Sink or link? The bacterial role in benthic carbon cycling in the Arabian sea oxygen minimum zone

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Abstract

The bacterial loop, the consumption of dissolved organic matter (DOM) by bacteria and subsequent transfer of bacterial carbon to higher trophic levels, plays a prominent role in pelagic food webs. However, its role in sedimentary ecosystems is not well documented. Here we present the results of isotope tracer experiments performed under in situ oxygen conditions in sediments from inside and outside the Arabian Sea Oxygen Minimum Zone (OMZ) to study the importance of the microbial loop in this setting. Particulate organic matter, added as phytodetritus, was processed by bacteria, protozoa and metazoans, while dissolved organic matter was processed only by bacteria and there was very little, if any, transfer to higher trophic levels within the 7-day experimental period. This lack of significant transfer of bacterial-derived carbon to metazoan consumers indicates that the bacterial loop is rather inefficient, in sediments both inside and outside the OMZ. Moreover, metazoans directly consumed labile particulate organic matter resources and thus competed with bacteria for phytodetritus.

1 Introduction

Isotope tracer experiments have been conducted in various settings to assess the processing of particulate labile organic matter (OM) by the benthic community (Blair et al., 1996; Levin et al., 1997; Moodley et al., 2002; Witte et al., 2003a; Witte et al., 2003b; Moodley et al., 2005b; Andersson et al., 2008; Woulds et al., 2009). These studies have revealed that all benthic size classes (bacteria, meiofauna, and macrofauna) were involved and took up some of the labeled OM. In some cases (e.g. in the deep Sognefjord and in the Porcupine Abyssal Plain), bacterial and meiofaunal response to a fresh input of OM was retarded when compared to macrofaunal reaction and it was suggested (Witte et al., 2003a,b) that the offered phytodetritus had to be processed in macrofaunal gut before being available to the micro and meio-biota. These

40 results, although very informative, left some questions largely unanswered, especially for
41 hypoxic areas: is organic matter assimilated directly by meio- and macrofauna feeding on
42 phytodetritus, or indirectly via ingestion of bacteria that in turn hydrolyzed particulate organic
43 matter (POM) into dissolved organic matter (DOM) and subsequently incorporated it? Are
44 bacteria therefore a major food source for sedimentary fauna or are they a minor resource? In
45 other words, are bacteria a link or a sink in the benthic food web?
46 Pomeroy (1974) suggested that bacteria and protozoa in pelagic ecosystems form an
47 important link between dissolved organic matter produced by primary producers and
48 metazoans higher up in the food web. Following studies confirmed this hypothesis and Azam
49 et al. (1983) named this pathway the "microbial loop" in pelagic ecosystems. Later, Jumars et
50 al. (1989) presented a provocative paper in which this theory was further elaborated: they
51 showed that heterotrophic bacteria were feeding not only on phytoplankton-derived dissolved
52 organic carbon but also on DOM derived from sloppy feeding by zooplankton.

53 Lee (1980) and Kemp (1988) were among the first to hypothesize that the microbial loop
54 might also be occurring in the sediment, although possibly in a more complex form.
55 According to these authors, sediment bacteria would incorporate DOM via their membranes
56 directly from the pore water or from polymeric materials and particles, after breaking them
57 down to more simple compounds using exoenzymes. Bacteria would then become food for
58 protozoans and metazoans and thereby play a key role in the transfer of carbon and nutrients.
59 Lee (1992) conducted ^{14}C incubation experiments using water samples from an enclosed
60 marine basin, to study carbon preservation under oxic and anoxic conditions. She found that
61 differences in OM decomposition rates between oxic and anoxic settings were very small, but
62 that anoxic sediments nevertheless showed high OM preservation. Lee (1992) suggested that,
63 where bacterial grazers are absent, organic matter in anoxic systems may be sequestered as
64 bacterial biomass, or as bacterially-derived products. Therefore, she concluded, that biomass

65 and biodiversity of bacterial grazers may explain part of the differences in carbon preservation
66 between oxic and anoxic settings, supporting the idea of a benthic microbial loop and offering
67 some insights in the relationships between bacteria and their grazers and a link with bottom-
68 water oxygen levels.

69 Recent observations on the trophic role of bacteria and bacterial-derived carbon in sediments
70 based on isotope tracer experiments revealed however that bacteria are not a major carbon
71 source for intertidal benthos (Van Oevelen et al., 2006b). Consistently, for deep-sea sediments
72 Nomaki et al. (2006) and Guilini et al. (2010) found limited to no bacterivory by meiofaunal
73 foraminifera and nematodes, previously thought to be major consumers of bacterial
74 production. If faunal grazing does not constitute a main controlling factor of the bacterial
75 community, then the latter must be controlled in a different way. Danovaro et al. (2008)
76 proposed viral infection as heterotrophic bacterial production controlling mechanism in the
77 sediment, asserting that at water depths beneath 1000m, viral lysis would be responsible for
78 nearly all prokaryotic mortality, transforming bacteria in detritus. The viral shunt hypotheses
79 and the results obtained in bacterivory studies suggest that the microbial loop in sediments is
80 a sink rather than a link in the benthic food web.

81 The importance of the microbial loop in deep-sea benthic food webs has however been
82 exclusively studied in sediments underlying oxic bottom waters. Oxygen minimum zones
83 (OMZ) are known for their different biogeochemistry as compared to regular oxic settings.
84 The naturally occurring OMZ in the Arabian Sea is an archetypical example: here OM
85 accumulates in the sediment (Cowie et al., 1999; Middelburg and Levin, 2009). Several
86 explanations have been given for this accumulation: low faunal biomass (Demaison and
87 Moore, 1980; Levin et al., 1991; Jeffreys et al., 2009) and faunal activity (Woulds et al.,
88 2007; Levin et al., 2009), lack of oxidants and interaction of the OM with inorganic material
89 (Hedges and Keil, 1995), incorporation of organic particles in geomacromolecules or humic

substances (Mayer, 1994), and the refractory nature of the OM deposited at the bottom (Henrichs, 1992). Focusing on the functioning of this ecosystem, results by Moodley et al. (2011), Pozzato et al. (2013) and Koho et al. (2013) showed that limited faunal activity, low oxygen concentrations and low faunal biomass cannot explain on their own the OM accumulation. Instead, community functioning (in terms of trophic relationships and food preferences) and efficiency in OM processing are more likely to be key factors in determining such phenomenon.

We investigated the specific role of bacteria in the sediments of the Arabian Sea OMZ to clarify if they are a sink or a link in the benthic food web and whether this differs between sediments underlying oxygenated or low-oxygen bottom waters. We amended sediment from inside and outside the Arabian sea OMZ with two different complex OM tracers, particulate organic matter (POM) and dissolved organic matter (DOM). This approach allowed us to follow the two main routes of OM degradation in the benthic food web: POM degradation by bacteria and metazoans, i.e. the detritivore pathway (Mayer, 1989; Chrost, 1991b, a), and DOM incorporation by bacteria which in turn are subsequently grazed by fauna, i.e. the microbial loop. While it is clear that anoxic sediments lacking metazoans do not support a microbial loop, it is unknown whether the benthic microbial loop is more or less efficient in OMZ sediment with substantial faunal biomass as found in the Arabian Sea. In such sediments the relatively high quantity of refractory OM (preferentially degraded by bacteria) support secondary production of microbes which then might be assimilated by the specialized fauna of OMZ sediments. Should the microbial loop play a key role in benthic ecosystems, one would then expect it to be prominent in fauna-bearing, low-oxygen, organic-rich sediments such as found in the Arabian Sea OMZ.

2 Material and Methods

2.1 Study area

The study focused on two stations on the Murray Ridge, situated in the northern Arabian Sea between the Indus River delta and the city of Muscat (Fig. 1) and was carried out in January 2009 therefore during the winter monsoon season. Station STOMZ lies in the core of the OMZ and was shallower (22°32.9' 64°02.4', 885 m) than the deeper station SToutOMZ (22°18.5' 63°24.5', 1791 m) at the lower boundary of the OMZ. The same stations have been investigated in detail for organic geochemistry (Koho et al., 2013), pore-water geochemistry (Kraal et al., 2012) and for particulate organic carbon processing (Pozzato et al., 2013). The latter companion study focussed on the effect of bottom-water oxygen manipulation on community processing of particulate organic carbon and the controls of this and that study are identical.

The Murray Ridge is located south west of the Pakistan margin and it starts about 100 km from the coast into the Arabian Sea; it is approximately 20 km wide and 750 km long. The Ridge is composed of three different parts: the southern crest at water depth <1000 m, the northern zone is subdued with water depths of 2000 m and the >4400 m deep Dalymple Trough (Gaedicke et al., 2002). The sedimentary cover of the Ridge increases in thickness towards the coast and it progressively widens. The Ridge is a perfect experimental setting to study marine OM processing because it is isolated and far away from shore (Shimmield et al., 1990), preventing confounding terrestrial input to this site. The OMZ starts approximately 150 meters below the sea surface and coincides with the seasonal thermocline. At STOMZ a surface mixed layer was recorded by our CTD at depth between 80 and 85 m, whereas the water column at SToutOMZ was fully mixed.

Three different methods were used to analyze the oxygen concentration in the water column: the oxygen sensor on the CTD, Winkler titration of the water sampled by the rosette sampler and an optode fitted to the multicorer. The CTD was equipped with a sea-Bird SBE43 sensor, detection limit 3 μmol L^{-1}, accuracy 2%, which was calibrated using Winkler titration of samples from outside the OMZ. One CTD cast was done per station. Sea surface temperature was 25.2˚C and below the mixed layer the temperature declined to a minimum of 2.1˚C at 2600 m. Salinity profiles showed a distinct maximum of 36.2 at 320 m, probably related to the Persian Gulf outflow. The salinity decreased below 400 m to a minimum of 34.7 at 3000 m.

2.2 Preparation of isotopically labeled substrates

POM and DOM were prepared in the laboratory from axenic cultures of the brown diatom *Thalassiosira pseudonana* that were grown in a 30% ^{13}C-$NaHCO_3$ enriched F2 medium. Algae were harvested via centrifugation (2000 g force), the pellet washed of any residual label with artificial seawater and centrifuged again. The pellets were frozen at -80˚C and freeze-dried. We harvested ~0.8 g dry algae from 9 L of cultured *Thalassiosira pseudonana*. Sterilized Milli-Q water was then added to the freeze-dried pallets to burst the algal cells and release the inner cytoplasm and exudates from the algal frustules. DOM was separated from POM by centrifugation (2000 g force). This procedure was repeated three times. The remaining pellet was used as POM substrate. The supernatant was taken as DOM substrate and was filtered through a GF/F pre-combusted filter, filtered through a 0.2 μm syringe filter before being stored in glass vials. Both POM and DOM were portioned, frozen and freeze-dried again before usage in the experiments. The ^{13}C content of the POM was 20% (equal to a $\delta^{13}C$ value of 21,500‰) and 18% of the DOM (equal to a $\delta^{13}C$ of 18,500‰) as measured by a

Thermo Electron Flash EA 1112 analyzer (EA) coupled to a Delta V isotope ratio mass spectrometer (IRMS).

2.3 Sediment core sampling

Intact 10 cm i.d. multicorer cores were retrieved. A total of ten cores per station was collected: two were used for sediment characteristics, four were incubated for seven days and four were used as control to sample for bacterial and fauna isotopic background values and biomass. Upon arrival on deck, the two cores used for sediment characteristics determination were sliced immediately, the others were used for incubation or as controls. They were transferred to a temperature-controlled laboratory and were allowed to acclimatize for 2 days in a water bath at in situ temperature. During this period, the overlying water of the cores was gently bubbled with pre-made air mixtures (certified O_2, CO_2 and N_2 gas mixture by Hoekloos BV, The Netherlands) to give oxygen concentrations of 125 μM O_2 for STOutOMZ and 6 μM O_2 for STOMZ (Pozzato et al., 2013); these conditions were maintained and monitored during incubation.

2.4 Sediment characteristics

Two cores per station were used to determine sediment characteristics. The upper 3 cm of each core was sliced off, homogenized and 10 cm^3 of each slice subsampled and used for analysis. The porosity was calculated on sediment wet/dry measurements using an average sediment density of 2.55 g cm^{-3}. Grounded freeze-dried sediment samples were measured for organic carbon content, molar C:N ratio and background $\delta^{13}C$ values with a Thermo Electron Flash EA 1112 analyzer (EA) coupled to a Delta V isotope ratio mass spectrometer (IRMS).

Sediment grain size distribution was assessed with a MALVERN Mastersizer 2000 on freeze-dried sediment samples.

2.5 Experimental incubations

Four cores per station were incubated for 7 days: from STOMZ, 2 with the equivalent of 400 mg C m^{-2} POM, 2 with the equivalent of 400 mg C m^{-2} DOM and from SToutOMZ, 2 with the equivalent of 100 mg C m^{-2} POM and 2 with the equivalent 100 mg C m^{-2} DOM. The selected tracer quantities represent 0.31 and 0.19% of the organic C present in the surface 3 cm of the sediment. The POM was resuspended in 0.2 μm filtered natural seawater and then gently delivered to the sediment surface of each core via a long glass pipette, paying attention not to disturb the sediment. The DOM was dissolved in 0.2 μm filtered natural seawater and subsequently introduced gently throughout the sediment profile down to approximately 4 cm, with a micro-glass syringe via numerous injections of 10 μL each, equally distributed over the surface of the core. The cores were sealed on the bottom and on the top with O-ring lids and incubated for 7 days under suboxic conditions (6 μM O_2 for STOMZ and 125 μM O_2 for SToutOMZ,), via bubbling with pre-made mixtures of air as done during pre-incubation (see above). To verify that the oxygen concentration in the core water of the different incubations was matching the desired ones, measurements were carried out with an oxygen-optode probe (Presens, Germany) following standard procedures of calibration, according to the optode manual, directly before and after the experiment. The out-flowing air of each core entered into two successively placed CO_2 traps, which were connected to each other and changed every 3.5 days to prevent oversaturation. The CO_2 traps were filled with a solution of Milli-Q water and NaOH (Kristensen et al., 1992) and were prepared in 500 mL sealed-off glass bottles with a screw-on septum cap. At the end of the incubation time, the cores were opened sequentially, oxygen concentration measurements done in the cores and water samples were taken.

209 Four cores per station were used to determine isotopic and biomass backgrounds. The natural
210 abundance and background data have been presented and discussed by Pozzato et al. (2013).
211
212 **2.6 Sample processing**

213 At the end of the incubation, the overlying water of the cores and the content of the CO_2 traps
214 were filtered on GF/F pre-combusted filters. The filters were then analyzed for phospholipid
215 fatty acids (PLFA) to estimate the water column bacterial contribution to OM degradation
216 (see below). The sediment cores where then frozen in their coring tube. While frozen, the
217 upper 10 cm of each core was cut off when still inside the coring tube with a hand saw for
218 later sub-sampling. This procedure was preferred over normal slicing due to the extremely
219 soft nature of the sediment. The intact core sections were stored in a -20 °C freezer and
220 transported to the laboratory at NIOZ-Yerseke.

221 After arrival in the laboratory, four weeks later, the background and incubation cores were
222 sliced frozen in 2 cm intervals: 0-2 and 2-4 cm with a circular electric saw. While frozen, each
223 slice was portioned as follows: one quarter (approx. 39 ml) was analysed of bacterial PLFA,
224 two quarters were used for faunal extraction and the remaining quarter was used for total
225 lipids extraction and archaeal biomarkers (Lengger, 2013). The fatty acid subsample was
226 freeze-dried and stored at -20°C until further processing. The frozen subsamples for faunal
227 analysis (for background and incubations cores) were immersed in a mixture of 40% buffered
228 formaldehyde stained with rose Bengal and allowed to thaw at room temperature. This was
229 preferred over fixing and staining after defrosting to better preserve the fauna. The stained
230 sediment was stored for 2 days at room temperature before sieving, to give the stain sufficient
231 time to colour the animals.
232
233 **2.7 $\delta^{13}C$ isotope measurements**

2.7.1 Bacteria

The bacterial tracer incorporation was estimated through the isotope enrichment of bacterial-specific PLFAs (Boschker and Middelburg, 2002). Two main biomarkers were chosen for the analysis: iC15:0 and ai15:0 because of their specificity for bacteria and presence in all samples. Fatty acids were extracted using the Bligh and Dyer extraction protocol and analyzed using GC-c-IRMS (Middelburg et al., 2000) and $\delta^{13}C$ values were corrected for the C addition during derivatization and also per PLFA-C content differences. Incorporation of ^{13}C into these bacterial PLFAs was converted to incorporation into bacterial biomass by assuming that the specific PFLAs represent 11% of all bacterial PLFAs, which in turn comprise 5.6% of total bacterial carbon (following Moodley et al. 2002). The conversion from PLFA gDW to bacterial biomass m^{-2} was done using determined sediment porosity values.

2.7.2 Fauna

Following Rose Bengal staining, the sediment was sieved on stacked 500 and 38 μm sieves to separate macrofauna (retained on 500 μm sieve) and meiofauna (retained on 38 μm sieve). The meiofauna fraction was then further treated with Ludox (colloidal silica) centrifugation (Burgess, 2001) to separate the nematodes from the sediment. The division between macro- and meiofauna was based on size and not on genera. Foraminifera are usually considered to be part of meiofauna, but in our samples of approx. 78 ml sediment, up to 100 specimens were retained on the 500 μm sieve. Residues retained on both mesh sizes, containing the fauna and some sediment particles, were then hand-picked under a binocular microscope and the fauna was divided in: branched and shelled Foraminifera, soft-bodied protists (both Gromiids and Allogromiids), Polychaetes (other than *Linopherus sp*. which is listed separately due to its predominance in biomass), *Linopherus sp*., nematodes and "Eukarya" (regrouping all other faunal species for which species determination was not done but

consisting mostly of crustaceans). The fauna was transferred into pre-weighed silver cups, oven-dried (50˚C), decarbonated with 10% HCl (slow addition for Foraminifera and checked under binocular to ensure that bubbling had finished) and again oven-dried. The silver cups were then weighed to establish the dry-weight of the fauna and pinched closed. The samples were then analysed for carbon and nitrogen concentration and $\delta^{13}C$ value with a Thermo Electron Flash EA 1112 analyzer (EA) coupled to a Delta V. Faunal biomass was determined via dry weight and C content values from the IRMS, combined with measurements of faunal abundance through counting all specimens in the faunal sediment sample (78 mL) under the binocular.

2.8 Uptake calculations

The relative uptake of the isotope tracer is presented in the $\Delta\delta^{13}C$ notation, which represents the enrichment in $\delta^{13}C$ of the sample in excess of its natural background value ($\delta^{13}C_{background}$), and is calculated as

$$\Delta\delta^{13}C_{sample} = \delta^{13}C_{sample} - \delta^{13}C_{background} \quad (1)$$

The ^{13}C enrichment in at % (E) is calculated as

$$E = F_{sample} - F_{control}, \quad (2)$$

$$\text{where } F = \frac{^{13}C}{^{13}C + ^{12}C} = \frac{R}{R+1} \quad (3)$$

$$\text{and } R = \left(\frac{\delta^{13}C}{1000} + 1\right) \text{x } R_{ref} \quad (4)$$

with $R_{ref} = 0.01118$. The uptake of ^{13}C tracer (I, µg tracer ^{13}C m-2) is then calculated as the product of E and biomass. The total uptake of the DOM and POM substrates (µg tracer C m^{-2})

for both bacteria and fauna was then calculated according to Moodley et al. (2005a), by dividing the total ^{13}C tracer uptake (I) with the fractional abundance of ^{13}C in the tracers (i.e. 0.2 for POM, 0.18 for DOM). Please note that the presented data on relative and total uptake are calculated for each individual experimental core and then averaged. Biomass values are however the average of all cores (i.e. all experimental and background cores per station) since the larger area covered implies a better biomass estimate.

2.9 Respiration measurements

Water samples of ~2 mL for analysis of DIC (dissolved inorganic carbon) from the CO_2-traps (changed every 3 days) and the overlying water of the incubated cores were taken with a sterile plastic syringe, filtered on a GF/F filter attached to the syringe and injected into helium pre-flushed 5 ml glass vials, were sealed with crimp-cap with rubber septum. DIC samples were immediately acidified with 99% H_3PO_4 (10 μL per ml sample) to stop biological activity and vials were stored upside down at 4°C until analysis in the laboratory (~four weeks after sampling). Shortly before analysis, each vial was further acidified to convert any residual bicarbonate into CO_2, which then accumulates in the vial headspace. The headspace was sampled using a glass syringe and CO_2 concentration and isotope ratio of the gas were measured on a Thermo Electron Flash EA 1112 analyzer (EA) coupled to a Delta V isotope ratio mass spectrometer (IRMS). Total respired ^{13}C-DIC was determined by summing the values obtained from the overlying water and CO_2 trap. The second CO_2-trap in succession showed no to very limited ^{13}C enrichment in the DIC, which indicated that all DIC had been trapped and that the data from the second trap could safely be ignored in the calculations.

3 Results

3.1 Sediment

The sediment was very different between the 2 stations. SToutOMZ was mainly composed of light brown-gray and very compact clay, whereas at STOMZ it was dark brown-black, watery and fluffy, poor in clay but with a high content of foraminiferan shells and diatom frustules. The layering and burrows were still intact, indicating that no alteration of the sediment occurred during sampling and retrieval. The total organic carbon content and C:N ratio were higher inside the OMZ, whereas the $\delta^{13}C$ and $\delta^{15}N$ of the sediment inside and outside the OMZ were comparable (Table 1).

3.2 Benthic biomass

Biomass data per layer and station of both incubated and background cores are shown in Fig. 2 as averages among treatments to account for sediment patchiness. For complementarity, depth (0-4 cm) integrated biomasses will be presented below. Bacterial biomass was 1,000±120 and 1,600±110 mg C m^{-2} respectively in SToutOMZ and STOMZ, meiofaunal nematode biomass was 19±1 and 13±2 mg C m^{-2}, meiofaunal-sized shelled foraminiferan biomass was 42±12 and 38±8 mg C m^{-2}. Macrofaunal-sized shelled foraminiferan biomass accounted for 118±58 and 183±100 mg C m^{-2} for SToutOMZ and STOMZ respectively; polychaetes biomass was 87±19 and 897±299 mg C m^{-2} with *Linopherus sp.* the most abundant species in STOMZ. Macrofaunal nematodes accounted for 17±0 mg C m^{-2} in SToutOMZ but were found only in background cores at low biomass in STOMZ, macrofaunal branched foraminifera and soft-bodied protists were found only in SToutOMZ where they accounted for 208±75 and 497±138 mg C m^{-2}, respectively. Other eukaryotes accounted for 0.2±0 mg C m^{-2} and were found only in SToutOMZ.

3.3 OM incorporation

329 Incorporation into biota of the two ^{13}C labeled OM types was evident, although the level of
330 enrichment differed per station, benthic group and depth layer (Table 2 and Fig. 3).
331 At STOMZ, the biota in the upper layer (0-2 cm) accounted for only a small part (3,247 µg C
332 m^{-2} or 12% of the total uptake) of the POM uptake: bacteria incorporated 2,181±1 µg C m^{-2},
333 meiofaunal nematodes 36.8±28 and shelled foraminifera 1,028±547 µg C m^{-2}, macrofaunal
334 shelled foraminifera incorporated 0.13±0.12 µg C $m^{-2}$and *Linopherus sp*. accounted for
335 1.18±1 µg C m^{-2}. Almost all the POM incorporation took place in the deeper 2-4 cm layer
336 (23,000±4,000 µg C m^{-2}or 87% of the total uptake). Meiofaunal nematodes accounted for
337 2.66±0.5 µg C $m^{-2}$and shelled foraminifera for 6.55±2.5 µg C m^{-2}, macrofaunal shelled
338 foraminifera accounted for 4.83±3.4 µg C m^{-2}, polychaetes for 6,133±3,050 µg C m^{-2} and
339 *Linopherus sp.* for 16,807±840 µg C m^{-2}. The bacterial contribution to POM uptake in this
340 deeper layer was negligible. DOM incorporation was strongly dominated by bacteria and
341 thereby clearly different from that of the POM substrate. In the upper layer, most of the total
342 uptake took place 12,314 µg C m^{-2} (87%): bacteria incorporated 12,312±7 µgC m^{-2}, followed
343 by meiofaunal nematodes with 2.33±0.28 µg C m^{-2}; macrofaunal shelled foraminiferans
344 incorporated 0.02±0 µg C m^{-2} and *Linopherus sp.* 0.05±0 µg C m^{-2}. Meiofaunal shelled
345 foraminiferan contribution was negligible. In the deeper layer, 2-4 cm, the uptake was much
346 lower (1,818±13 µg C m^{-2} or 13%): bacteria again clearly dominated uptake (1,803±1 µg C
347 m^{-2}), while meiofaunal shelled foraminifera accounted only for 8.4±0.5 µg C m^{-2}, meiofaunal
348 and macrofaunal nematodes had negligible uptake and macrofauna *Linopherus sp.* accounted
349 for 6.1±0 µg C m^{-2}.
350 At SToutOMZ, the largest part of the POM uptake was found in the upper layer: bacteria
351 incorporated 466±0.2 µg C m^{-2}, meiofaunal shelled foraminifera 2.06±0 µg C m^{-2},
352 macrofaunal nematodes 0.42±0.4 µg C m^{-2}, other groups showed negligible uptake. In the
353 deeper layer, only 0.6% of the total POM was taken up by meiofaunal shelled foraminifera

354 (1.47±1.4 μg C m^{-2}) and macrofaunal nematodes (1.80±1.8 μg C m^{-2}). DOM uptake in the
355 upper layer was dominated by bacteria (3,637±2 μg C m^{-2}), with negligible uptake by the
356 other groups (<0.01 μg C m^{-2}). This pattern was different in the deeper layer: bacteria
357 incorporating 805±0.8 μg C m^{-2}, meiofaunal shelled foraminifera 63.7±62 μg C m^{-2},
358 macrofaunal shelled foraminifera 1.94±1.8 μg C $m^{-2}$and polychaetes 2.63±2.6 μg C m^{-2}.
359
360 **3.4 Respiration**

361 Respiration of POM at STOMZ was twice as high as compared to DOM respiration, 54.5±10
362 versus 26.9±9.5 mg C m^{-2}, respectively. The same pattern was seen at SToutOMZ, where
363 respiration of POM and DOM were 6.82±1 and 2.07±1.1 mg C m^{-2}, respectively. Most of the
364 POM and DOM tracers was not processed within the 7-day incubation time, but respiration
365 was the dominant fate of processed DOM and POM at both stations (Fig. 4), accounting for
366 up to 13.6% and 6.73% of the added tracer on average for STOMZ POM and DOM treatment
367 and 6.80% and 2.07% for SToutOMZ (Fig. 4).
368
369 **4 Methodological issues**

370 Experimental research on ocean margin and deep-sea sediments is challenging because of the
371 limited amount of sample material available and the difficulties of mimicking in situ
372 conditions (temperature and pressure). Our experiments were performed at in situ
373 temperature, but on-board ship, implying that any differential pressure effect on prokaryotic
374 vs. eukaryotic compartments might have affected our results. Experimental research on
375 sediments underlying OMZ requires also incubation with controlled bottom-water oxygen
376 levels. Oxygen concentrations during incubations were successfully maintained close to in
377 situ conditions. The two substrates used were introduced in two different ways: POM was
378 distributed on the core surface, DOM was injected throughout the 0-4 cm depth. This strategy

was adopted to mimic best natural conditions, because POM is deposited on the sediment from the water column, while most DOM is generated within the sediments from sedimentary organic matter. However, this difference in spatial delivery patterns might have affected the availability of labelled DOM and POM to surface deposit feeders vs. subsurface feeders and microbes. We believe this to be a minor effect for macrofauna because macrofauna biomass (in particular *Linopherus sp.*) was higher in the 2-4 cm layer, but these animals were actively surfacing upon pulsed tracer delivery, hence making immediate use of the resource in the surface layer.

Our incubations lasted 7 days and this period might have been too short to allow complete upward transfer of bacterial-derived carbon to meio- and macrofauna in these sites. However, shorter experiments executed in similar settings did report significant tracer transfer to bacteria and fauna (Moodley et al., 2005a,b; Woulds et al., 2007; Guilini et al., 2010). Nevertheless, a 7-day period is relatively short if transfers from bacteria to metazoans consumers involve multiple benthic compartments (e.g. Nomaki et al., 2008). In that case longer lasting experiments might be needed to confirm our findings. Our community response study did not include archaea or mobile megafauna. No megafauna was found in the cores used for this study, therefore we cannot exclude potential bacterial ingestion by such deposit feeders (i.e. holothurians and echinoderms). Our analysis of the microbial community was limited to bacteria because in a companion study conducted at the same stations, Lengger (2013) showed that only bacteria and not archaea utilized the ^{13}C enriched POM and DOM, indicating bacteria as dominant active microbial group.

5. Discussion

In most environmental settings, the majority of the OM that reaches the ocean floor is used by the benthic community for biomass production and respiration (Burdige, 2006). This processing is thought to occur essentially along two pathways in the benthic food web. The

405 POM pathway involves metazoan ingesting of particulate detritus, while consortia of bacteria
406 hydrolyze POM to DOM first, before taking it up. The DOM pathway involves the microbial
407 incorporation of DOM directly, or after hydrolysis. Microbial carbon, produced through either
408 the POM or DOM pathway, can be transferred to higher trophic levels via predation and
409 grazing in accordance with the microbial loop concept. Woulds et al. (2009) reviewed isotope
410 tracer experiments and showed that all benthic size classes are involved in the POM pathway,
411 in shallow and deep ecosystems, spanning from low to high activity communities and from
412 oxic to suboxic environments. The POM uptake rates in our experiments (Table 2 and Fig. 3),
413 both here and in the companion study (Pozzato et al., 2013), confirm that all size classes take
414 up recently deposited phytodetritus in Arabian Sea sediments. The biomass values of bacteria,
415 protists and fauna in our stations (Fig. 2) were comparable to other deep-sea settings
416 (Moodley et al., 2002; Witte et al., 2003b; Moodley et al., 2005b; Woulds et al., 2009; Hunter
417 et al., 2012), which confirms that the benthic community in Arabian Sea sediments underlying
418 oxic and suboxic bottom water is well developed.
419 Although one decade of isotope tracer experiments has established that phytodetritus-derived
420 carbon eventually ends up in all benthic size classes (Woulds et al., 2009;), we have little
421 understanding whether this represents direct access of consumers to POM and therefore direct
422 incorporation of the tracer, or whether the POM uptake by fauna is mediated by bacteria. Here
423 we directly address the question by focusing on the carbon flow in parallel incubations using
424 complex ^{13}C enriched DOM and POM substrates and target both microbes and fauna.

425 In our experiments, the DOM tracer was quickly incorporated by bacteria but no appreciable
426 uptake was seen by benthic meio- and macrofauna (Fig. 3 and 4). This indicates not only
427 absence of direct exploitation of DOM but also limited bacterivory by these two biotic
428 compartments and the comparison of relative labeling ($\Delta\delta^{13}C$, Table 2) of bacteria versus their
429 potential consumers in other ecosystems (Hall and Meyer, 1998; Van Oevelen et al., 2006b)

supports this finding. Few studies (Lee et al., 1966; Delaca et al., 1981; Delaca, 1982) reported foraminifera feeding directly on dissolved organic carbon, but it was not clear whether such uptake was direct or mediated by symbiotic bacteria. Our StoutOMZ samples also showed some enrichment in foraminifera (Fig. 3), thus we cannot exclude direct DOM assimilation or transfer through bacterivory to protists, the latter also suggested by Van Oevelen et al. (2006b) although in relative low amounts. Our findings therefore show that, at least in the short term, in the DOM pathway bacteria were not a principal food source for the fauna and that bacterial carbon was not transferred up the food web. Consequently we infer that the fauna uptake in our POM experiments, and most likely in other isotope tracer experiments, must also be due to direct ingestion of the POM substrate rather than ingestion of bacterial biomass. In the POM treatment, the $\Delta\delta^{13}C$ values of fauna are much higher than those of bacteria (Table 2), further supporting that tracer incorporation occurred via direct substrate ingestion.

The two stations investigated differ not only in bottom-water dissolved oxygen concentrations, but also in grain size, sediment organic carbon, temperature (Table 1) and benthic community structure (Fig. 2). In the companion study (Pozzato et al, 2013) showed that the communities were most efficient in processing particulate organic matter at in situ oxygen conditions and that organic matter supply rather than oxygen and temperature governed the higher faunal activity at STOMZ relative to SToutOMZ. This study shows that the transfer of bacterial carbon to protists and metazoan consumers was very limited at both stations although one might have anticipated more carbon flow via the DOM-bacteria-metazoan pathway at STOMZ because of the higher carbon content and more active community (Pozzato et al., 2013).

Kemp (1990) reviewed the fate of benthic bacterial production in shallow/soft sediments and concluded that likely only a small fraction of bacterial production enters the macrofaunal food

455 web (directly and indirectly) and that most bacterial production is remineralized and respired
456 by a microbial food web. Later predation studies (Hondeveld et al., 1992; Starink et al., 1994;
457 Hamels et al., 2001) further debunked the applicability of the microbial loop theory and
458 Pomeroy's ideas for sediments, by not finding evidence of bacterial ingestion by their
459 "thought to be" main grazers, protozoa, suggested as link between bacteria and fauna.

460 The limited bacterial-derived carbon transfer was confirmed experimentally by Van Oevelen
461 and coauthors (2006a; 2006b) who used in situ stable isotope pulse-chase experiments and
462 modeling in a tidal mudflat. They concluded that some bacterial production was lost to the
463 sediment via exchange processes and grazing by meio- and macrobenthos, but that its major
464 fate was mortality (65%), which induced recycling of bacterial biomass in the sediment. The
465 same studies reported also that only a small portion (up to 11%) of the faunal carbon
466 requirements was met by bacterivory, irrespective of dwelling depth and organism size.
467 Hence, they concluded that intertidal meio- and macrofauna depend primarily on carbon
468 sources other than bacteria. Nomaki et al. (2006) conducted feeding experiments with deep-
469 sea foraminifera using ^{13}C-labeled bacteria and algae and found that these protozoa
470 selectively ingested algae or randomly ingested sedimentary organic matter. No selective
471 feeding on bacteria was observed, because bacteria were either not or to a very limited extent
472 assimilated. Guilini et al. (2010) conducted similar experiments in the Arctic targeting
473 nematode feeding habits and reached similar conclusions: bacteria were not a major food
474 source for nematodes and the very limited amount of ^{13}C incorporated could not be attributed
475 to bacterivory. These results by Van Oevelen et al. (2006a; 2006b), Guilini et al. (2010) and
476 Nomaki et al. (2006) support Kemp's conclusion that bacteria are not a major food source for
477 protists and meio- and macrofauna and that faunal carbon requirements are not met by
478 bacterial production. These and our experimental results disagree with other studies
479 (Vanreusel et al., 1995; Iken et al., 2001; Hoste et al., 2007) that suggested transfer of carbon

480 from bacteria to nematodes via direct predation from positive correlations between bacterial
481 density and nematodes abundance in the deep-sea. Along this line, Ingels et al. (2010) found a
482 preferential uptake of bacteria versus phytodetritus-derived carbon by nematodes, but the
483 isotope enrichment of the nematodes was so low that the authors concluded that the
484 contribution of nematodes to benthic mineralisation was limited in deep polar seas. Hence,
485 even though nematodes might be bacterivorous, upwards transfer of bacterial derived carbon
486 via nematodes appears to be minimal.

487 It has been shown that bacterial standing stocks along a depth gradient of 0 – 6000 m appears
488 quite stable in sediments (Rex et al., 2006). Hence, if it's not grazing controlling bacterial
489 populations in sediments, then some other mechanism must be responsible. Recently
490 Danovaro et al. (2008) suggested that viral infection controls bacterial production. In their
491 recent review Rowe and Deming (2011) finally proposed a paradigm shift that is in line with
492 most isotope tracer studies. Free-living heterotrophic microbes in marine sediments would
493 play only a minor role in metazoan food webs, providing only a small fraction of metazoans
494 nourishment.

495 Finally, although our data are consistent with and support many recent studies showing a
496 minor role for bacterivory in marine sediments, this does not apply to specific environments
497 such as hydrothermal vents, colds seeps and other chemosynthetic systems in which primary
498 production based on chemical energy provides the main energy source for entire food webs
499 (Levin, 2005).

500

501 **5 Conclusions**

502 Accumulating evidence from the literature and our experimental results suggest strongly that
503 bacteria are a carbon sink in the benthic food web of the Arabian Sea OMZ sediments because

no appreciable carbon transfer from bacteria up the food chain to meio- and macrofauna occurs via predation or grazing. This also implies that the POM and DOM processing pathways in this system are separated. The DOM pathway is limited to bacterial exploitation, since no other group showed any uptake of this resource, and no transfer of DOM-derived carbon via bacterial grazing is observed, thus making this processing route a dead end. Within the POM pathway, there is competition for food, since bacteria as well as various faunal groups take up this resource, as shown in many short-term experiments. Similarly to what happens with the DOM though, once POM is taken up by bacteria it is lost for faunal secondary production. Protists could have grazed upon bacteria to a minor extent but we could not investigate such predation, due to present lack of technical tools to do so in intact sediment cores. Our results from StoutOMZ indicate that direct DOM uptake by foraminifera may occur, as also found by others, which when predated by bigger fauna, might be an alternative route for carbon transport along the food chain. Evidently, there is great need for future investigation for the role of protozoans and protists in sedimentary food webs to be able to further constrain the apparently weak link between bacteria and protozoan and metazoan consumers in marine sediments.

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709 **Tables**

710

711 Table 1. Station locations, environmental parameters, water and sediment characteristics of

712 the two sampling stations STOMZ and SToutOMZ. After Pozzato et al. (2013).

	STOMZ	SToutOMZ
Position	22°32.9' 64°02.4'	22°18.5' 63°24.5'
Depth	885 m	1791 m
Bottom water		
Temperature ˚C (CTD sensor)	10	4
Salinity (CTD sensor)	34.8	34.9
Dissolved O_2 µmol kg^{-1} (CTD sensor)	2	45
Sediment		
Median grain size µm (0-3 cm)	35.4	16.5
% Total organic carbon (0-3 cm)	6.38	1.03
C:N ratio (0-3 cm)	9.75	7.87
$\delta^{13}C$ (‰)	-21.5	-20.0
$\delta^{15}N$ (‰)	8.04	8.68

713

714

715 Table 2. Relative uptake ($Dd^{13}C$) for POM and DOM treatment and natural abundances ($d^{13}C$)

716 of the background samples for comparison. "n.f." means that no specimen of the group was

717 found in the core layer. The terms "Soft bodied protists" represents Gromiids and

718 Allogromiids, "Polychaetes" regroup all polychaete species other than *Linopherus sp.*

719

	Relative Uptake $\Delta\delta^{13}C$ ‰	Relative Uptake $\Delta\delta^{13}C$ ‰	Natural Abundance $\delta^{13}C$ ‰
	STOMZ POM	**STOMZ DOM**	**Background**
Bacteria			
0-2	80±23	210±46	-25.4 ±3.1
2-4	0±0.0	65±26	-24.4 ±2.2
Meiofauna			
Nematodes			
0-2	69±46	5.9±10	-22.3 ±1.0
2-4	16±5.6	0.4±0.9	-22.2 ±1.1
Shelled foraminifera			
0-2	685±404	3.6±3.4	-23.7 ±0.1
2-4	23±6.6	9.3±0.8	-11.1 ±3.0
Soft-bodied foraminifera			
0-2	n.f.	n.f.	n.f.
2-4	n.f.	n.f.	n.f.
Macrofauna			
Shelled foraminifera			
0-2	18±17	5.2±2.7	-21.5 ±0.1
2-4	1.1±0.3	11±10	-20.8 ±0.7
Linopherus sp.			
0-2	44±44	1.5±1.5	-19.7 ±0.1
2-4	136±116	11±9.6	-20.0 ±0.0
Polychaetes			
0-2	185±167	n.f.	-16.9 ±0.0
	STOutOMZ POM	**STOutOMZ DOM**	**Background**
Bacteria			
0-2	13±2.0	40±3.3	-21.8 ±0.8
2-4	no uptake	26±26	-23.2 ±1.1
Meiofauna			
Nematodes			
0-2	no uptake	1.8±0.0	-23.2 ±0.0
2-4	no uptake	no uptake	-23.3 ±0.1

Shelled foraminifera			
0-2	3.9±1.8	24±20	24.2 ±0.0
2-4	0.6±0.6	40±39	-24.7 ±2.1
Soft-bodied foraminifera			
0-2	n.f.	n.f.	n.f.
2-4	n.f.	n.f.	n.f.
Macrofauna			
Nematodes			
0-2	0.8±0.2	2.2±2.0	-17.4 ±2.1
2-4	n.f.	2.5±1.6	-17.2 ±0.0
Shelled foraminifera			
0-2	0.2±0.0	0.4±0.2	-20.3 ±0.2
2-4	n.f.	0.5±0.5	-20.0 ±0.1
Branched foraminifera			
0-2	no uptake	0.8±0.8	-21.6 ±0.2
2-4	n.f.	n.f.	-20.9 ±0.0
Soft bodied foraminifera			
0-2	1.0±1.0	0.3±0.2	-20.6 ±1.0
2-4	n.f.	6.0±6.0	-19.5 ±0.0
Polychaetes			
0-2	1.0±1.0	0.3±1.0	-19,.4 ±0.1
2-4	7.3±7.3	0.5±0.4	-18.5 ±0.0

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722 **Figure 1.** The Northern Arabian Sea (A) and the sampling areas STOMZ and SToutOMZ

723 indicated on the Murray Ridge (B) after Pozzato et al. (2013).

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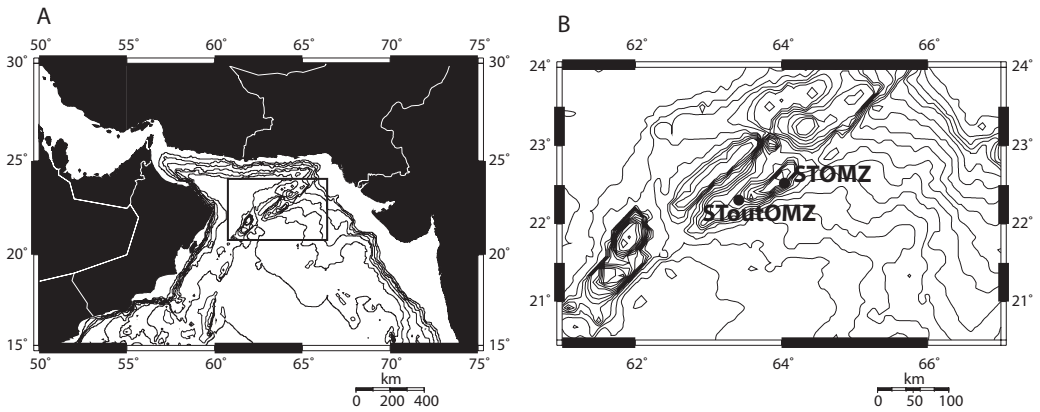

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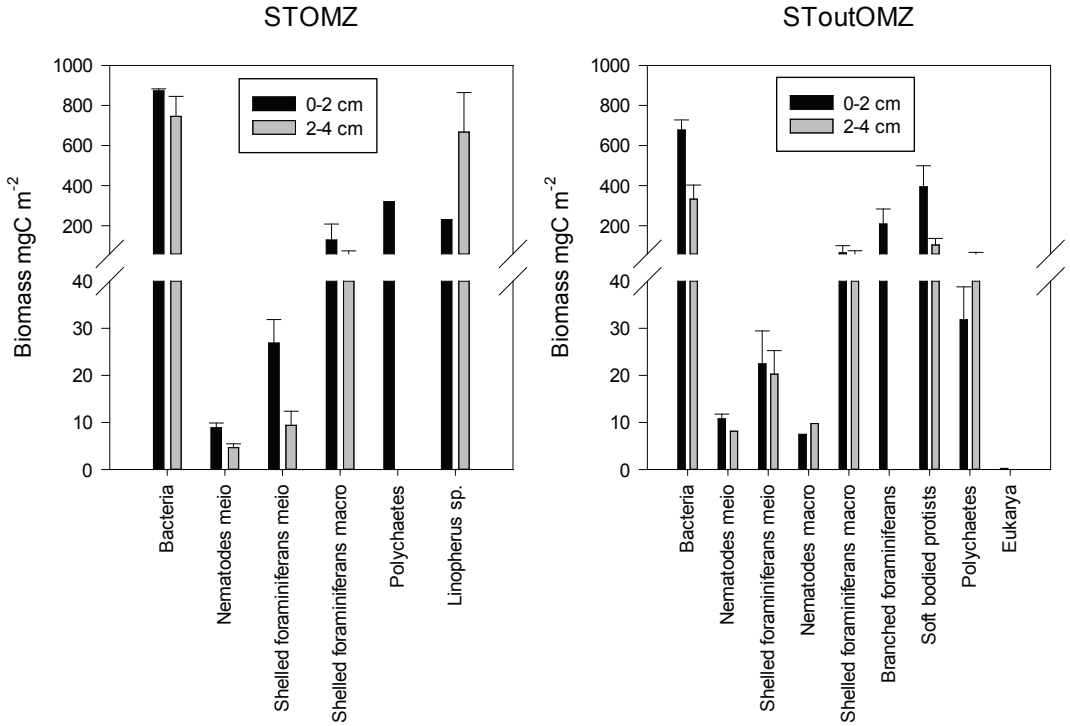
729 **Figure 2.** Group-specific biomass presented per station. Values are averages of all cores,
730 background and incubated ones, both treatments, to better account for heterogeneity.
731 "Polychaetes" regroup all polychaete species other than *Linopherus sp.,* "Eukarya" regroup all
732 other faunal groups besides Nematodes, Foraminifera and Plychaetes, for which no
733 determination was done.



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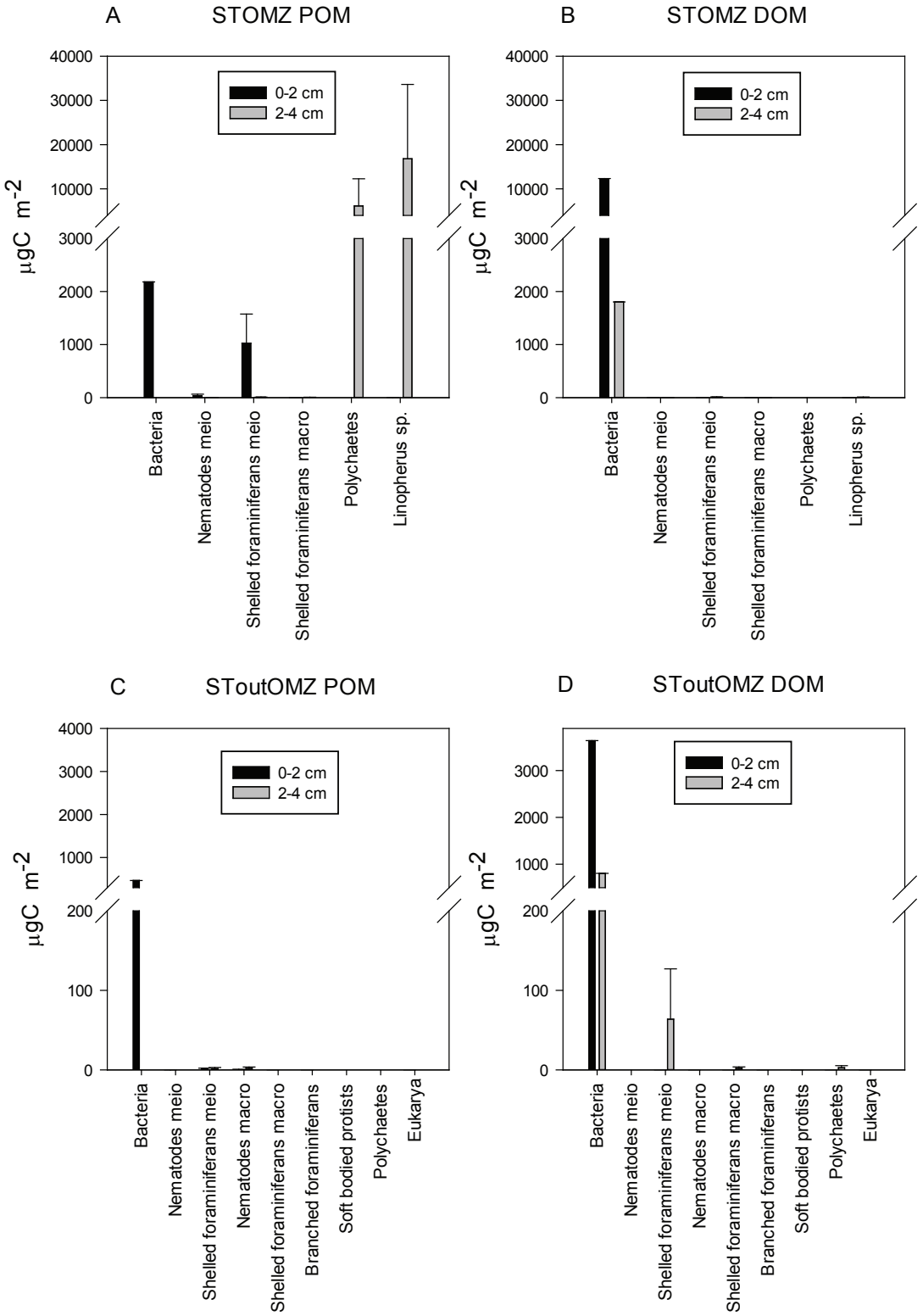
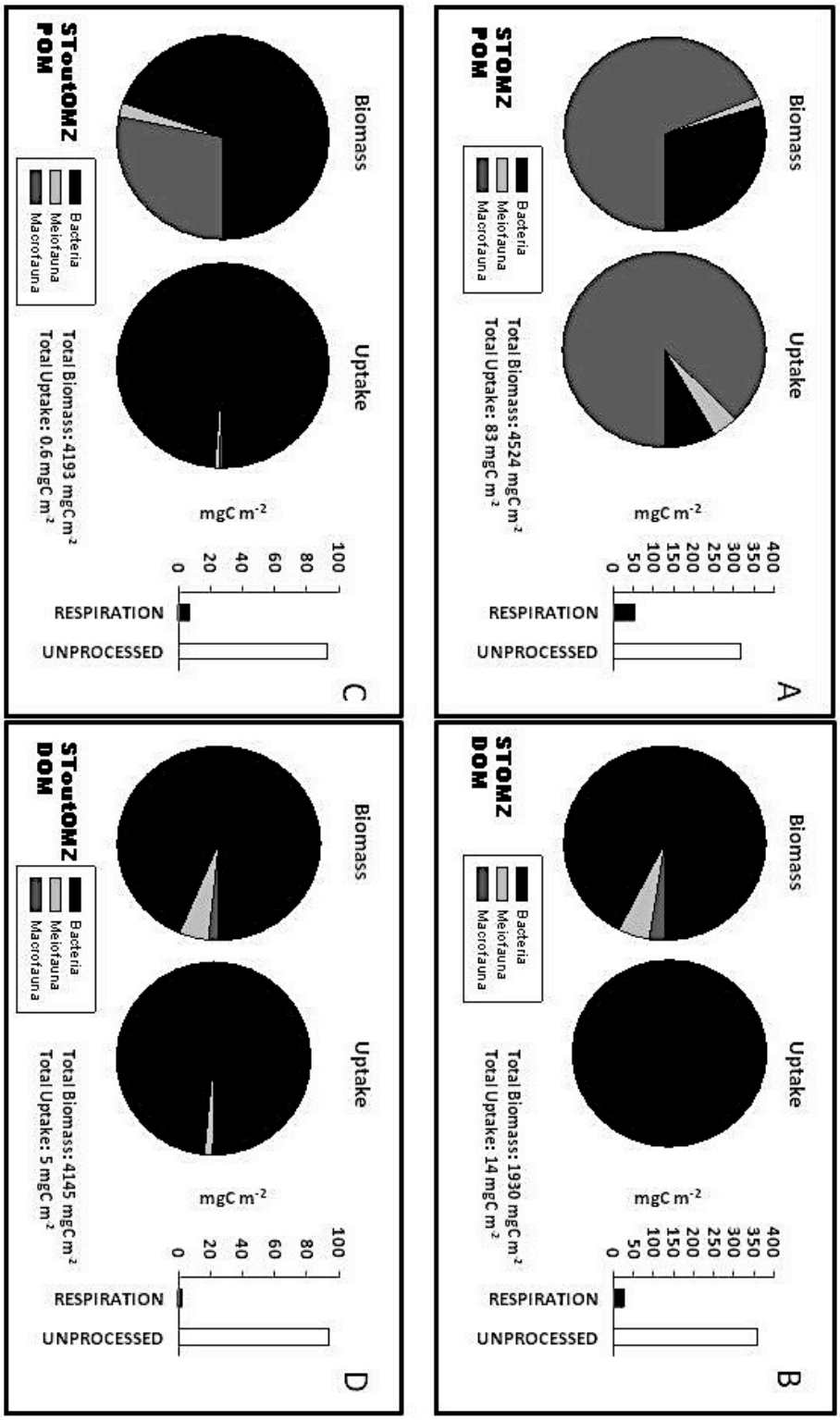
744 **Figure 3.** Total tracer C incorporation in the stations divided per treatment. The term "Soft
745 bodied protists" represents Gromiids and Allogromiids. "Polychaetes" regroup all polychaete
746 species other than *Linopherus sp.,* "Eukarya" regroup all other faunal groups besides
747 Nematodes, Foraminifera and Plychaetes, for which no determination was done.



748

749 **Figure 4.** Comparison between stations and treatments and overview of the benthic
750 community structure and functioning. Biomass values are calculated per treatment and whole
751 core, to allow direct comparison with uptake values.

752


STOMZ POM
Biomass
Uptake
Bacteria
Meiofauna
Macrofauna
Total Biomass: 4524 mgC m-2
Total Uptake: 83 mgC m-2
mgC m-2
400 350 300 250 200 150 100 50 0
RESPIRATION
UNPROCESSED
A
STOMZ DOM
Biomass
Uptake
Bacteria
Meiofauna
Macrofauna
Total Biomass: 1930 mgC m-2
Total Uptake: 14 mgC m-2
mgC m-2
400 350 300 250 200 150 100 50 0
RESPIRATION
UNPROCESSED
B
STOutOMZ POM
Biomass
Uptake
Bacteria
Meiofauna
Macrofauna
Total Biomass: 4193 mgC m-2
Total Uptake: 0.6 mgC m-2
mgC m-2
100 80 60 40 20 0
RESPIRATION
UNPROCESSED
C
STOutOMZ DOM
Biomass
Uptake
Bacteria
Meiofauna
Macrofauna
Total Biomass: 4145 mgC m-2
Total Uptake: 5 mgC m-2
mgC m-2
100 80 60 40 20 0
RESPIRATION
UNPROCESSED
D