictability of sedimentation events severely hampered an in-depth understanding of the subject.

Advances were made with studies that experimentally simulated a settling phytoplankton bloom in situ (e.g., Moodley et al. 2000; Witte et al. 2003*b*). With tracer experiments inside the chambers of benthic landers, these investigations used artificial food pulses of known quality, quantity, and timing to investigate the reaction of deep-sea communities to a food pulse. Although clearly demonstrating that the benthic community rapidly process incoming carbon, observations were sometimes contradictory. For example, bacterial assimilation of tracer carbon at 4,800-m water depth at the Porcupine Abyssal Plain showed a delay of 8 d (Witte et al. 2003*b*), whereas investigations of Moodley et al. (2002) revealed bacteria reacting within 36 h at a 2,200-m-deep station northwest of Spain. Additionally, in general, these investigations were not laid out to closely match natural conditions. To not miss a potential benthic response, the authors added relatively high amounts of organic carbon that were unlikely to settle within a few days. Here, we examine the response of an impoverished benthic community to an input of low versus high organic matter (OM) input. The deep Cretan Sea (northeast Mediterranean) is one of the most oligotrophic deep-sea areas of the world's oceans (Tselepides et al. 2000*b*; Lykousis et al. 2002). Because of low terrestrial runoff and large volume, the Mediterranean is an oligotrophic ocean basin, with increasing nutrient depletion from west to east (Turley et al. 2000). Integrated production in the Aegean Sea is ~15.2 g C $m^{-2}$ $yr^{-1}$ (Lykousis et al. 2002), with only 2–3% of the export production reaching the sediment (Danovaro et al. 1999), resulting in a highly food-limited deep-sea environment. The bacterial biomass (0.4 g C $m^{-2}$; Duineveld et al. 2000) and macrofaunal biomass (0.06 g C $m^{-2}$; Tselepides et al. 2000*a*) in 1,500-m water depth in the Cretan Sea are comparable to the Porcupine Abyssal Plain in the North Atlantic at 4,800 m (bacterial biomass 0.25 g C $m^{-2}$, macrofaunal biomass 0.12 g C $m^{-2}$; Witte et al. 2003*b*), underlining the oligotrophy of the eastern Mediterranean Sea.

The aim of this study was to investigate whether the benthic response to a food pulse varies—both qualitatively and quantitatively—with the amount of carbon settling to the seafloor. An addition of 250 mg C $m^{-2}$, equivalent to approximately half of the yearly export production in the area of investigation (300–400 mg C $m^{-2}$ $yr^{-1}$, calculated after Lykousis et al. [2002] and Danovaro et al. [1999]), and an addition of 25 mg C $m^{-2}$ were performed, which is $\frac{1}{20}$ of the annual food load and an amount that can easily be deposited within 1 d. The leading hypothesis for our investigation was that a higher amount of food provided to the benthos will result in a corresponding increase in overall carbon turnover rates that could induce different reaction patterns within the benthic community.

Simulated pulses of OM, consisting of the $^{13}C$-labeled diatom *Thalassiosira rotula*, were created in the chambers of an autonomous benthic lander system. The benthic response was followed in total community respiration and bacterial assimilation. Respiration of tracer carbon might not always be evident in sediment community oxygen consumption (SCOC) enhancement (Moodley et al. 2002; Witte et al. 2003*a*); here, we compared respiration of the added carbon estimated indirectly through enhancement in SCOC and directly through $^{13}C$ enrichment in $CO_2$. Bacterial assimilation was assessed by incorporation of tracer into bacterial fatty acids.

## Materials and methods

*Study site*—The experiments were carried out in June 2002 with RV *Philia* at 1,540-m water depth in the southern Cretan Sea off Crete (35°44.15′N, 25°8.40′E; eastern Mediterranean). Our experimental site is located at the southern boundary of the Cretan Sea in 1,540 m of water depth and is a site that was intensely studied during the CINCS project (pelagic–benthic coupling in the oligotrophic Cretan Sea; Tselepides and Polychronaki 2000).

*Culture of axenic diatoms*—Before the experiments, an axenic clone of the diatom *T. rotula* (*Bacillariophyceae, Thallassiosiraceae*) was cultured at 16°C in artificial seawater (Grasshoff 1999) with a salinity of 35, enriched with f/2 medium (Guillard and Ryther 1962). The medium contained 25% $^{13}C$-bicarbonate (99% $NaH^{13}CO_3$, Cambridge Isotope Laboratories). The algal material was harvested by centrifugation (404 × g, 4 min), rinsed three times with an isotonic sodium chloride solution, and dried in vacuo. The axenic state of the algae was verified by microscopic observations and by incubation of a few drops of the culture, in the dark, on agar plates containing the culture medium and glucose. The produced algae contained 20 atom% $^{13}C$ (measured directly on an isotope ratio mass spectrometer [IRMS]; Fa. ThermoFinnigan). Before injection, the dried algae were brought into suspension with 0.2-$\mu$m filtered seawater.

*Experiments*—A deep-sea benthic chamber lander (for further details, *see* Witte and Pfannkuche 2000) was used for the experiments. It was equipped with three benthic chambers of 0.04 $m^2$ (20 × 20 cm) each. The injection of the labeled algae was achieved with an injection unit integrated into the lid of each chamber. Additionally, a small plastic piece was added to the algal solution to check for successful injection. One hour after insertion of the chambers into the sediment, 1.01 and 10.14 mg C of algae, corresponding to an addition of 25 and 250 mg C $m^{-2}$, respectively, were injected. Experiment duration was 36 h. Control experiments ran without algal injection. At the end of the incubations, a shutter at the bottom was closed to retrieve the sediment. Once the shutter was closed, the chamber was slowly heaved out of the sediment and the lander could be called back to the surface, where it arrived about 30 min later.

A stirrer suspended the injected algae in the chamber to achieve homogeneous distribution before sedimentation and ran throughout the duration of the experiment. Each chamber was equipped with a syringe water sampler, which was preprogrammed to take seven water samples of 50 mL every 6 h during the incubation. The first sample was taken 12 min after algae injection and was used as the 0-h incubation, correcting directly for possible contamination of $^{13}C$-bicarbonate still present in the diatom mix. SCOC was calculated

from the results of Winkler titration of water samples during the period of linear decrease of oxygen concentration. From each 50-mL syringe water sample, two replicate Winkler titrations were carried out. In total, nine syringe sample sets were analyzed for oxygen consumption, of which four were incubated with algae (two for the L and two for the H experiments).

Water samples for $\delta^{13}CO_2$ were filtered directly after lander recovery into 12-mL gas-tight glass scintillation vials sealed with a rubber septa and containing mercury chloride in an end concentration of 0.2% to stop bacterial activity. The samples were refrigerated until analysis.

At the end of the experiments, the chambers were processed (within 2 h) on the deck of the ship. Sediment samples were analyzed from three deployments: the first one resulted in one successful L experiment, the second one in one successful H experiment, and the third one for L and H experiments and a control chamber, whereas two of the three chambers during the first two deployments were control chambers not analyzed for sediment parameters. Five chambers were sampled in total (four experimental chambers, with two parallels each, for low (L)- and high (H)-carbon load experiments, and one control chamber) for the analysis of pore-water $\delta^{13}CO_2$ and bacterial biomarkers. Sediment was sliced in 1-cm intervals down to 5 cm and then in 2.5-cm intervals below that. Each sediment horizon was carefully homogenized before subsampling for lipid analysis and pore-water $\delta^{13}CO_2$. The samples for lipid analysis were stored frozen at −20°C until analysis, and the samples for $\delta^{13}CO_2$ were refrigerated until pressure filtration of the pore water. Teflon squeezers were used for pressure filtration. The squeezers were operated with argon at a pressure that was gradually increased up to 5 bars. The pore water was retrieved through 0.2-$\mu$m cellulose acetate membrane filters. A 2-mL aliquot of the pore water was kept at 4°C in a nitrogen-flushed 4-mL gas-tight glass scintillation vial containing mercury chloride (end concentration 0.2%).

*Lipid extraction and biomarker analyses*—The lipid extraction was performed according to Elvert et al. (2003). Concentrations were determined by gas chromatography (GC)–flame ionization detection and their stable isotope composition by GC-IRMS. Unknown compounds were investigated with the use of GC–mass spectrometry (for a detailed description, *see* Bühring et al. [2005]). The carbon isotopic ratios were corrected for the one methyl group inserted during derivatization.

*$^{13}CO_2$ analyses*—The determination of $^{13}C$ enrichment in $\Sigma CO_2$ was performed according to the method of Moodley et al. (2000). For the $\delta^{13}CO_2$ measurements, a head space was created in the scintillation vials by injecting nitrogen gas, and the sample was then acidified with 100 and 20 $\mu$L of sulfuric acid (20%) for the chamber and pore-water samples, respectively. The $\delta^{13}CO_2$ and concentrations of $CO_2$ in the headspace were measured with a Carlo Erba 1106 elemental analyzer coupled online with a Finnigan Delta S isotope ratio mass spectrometer. Reproducibility of the measurements was ~0.10‰.

*Calculations*—The carbon isotope ratios are expressed in the delta notation ($\delta^{13}C$) relative to Vienna Pee Dee belemnite (VPDB): $\delta^{13}C$ (‰) = $[(^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{reference} - 1] \times 1{,}000$. Incorporation of $^{13}C$ is reflected as excess $^{13}C$ (above background, measured in the control chambers) and is expressed in terms of specific incorporation ($\Delta\delta^{13}C = \delta^{13}C_{sample} - \delta^{13}C_{background}$) as well as total incorporation $I$. Specific incorporation is a clear indication of uptake of labeled algae but remains a qualitative measure because it is based on isotope ratios. Total incorporation $I$ is a quantitative measure of incorporation, and for the $CO_2$, is calculated as the product of excess $^{13}C$ ($E$) and the $CO_2$ concentration (Moodley et al. 2002). $E$ is the difference between the labeled fraction $F$ of the sample and background: $E = F_{sample} - F_{background}$, where $F = {}^{13}C/({}^{13}C + {}^{12}C) = R/(R + 1)$ and $R = (\delta^{13}C/1{,}000 + 1) \times R_{VPDB}$, with $R_{VPDB} = 0.0112372$. For bacteria, $I$ was calculated after Moodley et al. (2000) from label incorporation into bacterial fatty acids ($i$C$_{15:0}$, $ai$C$_{15:0}$, $i$C$_{16:0}$, $i$C$_{17:1\omega7}$, 10-*methyl*-C$_{16:0}$, $i$C$_{17:0}$, $ai$C$_{17:0}$, and C$_{17:1\omega6}$; *see*, e.g., Boschker and Middelburg 2002; Rütters et al. 2002) as $I_{bact} = \Sigma\, I_{bact\ fatty\ acids}/(a \times b)$, where $a$ is the average phospholipid-derived fatty acid (PLFA) concentration in bacteria of 0.056 g C PLFA g$^{-1}$ biomass (Brinch-Iversen and King 1990), $b$ is the average fraction-specific bacterial PLFA encountered in sediment dominated by bacteria (0.19; calculated after Findlay and Dobbs 1993; Rajendran et al. 1994; Guezennec and Fiala-Medioni 1996), and the prefixes "*i*" and "*ai*" mean "*iso*" and "*antiso*," respectively.

An unbalanced one-way analysis of variance (ANOVA) was used to test for differences in SCOC because of algal addition.

## Results

*Sediment community oxygen consumption*—The bottom water oxygen content obtained from the first syringe samples taken before addition of the algal material averaged 244 ± 1.5 $\mu$mol L$^{-1}$ ($n$ = 9). Background SCOC, as determined in five control chambers, averaged 0.63 ± 0.06 mmol m$^{-2}$ d$^{-1}$ ($n$ = 5).

L experiments did not induce a significant increase in SCOC, but the SCOC measured in the H experiments were significantly higher than the background and L experiments (unbalanced one-way ANOVA, $p < 0.001$; Fig. 1). By converting excess SCOC above background and with a respiratory quotient (RQ) of 1 (Witte et al. 2003*b*), approximately 15 mg C m$^{-2}$ of the added carbon was respired in the 36 h of the H experiment.

*Mineralization of added carbon*—The mineralization of the added carbon in the overlying water is given in Fig. 2. In the L experiments, we measured a $\Delta\delta^{13}C$ of 7.1‰ ± 3‰ in $\Sigma CO_2$ in the chamber water after 36 h. In contrast, in type H experiments already after 6 h, values of 145‰ ± 21‰ were reached, further increasing to 232‰ ± 2‰ after 36 h. The mineralization of added carbon in the pore water was detectable down to 3-cm depth for the L and 5-cm depth for the H experiments (Fig. 3), with $\Delta\delta^{13}C$ values reaching 2.5‰ and 17.1‰ for L and H experiments, respectively (mean value, 0–7.5-cm depth).

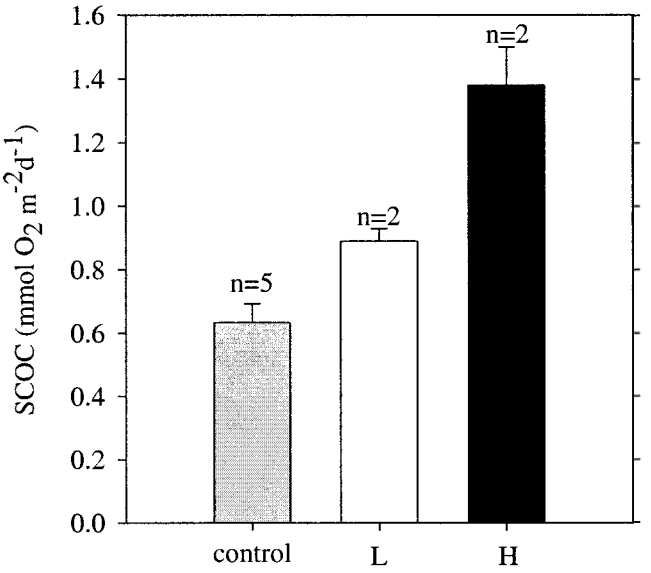


Fig. 1. Mean sediment community oxygen consumption (SCOC) for the control chambers and the L and H experiments. Error bars represent ±SD for the control and range for the experimental chambers.

In total (sum of overlying and pore water), 6% ± 2.7% (1.5 mg C $m^{-2}$) and 21% ± 1.1% (53.5 mg C $m^{-2}$) of added carbon was remineralized within 36 h in the L and H experiments, respectively.

*Relative abundances of bacterial fatty acids*—Figure 4 displays the relative abundance of all 23 fatty acids detected at our study site ($n$ = 5). Highest relative abundances were found for $C_{16:0}$, with 17.1% ± 0.8%, followed by $C_{18:1\omega9}$ (10.6% ± 7.4%) and 10*Me*-$C_{16:0}$ (9.5% ± 1.6%). $C_{18:0}$, $C_{18:1\omega7}$, $C_{16:1\omega7}$, *i*$C_{15:0}$, and *ai*$C_{15:0}$ follow in abundance. The other fatty acids contribute 4% or less.

*Incorporation of $^{13}C$ into bacteria*—Incorporation of our deliberate tracer was detectable in all bacterial fatty acids but in different amounts. Figure 5 shows the total incorporation of label into bacterial fatty acids ($C_{16:1\omega7}$ and $C_{18:1\omega7}$ are not shown because they are not bacteria specific and could be typical components of diatoms as well). In L experiments, most label was incorporated into *i*$C_{16:0}$ and *ai*$C_{15:0}$, with 7.8 and 4.8 μg $^{13}C$ $m^{-2}$, respectively, followed by *ai*$C_{17:0}$ with 1.2 μg $^{13}C$ $m^{-2}$. In other bacterial fatty acids, we found <1 μg $^{13}C$ $m^{-2}$. The H experiments were characterized by elevated incorporation into all bacterial fatty acids. Most label was again incorporated into *i*$C_{16:0}$ and *ai*$C_{15:0}$, followed by *ai*$C_{17:0}$, with 12.3, 6.9, and 4.3 μg $^{13}C$ $m^{-2}$, respectively. In total, twice as much label was incorporated into bacteria in H than in L experiments, accounting for 1.59 mg $^{13}C$ $m^{-2}$ (31.7% of the added label) and 3.43 mg $^{13}C$ $m^{-2}$ (6.8% of the added label) for the L and H experiments, respectively.

Incorporation into selected bacterial fatty acids versus sediment depth for L and H experiments is displayed in Fig. 6. For low carbon addition, although bacterial assimilation was evident down to 3.5 cm, the majority of bacterial assimilation was in the uppermost sediment layer. In contrast, for the H experiments, several investigated fatty acids displayed incorporation at deeper sediment depths. Comparing *i*$C_{15:0}$ and *ai*$C_{15:0}$, higher labeling and deeper penetration depth was measured for *ai*$C_{15:0}$. The incorporation into *i*$C_{16:0}$ displayed a subsurface labeling maximum between 3.5 and 4.5 cm. Incorporation into *i*$C_{17:1\omega7}$ during the H experiment showed low values at the surface and highest values in 1.5 cm of sediment depth. High incorporation into 10*Me*-$C_{16:0}$ could be observed at the surface and at 2.5-cm depth. The labeling pattern for *i*$C_{17:0}$, *ai*$C_{17:0}$, and $C_{17:1\omega6}$ were comparable with highest incorporation at the surface.

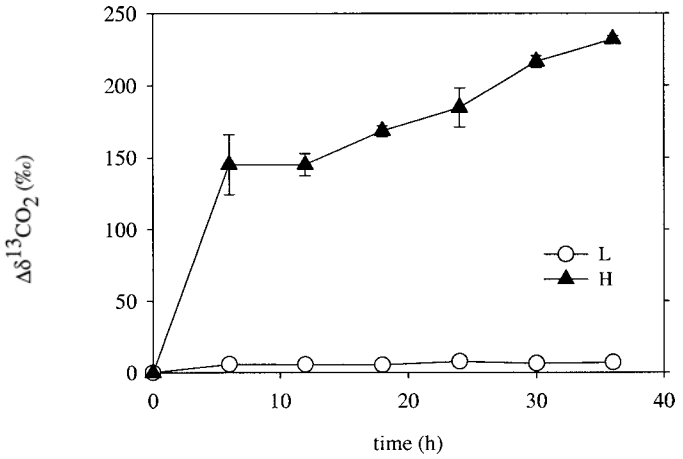


Fig. 2. $^{13}C$ enrichment in $\Sigma CO_2$ [‰] in the overlying water versus time for the L and H experiments. Error bars represent the range of two replicates.

## Discussion

The SCOC in the control chambers was 0.633 ± 0.06 mmol $O_2$ $m^{-2}$ $d^{-1}$, which is slightly higher than in investigations of Lykousis et al. (2002), who found 0.432 mmol

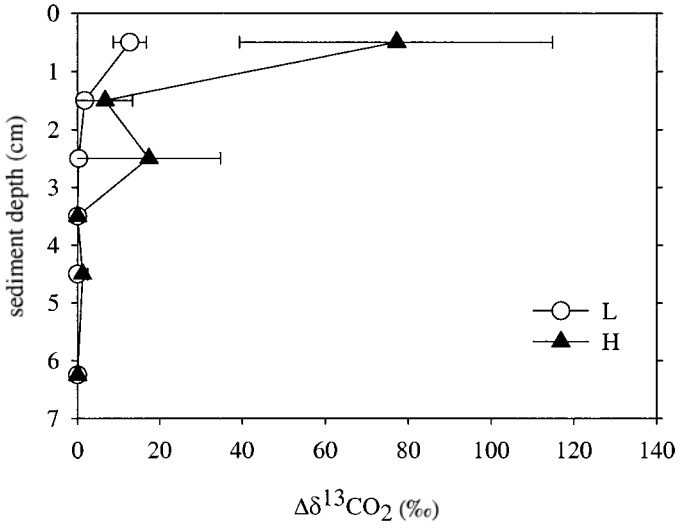


Fig. 3. $^{13}C$ enrichment in $\Sigma CO_2$ [‰] in the pore-water versus sediment depth for the L and H experiments after 36 h. Horizontal bars indicate the range of two replicates.

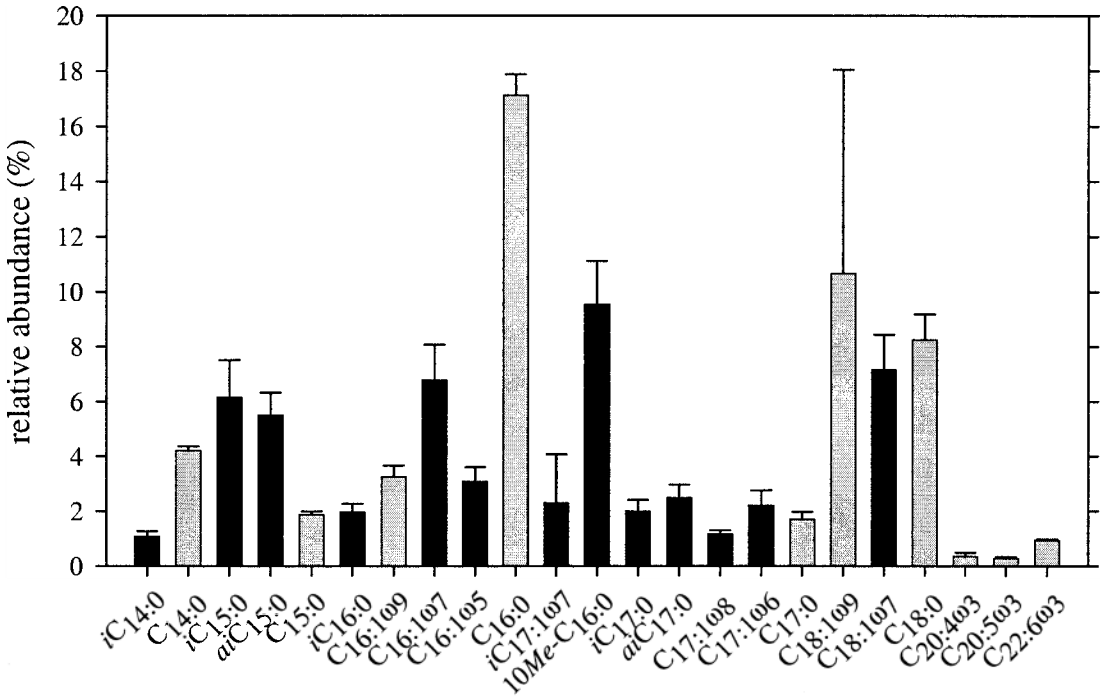


Fig. 4. Relative abundances of all fatty acids detected in all experimental ($n$ = 4) and one control chamber (0–3-cm depth; error bars indicate ±SD). Black bars indicate fatty acids of bacterial origin.

$O_2$ $m^{-2}$ $d^{-1}$ during Summer 1997 in the southern Aegean Sea. These values, measured at 1,500-m water depth, are lower than SCOC at abyssal sites of 4,000–5,000-m water depth in the northeast Atlantic or the Arabian Sea (0.6–1.4 and 0.9–6.3 mmol $O_2$ $m^{-2}$ $d^{-1}$, respectively; reviewed by Witte and Pfannkuche [2000]). Several investigations of SCOC on continental margin sediments revealed higher values as well (e.g., 3.6 mmol $O_2$ $m^{-2}$ $d^{-1}$ found in the deep Sognefjord by Witte et al. 2003*a*), confirming the oligotrophic status of our experimental site.

A striking observation is that the fatty acid 10*Me*-$C_{16:0}$ had the highest relative share of all bacterial fatty acids (Fig. 4). This fatty acid is described as a biomarker for *Desulfobacteriaceae* (Rütters et al. 2002), which is evidence for the importance of this group at our study site. The *Desulfobacteriaceae* belong to the sulfate-reducing bacteria (SRB),

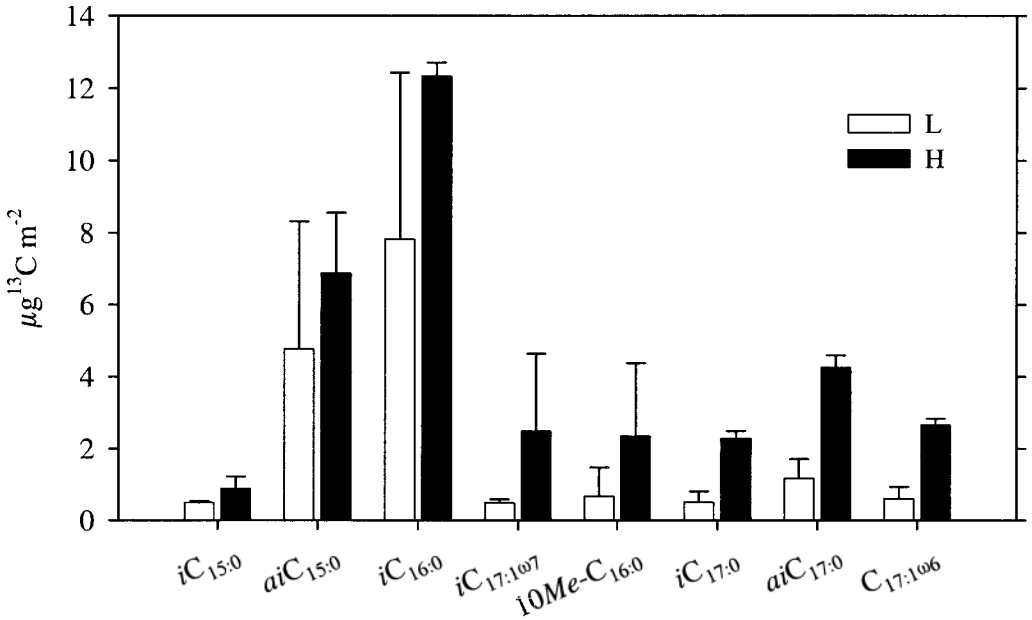


Fig. 5. Total incorporation of label into bacterial fatty acids ($\mu$g $^{13}C$ $m^{-2}$, sampling depth 0–7.5 cm). Error bars indicate the range of two replicates.

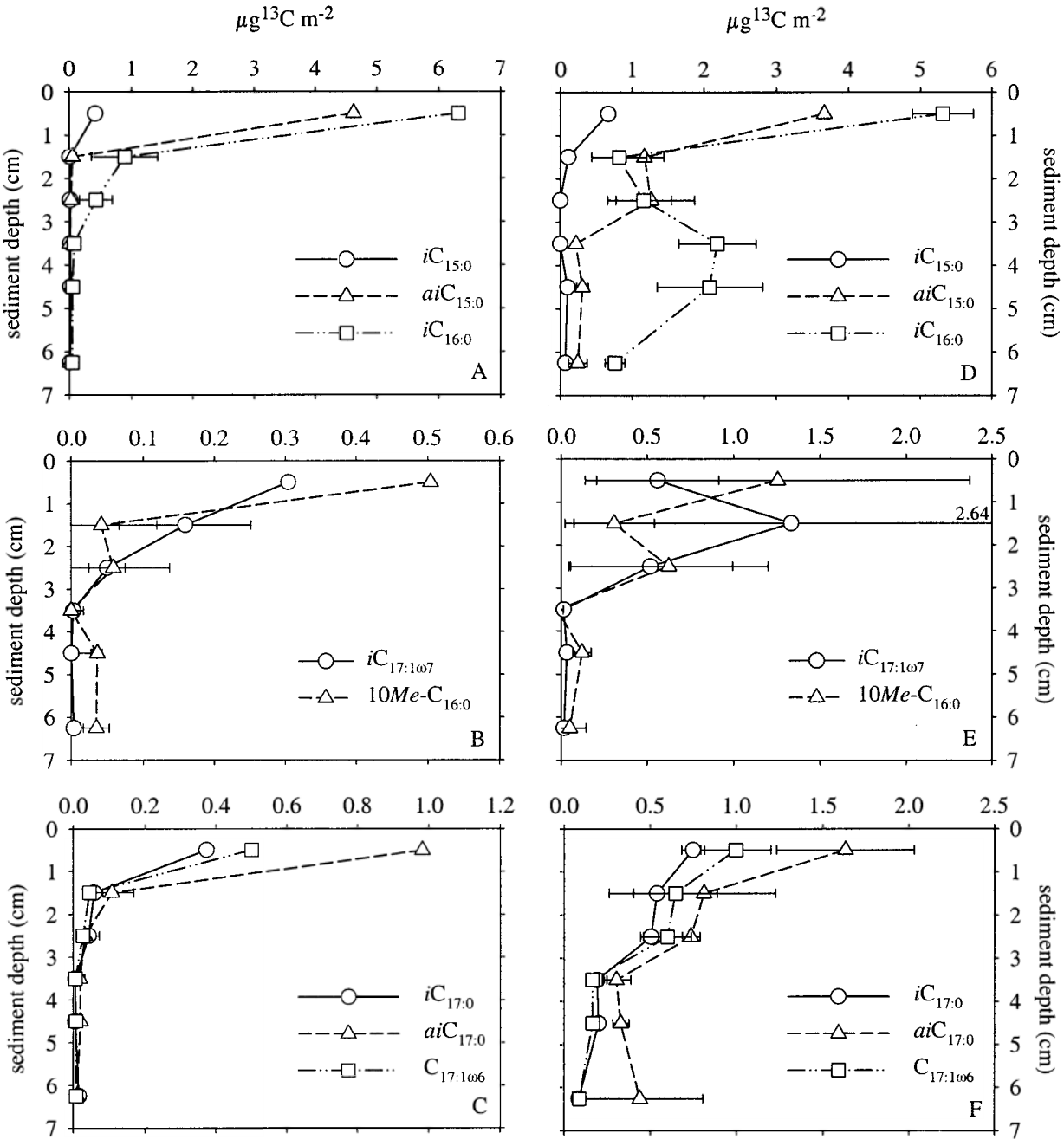


Fig. 6. Total incorporation of tracer $^{13}C$ into bacterial biomarkers versus sediment depth from the low-carbon load experiments (A–C) and high-carbon load experiments (D–F). Horizontal lines indicate the range of two replicates.

which form a coherent group within the δ-subdivision of the proteobacteria. Several other biomarkers confirm the existence of SRB. The fatty acids $iC_{17:0}$ and $aiC_{17:0}$ are generally considered to be of SRB origin (Findlay and Dobbs 1993), and $iC_{17:1\omega7}$ is very common in *Desulfosarcina* (Rütters et al. 2001). Considering the generally lower sulfate reduction activity in deep-sea sediments compared with shelf areas (Jørgensen 1982), the high relative abundances of SRB biomarkers is noteworthy. Only recently, Sass et al. (2002) isolated a new SRB from Mediterranean deep-sea anoxic sediments, *Desulfobulbus mediterraneus,* that is able to grow on carbohydrates. Its fatty acids were dominated by $C_{16:1\omega7}$, $C_{16:1\omega5}$, $C_{17:1\omega6}$, and $C_{18:1\omega7}$, which were all found at our study site, supporting the occurrence of this or a related species at our station.

Bacterial fatty acid concentrations did not increase significantly with time (data not shown). This is probably because of the short incubation time of 36 h, but it can also be speculated that the bacteria predominantly use the new material for energy-yielding processes, as shown by Bianchi et al. (2003) through glutamate uptake experiments in the southern Aegean Sea. We also have to keep in mind that we can only

monitor the "net biomass" of a microbial community that is constantly grazed upon.

The fatty acids $i\mathrm{C}_{16:0}$ and $ai\mathrm{C}_{15:0}$ showed the highest incorporation rates, corresponding to 13% (L) or 19% (H) of total incorporation into fatty acids, but on average, they contributed only 2–6% to total fatty acid concentrations. $i\mathrm{C}_{16:0}$ and $ai\mathrm{C}_{15:0}$ together with $i\mathrm{C}_{15:0}$ are very common in gram-positive prokaryotes (White et al. 1996), indicating that these bacteria must have processed the algal material immediately by either taking up DOC released from the algae or by uptake following a rapidly induced production of exoenzymes. Low incorporation patterns were found for biomarkers of SRBs, like $10Me\text{-}\mathrm{C}_{16:0}$. SRB usually take up secondary carbon products, which differ between the species. Some are even capable of using autotrophic pathways. The low incorporation of our deliberate tracer is consistent with the theory that the SRB have not directly processed the added POC during the course of our experimental duration but probably rely on the delivery of secondary carbon products such as short-chain fatty acids released by fermentative bacteria. It can be assumed that longer incubation times would probably lead to higher incorporation of label into the SRB biomarker.

Although label incorporation was higher when more carbon was available, there clearly is no 1 : 1 relation between the amount of fresh carbon available (L : H = 1 : 10) and bacterial carbon assimilation (L : H = 1 : 2). In L experiments, the incorporation of label into bacterial fatty acids was concentrated at the surface sediment (Fig. 6). This is in good agreement with the pore-water $\Delta\delta^{13}CO_2$ profiles (Fig. 3). During the H experiments, however, more pronounced subsurface bacterial label incorporation was evident (Fig. 6), again in accordance with the $\Delta\delta^{13}CO_2$ profiles. This indicates that mixing and penetration depth of OM is strongly related to the amount of OM entering the sediment. Graf (1989) observed the rapid subduction of fresh OM by bioturbating animals and proposed that this process provides deeper living organisms with relatively fresh material. In oligotrophic sediments, infauna often pull settled food material to deeper sediment layers to avoid competition. A rapid downward transport of food material down to 10-cm depth in the Cretan Sea was observed by Danovaro et al. (1999), as well as high bioturbation activity of infauna at our study site (Tselepides et al. 2000*a*). Witte et al. (2003*b*) found considerable incorporation of labeled algal material by macrofauna organisms at up to 10-cm depth already after 2.5 d by performing in situ experiments at a 4,800-m-deep station in the northeast Atlantic. They found that the subducting activity of macrofauna is related to or triggered by the amount of food available. In comparison to our study, Witte et al. (2003*b*) found a time lag of 8–23 d before incorporation into bacteria took place, with restriction to the upper sediment horizons, which could be a result of the lower water temperatures in their study. During gut passage of larger animals, the algal material is altered and secondary carbon products that could be taken up by SRB can become available.

As stated above, SCOC differed significantly between L and H experiments, indicating that it is modified by the amount of "fresh" organic carbon available in this oligotrophic deep-sea sediment. However, there is no 1 : 1 correlation between the variation in the amount of carbon added (1 : 10) and the increase in SCOC (1 : 1.5). $^{13}CO_2$ enrichment in $\Sigma CO_2$, on the other hand, is a very good tool to use to follow the mineralization of the added tracer carbon (Moodley et al. 2002) and proved to be highly sensitive to the different experimental conditions. Our experiments revealed a rapid benthic mineralization of the added carbon in the L and H experiments, as reflected in the increase in $\Delta\delta^{13}C$ in $\Sigma CO_2$ during the investigated time period (Fig. 2). However, the values for the H experiments were >30-fold increased compared with the L setup. Obviously, the addition of the large quantity of organic carbon boosts the mineralization; after only 6 h, values as high as 145‰ ± 20‰ were reached in the overlying water.

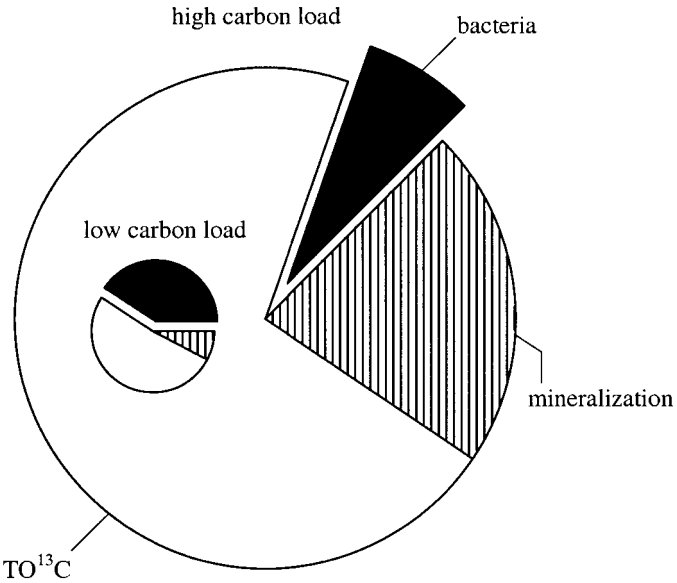


Fig. 7. Comparison of total incorporation of label into bacteria, mineralization of tracer POC to $^{13}CO_2$, and recovered total $O^{13}C$ ($TO^{13}C$) for the L and H experiments. The size of a pie's slice corresponds to the total amount of recovered label. The total organic carbon (TOC) values are corrected for the $^{13}C$ found in bacterial biomass.

Furthermore, the carbon pathways differed between the two experiments. Whereas in the L experiments the label was preferentially assimilated into bacterial fatty acids and only low $^{13}C$ enrichment in $CO_2$ was detected, the majority of the processed carbon was remineralized during the H experiments. This is probably caused by higher respiration activity of macrofaunal organisms. The low amount of label recovered in $CO_2$ compared with incorporation into bacteria during the L experiments (Fig. 7) indicate an additional respiration of "bulk" organic carbon already present in the sediment.

A comparison of carbon equivalents calculated from the SCOC (assuming a RQ of 1; *see Table 1*) reveals that 10% of carbon mineralization (1.5 mg C $m^{-2}$) came from the addition of the low carbon load (L experiments). During the H experiments, mineralization of the added carbon exceeded the SCOC by a factor of two (53.5 mg C $m^{-2}$). By subtracting the background oxygen consumption (no added algae), mineralization of the added carbon even surpasses the SCOC by a factor of four. Discrepancies in oxygen versus carbon dioxide budgets are reported frequently. Ferguson et al.

Table 1. Comparison of the amount of added carbon respired within 36 h on the basis of two methods: directly via $^{13}C$ enrichment in $\Sigma CO_2$ and via excess SCOC above background values (assuming an RQ of 1). BCL, benthic chamber lander.

| Deployment | Load | C respired via $^{13}CO_2$ (mg C $m^{-2}$) | Total SCOC (mg C $m^{-2}$) | C respired via excess SCOC above background (mg C $m^{-2}$) |
|---|---|---|---|---|
| BCL 1, chamber 2 | L | 0.83 | 16.54 | 5.15 |
| BCL 4 chamber 1 | L | 2.17 | 12.95 | 1.56 |
| BCL 3, chamber 2 | H | 56.15 | 24.93 | 13.54 |
| BCL 4, chamber 2 | H | 50.78 | 27.01 | 15.62 |

(2003) found uncorrelated $O_2$ and total $CO_2$ respiration rates in subtropical estuaries and suggested that benthic metabolism is characterized by periods of anaerobic mineralization during periods of high OM supply. It could therefore be concluded that the SCOC might underestimate the total turnover depending on the available amount or quality of OM.

Several earlier investigations revealed an increase in SCOC because of POM addition of ~50% (e.g., Moodley et al. 2002). These investigations led to the speculation that deep-sea benthic communities can only switch between a "low-turnover" and a "high-turnover" mode. Here, we demonstrated the capability of different responses as a result of the amount of algae added. Our results clearly show that both the whole and the microbial benthic community at our deep-sea study site are able to calibrate their reaction depending on the amount of settling phytoplankton.

The amount of processed carbon (incorporation into bacteria plus $CO_2$ produced from added carbon) was 6 mg C $m^{-2}$ $d^{-1}$ and 47 mg C $m^{-2}$ $d^{-1}$ for the L and H experiments, respectively. Taking into account that, for the H experiments, the amount of added algae (250 mg C $m^{-2}$) corresponds to half of the yearly export production to the deep Cretan Sea, the carbon processing rate of 47 mg C $m^{-2}$ $d^{-1}$ is comparatively high. Witte et al. (2003*b*) investigated the response of a benthic community in 4,800-m depth in the northeast Atlantic to algae addition and found processing rates of 4 mg C $m^{-2}$ $d^{-1}$. The addition of 1 g C $m^{-2}$ corresponds to the annual input at that study site. Moodley et al. (2002) used a diatom pulse of 229 mg C $m^{-2}$ at 2,150-m water depth in the northeast Atlantic (~0.7% of the OC content of the sediment) and found processing rates of 4–6 mg C $m^{-2}$ $d^{-1}$ of added carbon. Moodley et al. (2002) suggested that the prevailing conditions of low temperature in combination with low biomass concentrations and composition slow down the recycling of OM at this deep-sea study site. Following this hypothesis, our high processing rates could also be due to the higher bottom water temperature in the deep Cretan Sea. A direct comparison is hampered by our failure to include incorporation of macro- and meiofauna into our investigations. However, because bacteria are described as the primary agents for degrading settling particles arriving at the deep-sea floor (Danovaro et al. 1999), the different observations are comparable. The relatively high water temperature, with >13°C at bathyal depth (Danovaro et al. 2000), can be expected to lead to intense bacterial degradation of sinking OM (Legendre and Le Fèvre 1995). The general scarcity of food leads to a predominance of the microbial food web in the entire Aegean Sea (Lykousis et al. 2002), and the predominance of higher water temperatures could give the microbial community a competitive advantage over larger benthic fauna (Duineveld et al. 2000).

It can furthermore be presumed that the benthic community is able to increase its reaction when large quantities of food arrive. High bacterial community activities have previously been documented by Bianchi et al. (2003). They found hydrolytic activity of benthic bacteria enhanced in the southern compared with the less oligotrophic northern Aegean Sea. They concluded that the low availability of usable organic compounds in the southern Aegean Sea leads to bacterial communities well equipped for biopolymer hydrolysis. This could be an explanation for the fast bacterial reaction capacity, even if the amount of arriving food is comparably low, which we were able to observe in this study. A rapid production of exoenzymes induced when settling particles arrive could also be an explanation for the additional respiration of "bulk" material proposed for the L experiments.

The benthic community in the deep Cretan Sea evidently reacted rapidly to a food pulse provided experimentally on the seafloor. This concentration-dependent response was clearly reflected in carbon turnover, which increased significantly from the L to H experiments, whereas the SCOC proved to be a less sensitive tool for investigating the microbial reaction. The tracing of carbon revealed different pathways of processing for the two experimental designs, with a preferential incorporation into bacterial fatty acids in the L experiments and greater remineralization in the H experiments. The H experiments, furthermore, clearly illustrated labeled carbon processing deep in the sediment, probably reflecting food-stimulated bioturbation activity of the macrofaunal community. Several investigations revealed significant increases in SCOC because of POM addition (e.g., Witte et al. 2003*a*,*b*; this study). However, it is clear that benthic communities might not react in an on–off manner. Here, we demonstrated the capability of different responses as a result of the amount of added algae. Our results suggest that the response of both the whole and the microbial benthic community at our deep-sea study site could be modified according to the amount of settling phytoplankton. Clearly, more observations are required, and the experimental design combining in situ technology and stable carbon isotope labeling proved to be very useful in investigating benthic carbon turnover on community level.

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