Prokaryotic, protozoan and metazoan processing of organic matter in sediments: a tracer approach

Verwerking van organisch materiaal in sedimenten door prokaryoten en eencellige en meercellige eukaryoten: een tracer studie

(met een samenvatting in het Nederlands)

Proefschrift

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door

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Does the walker choose the Path

or the Path the walker?

From "Sabriel" by Garth Nix

1 INTRODUCTION

The circle of life

Generalities

The circle is completeness, union, traditionally it symbolizes what has no beginning and no end. According to Plato, the circle is perfection; in Zen Buddhism it means illumination, perfection of the man in synch with the primordial Principle. The Shahqt-mar, or Celtic wheel, is symbol of peace, freedom and knowledge. The circle is the Ariadne's thread of this thesis, connecting all the main characters that you'll find in this work. It's the circle of life, a perpetual feedback system, the continuous recycling of matter and energy that Antoine Lavoisier defined in his principle of mass/matter conservation.

The *Challenger* Expedition between 1873 and 1876 is considered to be the beginning of Deep-sea Biology. This thesis mainly concerns deep-sea ecology and ecosystem functioning.

Since the *Challenger*, many scientific projects have focused on this little known, dark world that makes up about 94% of the ocean bottom beyond the permanent thermocline (Sanders and Hessler 1969). In particular, coming back to the circle symbolism, not much is known about the deep-sea benthic cycle of one of the most important chemical elements on our Planet: Carbon. Carbon is the 15^{eth} most abundant element constituting the Earth's crust, the fourth most abundant element in the universe by mass, and the main constituent of organic molecules. The large quantity and the unique diversity of organic compounds and their unusual polymer-forming ability at the temperatures commonly encountered on

Earth, make carbon the chemical basis of all known life. In the human body carbon is the second most abundant element by mass (about 18.5%) after oxygen. The Earth is ~ 70% covered by ocean and beyond the threshold depth of 500 m (Levin and Gooday in Rowe and Pariente 1991) extends the deep sea (but researchers still debate on what depth to use as a limit and many consider deep sea starting after the twilight zone, approx 1000 m depth): it is therefore easy to see that deep-sea ecology and biology are an extremely important facet of marine processes and, carbon being the most important element for life, studying carbon cycling in the deep sea assumes a whole new importance and urgency.
From the minuscule bacteria to the huge blue whale all life forms in the ocean survive and thrive thanks to carbon: their food is made by carbon, their respiration is possible thanks to carbon and even if some groups and species have developed alternative pathways for their metabolism and can rely on other energy sources, carbon remains the unquestioned main actor in the play of life. Carbon, as many other elements on our planet, exists in a cycle that links all living organisms together in life and death.

Carbon cycle

In the ocean there are distinct pathways that carbon follows that have been unravelled in the last two centuries. A simplified scheme is offered in Fig. 1.

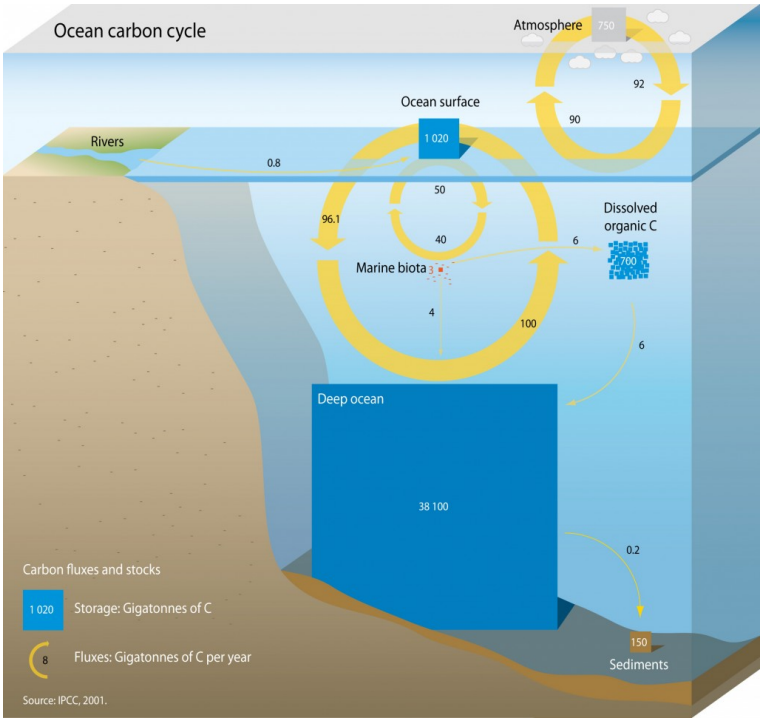

Fig 1: Ocean carbon cycle. IPCC report 2001

According to the numbers reported in this picture, the deep ocean represents a huge storage facility for carbon and marine sediments are the end point of a portion of the carbon input. Carbon enters the cycle via riverine input or land run-off (0.8 Gt y^{-1}), with raindrops and snowflakes, dissolving in the water directly from the atmosphere or literally falling from the sky (marine birds, organic aerosols, dust and human transport). Along this cycle, carbon can exist in different forms. Organic carbon can be found as particles (POC) or in dissolved form (DOC), it makes up the cells of all organisms being packed in the different tissues and organs. Inorganic carbon is normally found as constituent of the crystalline structure of minerals, both chemically- and biologically- deposited (Particulate Inorganic Carbon, PIC) or dissolved (Dissolved Inorganic Carbon, DIC). Near the ocean surface, inorganic carbon is utilized by primary producers via photosynthesis for biomass creation and produced via respiration. Phytoplankton is then grazed upon by zooplankton,

attacked by bacteria and viruses or simply dies and flocks to sink towards the deep. Zooplankton is preyed upon by carnivorous organisms, which in turn are food for bigger animals or die of infections or age, becoming food supply for smaller organisms and bacteria. Whatever carbon escapes water column remineralisation and deposits on the ocean floor, its fate is either fuelling the benthic community growth or being sequestrated into the sediment via a mechanism known as burial. According to the IPCC 2001 estimates, 150 Gt of carbon are stored in the sediment and a flux of 0.2 Gt per year is buried in oceanic sediments.

Organic Matter

This work focuses on the organic form of carbon as organic matter (OM). Here I define organic matter as material that once constituted living organisms or was expelled by them, made of organic compounds and subject to decay. It is a heterogeneous and very complex mixture of compounds and many of its constituents have not yet been characterized (Lee et al. 2004). In other words, a substance can be classified as organic matter when made of complex carbon based-molecules that can be "eaten" or else decomposed, and used as energy source.

OM in the ocean can be found in various particle sizes but traditionally it is divided in particulate organic matter (POM) and dissolved organic matter (DOM), This distinction is somehow artificial, DOM is defined as everything that passes through a 0.45 - 1.0 μm filter, POM everything that does not pass (Hansell 2002). POM sinks to the bottom of the ocean and deposits on the sediment surface where is utilized (by benthic deposit feeders and microbes) and transported to the deeper layer of the sediment by sediment dwelling species, to be accessible by others (Graf 1987; Graf 1989). OM can be degraded following two main pathways, aerobic and anaerobic metabolism (Burdige 2006). OM can be divided in high and low quality. High-quality OM is normally fresher, rapidly consumed and preferred by most organisms because its nutritional value is higher. Poor-quality OM is normally more refractory, older and more difficult to digest containing less energy and therefore being less palatable (Dauwe et al. 1999). It is generally believed that OM that

reaches the deep-ocean is of poor quality, due to the long time it took to sink and the ample usage that the water-column biota already made of it (Cowie and Hedges 1994; Wakeham et al. 1997). Normally, only what's left over from the upper layers and what remains after faunal gut passage manages to reach the benthic realm and biota have to cope with such food (Lopez and Levinton 1987). There are occasions though in which freshly produced OM does arrive to the sediment (Billett et al. 1983; Graf 1989). This may happen when OM particles form aggregates and sink very rapidly to the bottom, (mainly because of their weight) or when the production is so extensive that the water-column organisms do not manage to feed on all of it. Dead carcasses from large organisms such as whales also are a periodic input of fresh OM to the sediment food web (Smith and Baco 2003).

Benthic food web

The modern concept of food web developed from the initial ideas of Charles Darwin, who described the complex relations among biotic groups as "web of life" and "web of complex relations". Charles Elton is considered the father of the concepts of food cycles, food chains, and food size that he explains in his book "Animal Ecology" (1927). Later on, the name "food cycle" was replaced by "food web", which is the currently used terminology to define the structure of the interactions (mainly feeding relationships) among biota. Raymond Lindeman's paper (1942) introduced the idea of trophic dynamics in ecosystems, and nowadays a food web is considered to be the sum of the trophic interactions occurring between different organisms.

Generally a community can be divided into resources or primary producers and consumers: autotrophs and heterotrophs.
Autotrophs are organisms using energy derived from light (photosynthesis) or from inorganic chemical reactions (chemosynthesis) to produce complex organic molecules and therefore "fix" inorganic carbon. They are considered the primary producers of the food web and the basal step of the ecological pyramid. Algae, facultative autotrophic

flagellates, different groups of bacteria and archaea constitute this category.
Heterotrophs are organisms that cannot synthesize organic compounds using inorganic carbon and need to use already created organic carbon for growth, either predating other organisms or feeding on detritus and other OM. Metazoans and protozoans, big and small, from the microscopic heterotrophic flagellates to fishes fall under this second category. In this second category we find benthic meiofauna, eukaryotes ranging between 38 and 500 µm size and macrofauna, all eukaryotes ranging between 500 and 3000 µm size (Rowe 1983).

Primary production in the deep-sea sediment is limited to chemoautotrophy because of total absence of light; therefore the majority of the biota depends on the OM raining down from surficial waters (the so-called marine snow) and from predating/feeding on the local biomass. A simplified version of a deep-sea benthic food web is shown in Fig. 2.

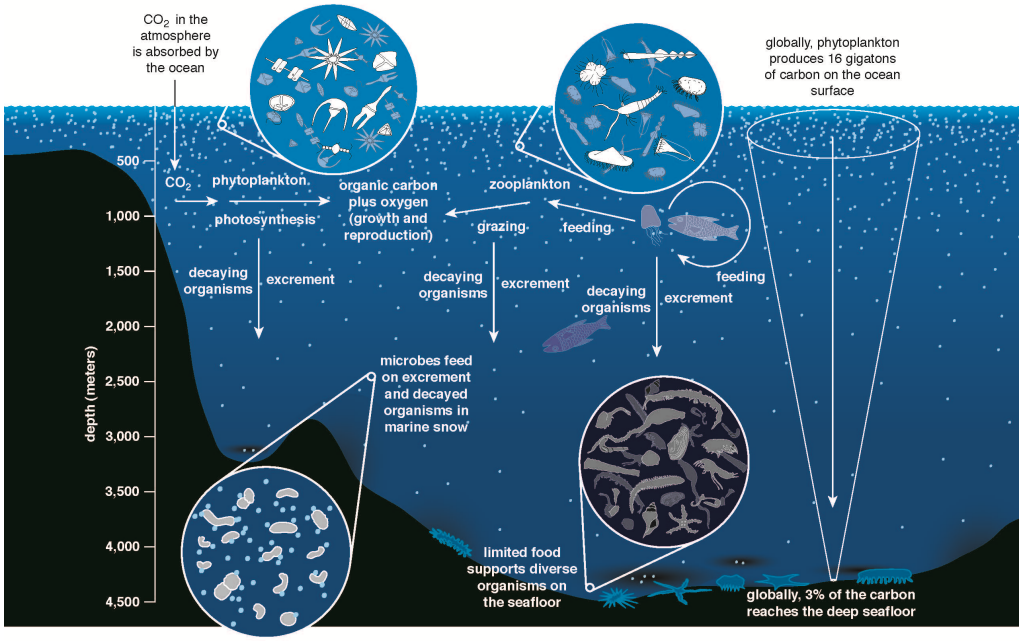

Fig 2. Deep-sea food web. From McClain et al. 2011 and courtesy of the American Scientist

There are deep-sea sediments where the bacterial community prevails in terms of biomass and carbon processing (Moodley et al. 2002), in other sediments metazoans are more abundant and most of the OM processing is carried out by them (Woulds et al. 2009). Many studies have been carried out on deep-sea bacteria and meio- and macrofauna (Aberle and Witte 2003; Levin et al. 2000; Moodley et al. 2000) but still much needs to be researched, especially concerning specific groups (like protists) and overall carbon assimilation, remineralisation and reworking which are the main topics addressed in the first three chapters of this thesis.

How organisms affect the deep-sea food web depends a.o. on their physiology. However, even for the larger groups, the megafauna, we have rather limited knowledge regarding how they utilize food, (in particular on which fraction they allocate to storage), their respiration and their tissue- biochemical composition (Smith et al. 2009; Tecchio et al. 2011). As due to the lethal effects of depressurization on living megafauna, in this thesis I had to use shallow-water species as analogue for deep-sea megafauna. I chose to use *Cerastoderma edule*, known also as "cockle". This species is well suited to serve as a model organism to elucidate metabolic use of food and carbon assimilation/storage/respiration. It may also be relevant to deep sea benthic megafauna because of its similarities with deep-sea cousin species (such as many *Lucinidae* and *Thyasiridae*, and *Amygdalum politum* found in the Arabian sea (Levin et al. 2000) but also in the Mediterranean (Wwf/Iucn 2005) and in the Atlantic (Payne and Allen 1991)), its ubiquity around the globe (Cardoso et al. 2009) and its ecological role (Iglesias et al. 1996).

Biomarkers and tracers

Many approaches have been used to study deep-sea sediment ecosystems, ranging from box coring and trawling (Rowe 1983) to *in situ* (Sweetman and Witte 2008; Witte et al. 2003a) and on-board ship experiments (Moodley et al. 2002; Woulds et al. 2009) to video footage data (Bett et al. 2001; Murty et al. 2009; Ruhl and Smith 2004) and remote sensing (Duineveld et al. 2012). As the benthic realm lays on the bottom and therefore, in the deep sea, many hundreds of meters below sea surface, it is notoriously difficult to sample. Therefore machinery

needs to be used and lowered via steel cables in order to retrieve samples, intact sediment cores, bottom-water or to deploy instruments, chambers or other devices for *in situ* operations. Getting proper samples is therefore already a challenge but it's not the only one that deep-sea researchers have to face. Once the intact sediment cores have been collected there is the need to find ways to study the community that lives in them. Biodiversity and biomass studies can be done by sieving the sediment and directly collect the animals: this will provide very useful information on the composition of the fauna but not on bacteria, archaea and viruses. Moreover, very limited data can be collected on biotic metabolism, OM processing and respiration by analysing only the organisms.

To fill these gaps, researchers make broad use of biomarkers, proxies and tracers. Biomarkers are compounds that have a biological specificity in the sense that they are produced only by a limited group of organisms (Boschker and Middelburg 2002). Examples are lipids, including fatty acids. These characteristics allow us to use them to estimate biomass, biogeochemical composition, component concentrations, assimilation, storage and processing. In this work we used phospholipid derived fatty acids (PLFA) (Middelburg et al. 2000) and amino acids (Veuger et al. 2012; Woulds et al. 2012) as biomarkers.
Proxies are measured variables used to infer the value of a parameter of interest that cannot be directly assessed (IPCC Third Assessment Report 2001). An example of proxy use is measuring dissolved inorganic carbon (DIC) in sediment pore-water and overlying core water to quantify CO_2 release and therefore respiration (Emrich and Vogel 1970; Miyajima et al. 1995). In this work we used DIC to estimate benthic respiration.
Tracers are compounds that can be used to follow the flow of these substances and quantify their concentration without affecting the process. Stable isotope tracers have shown to be very useful in ecology and in particular in ecosystem studies (Peterson and Fry 1987) and when combined with biomarkers, such as fatty acids, can provide important information on OM processing, uptake, incorporation and respiration.
This thesis will focus on ^{13}C-labelled algal derived OM material, used as tracer to evaluate deep-sea benthic prokaryotic and metazoan OM processing, storage in tissues, respiration. The tracers used are of three different kinds: particulate organic matter (POM), dissolved organic matter (DOM) and living algae.

The combined approach, on-deck plus laboratory experiments, offered some insights into (deep-sea) food-web functioning and alteration due to hypoxia, and on carbon cycling, as well as on carbon allocation into specific biochemical compounds such as phospholipid fatty acids (PLFA) and amino acids in different bivalve tissues.

Embedding

The research in this thesis has been conducted within two main cooperation projects: BIOFUN and PASOM.

BIOFUN stands for Biodiversity and ecosystem functioning in contrasting southern European deep-sea environment and is an European Science Foundation EuroDEEP project conducted in the period 2008-2011. Aims of the program were to characterize, through an ecosystem approach, two deep-sea habitats – the mid-slope and abyssal plain – to understand the linkages between biodiversity patterns and ecosystem functioning in relation to environmental conditions along a trophic gradient, from Eastern Atlantic to the Western, Central and Eastern Mediterranean, enabling the simulation of their potential response to changing trophic conditions. The BIOFUN project is part of the European Science Foundation EuroDEEP program.

PASOM is a process study on the impact of the Arabian Sea oxygen minimum zone on organic matter degradation, nutrient regeneration, trace metal cycling and foraminiferal proxies and is a NWO-ALW project conducted in the period 2008-2011. The project investigates the impact of oxygen depletion on organic matter processing and preservation and the recording of proxies. The PASOM cruise was funded by the Netherlands Organization for Scientific Research (NWO) grant number 817.01.015

All the field data and deep-sea samples were collected during three cruises, the 2008 BIOFUN cruise in the NE Atlantic Ocean Galicia Bank

and the 2009 BIOFUN cruise in the Western Mediterranean-Balearic Sea, on the Dutch vessel RV Pelagia and the 2009 PASOM cruise in the Arabian Sea on Murray Ridge, again on RV Pelagia.

My PhD appointment was possible thanks to the co-funding received from the Darwin Centre for Biogeosciences and the research was one of two twin research projects constituting the larger proposal "Impact of benthic processes on biogeochemical organic carbon cycling and organic proxy records in marine sediments".

The rationale of the larger program

When organic matter (OM) reaches surface marine sediments, its processing results in degradation and in major compositional changes. Bacteria, protists and eukaryotes are the key actors in this process but the knowledge on their relative importance and impact on the composition of sediment OM is still limited. Research approaches so far have been generally monodisciplinary which, besides holding back progress, provided a biased view of sediment OM processing. Organic geochemists focussed on the composition of OM neglecting respiration processes and the biota; biogeochemists and ecologists investigated carbon transfers, respiration and food web structure, ignoring OM's nature. This work attempts to approach the sedimentary OM processing in an integrative and comprehensive way. We focussed on the effect of oxygen and organic matter form on isotopically enriched algal tracers OM degradation and transformation by deep-sea biota, trying to identify their role in the food web. Furthermore, to assess the carbon metabolic pathways and tissue storage of the main biochemical compounds (phospholipid fatty acids and amino acids) in megafauna, we selected a shallow-water species of bivalve as an analogue for deep-sea megafauna and subjected it to feeding experiments with live isotopically enriched algae.

Major aims of this PhD study were:

1. To study the effect of oxygen availability on deep-sea OM degradation, transformation, preservation and composition.

2. To use ^{13}C labelled algae to trace the involvement of prokaryotes, protozoans and metazoans in phytodetritus deep-sea processing.

3. To investigate the use and transformation of natural algae assemblages by a selected species of metazoan suspension feeder (*Cerastoderma edule*).

Thesis outline

The second and third chapters of this thesis present the work I did in the Arabian Sea, on the Murray Ridge, Pakistan-Oman margin. The Arabian Sea is famous among marine researchers for its permanent Oxygen Minimum Zone (OMZ), its peculiar fauna assemblages and the lack of hydrogen sulfide that normally characterizes anoxic sediments. But the most striking characteristic of this area, which it shares with other OMZs around the globe, is the OM accumulation. Our work aimed to unravel the reasons for this accumulation: is it because of the limiting redox condition, or because of lack of processing fauna, is it maybe because the OM is too refractory to be utilized further or are there other causes?

Chapter 2 "Carbon processing at the deep-sea floor of the Arabian Sea Oxygen minimum Zone: a tracer approach" focuses on the effects of oxygen depletion on algal-derived particulate organic matter processing by sediment biota in the OMZ. This study confirms abundant faunal presence and activity, despite the scarcity of oxygen.

Chapter 3 "Dissolved and particulate organic matter processing by the deep-sea benthic community inside and outside the Arabian Sea oxygen minimum zone" focuses on the effect of OM quality on its degradation, with particular attention to the different particulate versus dissolved organic matter processing food web pathways in the OMZ. This study reports on the different fate OM depending on its quality: the rich DOM and the poorer POM and shows how both are promptly degraded and the biotic groups that carry out the processing.

The next two chapters push the boundaries of my interest a bit further, in terms of geographic areas and water depth.

Chapter 4 "Organic matter processing in the deep sea: a comparison between North-East Atlantic and Mediterranean sediments" focuses on benthic phytodetritus processing and assimilation in two different settings, with particular attention to the fate of the amended particulate and dissolved OM: assimilation and respiration by biota under manipulated oxygen concentrations. Emphasis was also given to tracer

quality and quantity in the interpretation of the experimental results. This study reports on different ecosystems in different geographic areas. The comparison between North-East Atlantic and Mediterranean Sea allows a broader generalization of my findings and emphasizes differences and similarities in ecosystem functioning and OM processing due to physical conditions such as temperature, oxygen concentration, OM quality.

Chapter 5 "Food assimilation from a mixed diatom/flagellate diet by *Cerastoderma edule*: you are what you eat or not?" aimed at investigating megafauna food incorporation, respiration and biochemical composition. The estuarine cockle can be used as analog for deep-sea cousin species that could not be used due to depressurization issues, providing experimental ease, more controllable conditions such as temperature and food intake, more literature knowledge.

Chapter 6 is a synthesis and summary of the collective results of the studies. I draw general conclusions, assess the methodological flaws and offer few follow up suggestions on deep-sea benthic food web studies.

2 Carbon processing at the deep-sea floor of the Arabian Sea Oxygen Minimum Zone: a tracer approach

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In revision for Journal of Sea Research

Abstract. *In this study we aimed at elucidating the trophic interactions in the food web of sediments from and close to the Oxygen Minimum Zones (OMZ) of the Arabian Sea. Sediment cores from inside (885 m depth) and outside (1791m depth) the OMZ were manipulated onboard by adding ^{13}C-enriched phytodetritus. The incorporation of phytodetritus by the benthic community was quantified after incubating for 7 days. To assess the effect of bottom-water oxygenation on the processing of organic matter, the oxygen concentration in the overlying water of the incubated cores was also manipulated. Biomass values inside and outside the OMZ were comparable for bacteria (1068 and 1276 mg C m^{-2}) and macrofauna (2528 and 3263 mg C m^{-2}), but not for meiofauna (63 and 1338 mg C m^{-2}). Uptake values, in percentage on total added tracer, were: 0.8% suboxic treatment and 0.5 oxic treatment inside the OMZ 0.5% suboxic treatment and 1.2 oxic treatment outside the OMZ for bacteria, 0.4% suboxic treatment and 0.3% oxic treatment inside the OMZ and 0.06%, 0.008% respectively outside the OMZ for meiofauna, 17.4% suboxic treatment and 4.4% oxic treatment inside the OMZ, 0.1% and 1.3% respectively outside the OMZ for macrofauna. Respiration, in percentage on total added tracer, accounted for 13% inside the OMZ for both treatments, 4.6 and 6.8% outside the OMZ respectively for oxic and suboxic treatment. Our results show that phytodetritus are most efficiently processed at in situ oxygen conditions, and that foraminifera and bacteria remain active both under elevated and lowered bottom-water oxygen levels.*

1. Introduction

The oxygen concentration of water bodies is controlled by the balance of supply and consumption. Below the photic zone, oxygen supply is mainly controlled by physical processes. Oxygen consumption in the water column relates primarily to the degradation of dissolved and particulate organic matter. Oxygen consumption may outbalance supply in water bodies that receive high loads of nutrients and organic matter through river run-off (e.g. the Po river outflow) or those underlying upwelling areas (e.g. Arabian Sea), especially when external factors such as high temperatures intensify the degradation of phytoplankton blooms, or when water-column stratification prevents oxygen replenishment. When these combined effects result in oxygen concentrations below 63 μM, aerobic degradation processes may become significantly impaired. Such water bodies are then described as "hypoxic" or "low-oxygen zones", or in case of open ocean settings "oxygen minimum zones"(OMZ) (Levin 2003; Middelburg and Levin 2009). Helly and Levin (2004) report that there are over one million km^2 of permanently hypoxic shelf and bathyal sea floor, distributed all around the globe. Some OMZ areas impinge on coastal areas (Stramma et al. 2008) and due to global changing could become ever more pronounced, leading to permanent oxygen deficiency and ecosystem perturbation (Stramma et al. 2010).

Sediments underlying OMZs typically have a specialized protozoan and metazoan fauna that is dominated by few species. Macrofaunal biomass is higher in OMZ boundary sediments, which is thought to be an OMZ edge effect (Levin 2003), and occurs because organic matter is abundant and oxygen concentrations are physiologically tolerable to metazoan macrofauna (Woulds et al. 2007). Fauna inhabiting these sediments have specialized adaptations, for example foraminifera can sustain low metabolic activity through denitrification (Piña-Ochoa E. 2009; Risgaard-Petersen et al. 2006) and polychaetes have large external gills (Lamont and Gage 2000; Levin et al. 2009b) or high concentrations of oxygen-binding proteins and pyruvate (Gonzalez and Quinones 2000; Pals and Pauptit 1979; Ruby and Fox 1976).

Demaison and Moore (1980) were among the first to suggest that oxygen depletion is responsible for organic matter (OM) accumulation in sediments, due to inefficient carbon processing and the lower oxidative power of anaerobic degradation pathways. This assumption has been debated: Calvert et al. (1995) and Pedersen and Calvert (1990), argue

that high organic matter delivery (export production), sediment texture and dilution by other sedimentary components (such as relatively organic-poor terrestrial clays and the inorganic sediment matrix) rather than water-column anoxia control accumulation of organic matter in sediments. Other, non-oxygen related factors such as OM quality and sorption to the inorganic mineral matrix have also been suggested as primary causes for the OM enrichment in OMZ sediment (Hedges and Keil 1995a). Smallwood et al. (2000; 1999) and Jeffreys et al. (2009a) agree that mega, macro e meiofauna in the OMZ core affect OM quality, stripping away the more labile compounds and determining a change in OM quality. Wakeham et al. (2002) report that mid-water column fluxes are not consistent with continual degradation of OM as it sinks in the Arabian Sea: in fact, despite temporal and spatial variability in amount and composition of OM reaching their sediment traps, fluxes towards the bottom were always abundant, but accumulation rates and concentrations in surface sediments were substantially lower than the fluxes recorded in the traps, implying relative little remineralisation occurring in the water column. This could then imply that the OM in the water column is already of poor quality as it sinks. All these processes going on both in the water column and in the sediment make it difficult to separate the single effects of oxygen concentration and OM quality on sedimentary biotic processing. This work aims at discriminating between OM quality and oxygen depletion as factors determining biota OM processing, analysing in particular how oxygen concentration inside and outside the OMZ affects biotic carbon uptake and respiration.

The mid-water OMZ in the Arabian Sea is permanent, well delineated and the largest on Earth (Helly and Levin 2004). This oxygen depletion is due to respiration of high concentrations of OM produced during monsoon-driven upwelling of nutrient rich waters, input of intermediate low-oxygen waters from the Southern Hemisphere and strong stratification (Andersson et al. 2008) and increased OM residence times caused by reversal of surface and intermediate water currents (Wyrtki 1961). The Arabian Sea OMZ has persisted for many decades and has been reported for the first time in the early 20th century (Cowie 2005). This makes it a natural field-laboratory to better comprehend the processes and the dynamics occurring in highly oxygen depleted areas. Studies have reported oxygen concentrations of < 9 μM between 150 m and 1000 m water depth, which coincide with the maximal values for sediment OM quantity (Brand and Griffiths 2009; Breuer et al. 2009; Cowie et al. 2009). The OMZ in the Arabian Sea persists roughly between

~100 and ~1000 m depth, although the boundaries fluctuate up to between 60 to 80 m and down to 1200 m depending on season and location (Brand and Griffiths 2009).

Studies based on ^{13}C and ^{15}N labelling of food sources can provide a quantitative indication of the OM remineralisation and assimilation by biota and therefore can indicate their activity (Blair et al. 1996; Hunter et al. 2012a; Hunter et al. 2012b; Middelburg et al. 2000). This methodology has also been applied to better understand the OM processing in the Arabian OMZ. Short-term incubations using lander chambers *in situ* or on-deck using controlled conditions carried out in the Pakistan Margin of the Arabian Sea (Andersson et al. 2008; Cowie and Levin 2009; Woulds et al. 2009; Woulds et al. 2007) have shown to be valuable in investigating the benthic response to a fresh OM deposition event and the fate of the OM added to the system. Already after 2-5 days incubation period these authors found bacterial, foraminiferal and metazoan macrofaunal uptake to account for 2–32%, 1–17% and 0–46% of total OM processing, respectively. At some sites metazoan uptake was similar in magnitude to that of bacteria and/or total respiration, although in most instances bacteria processed most of the carbon. These studies also confirmed that the benthic community is active in low-oxygen conditions and that OM is utilised and processed both for respiration and biomass production.

In those studies, the sediment core incubations were however conducted at ambient oxygen concentrations. In an area which experiences temporary migrations of the OMZ zone, it makes sense to look at the response of the biota under short-term changes in oxygen concentration, in particular for the lower OMZ boundary exposed to such fluctuations. In our study on the Murray Ridge, we manipulated the oxygen concentration of sediments taken from inside and outside the OMZ so that the direct control of oxygen concentration on the short-term processing of phytodetritus can be identified. We hypothesized a higher response of the benthic community when exposed to higher oxygen levels. We also expected that when sediment underlying oxygenated waters, from the lower boundary of the OMZ, was exposed to oxygen concentrations as found inside the OMZ, the response in terms of uptake and respiration by benthic community would be impaired. Our focus was on the main benthic compartments based on traditional size-classes bacteria and meio- and macrofauna.

The aims of this study therefore are:

1. to quantify the effect of O_2 depletion on biotic processing of fresh particulate organic matter (POM) inside and outside the OMZ
2. to quantify benthic group-specific fresh carbon incorporation and total benthic respiration
3. to compare fresh organic matter processing efficiency of the biota inside and outside the OMZ
4. to find evidence that scarce remineralisation of OM inside the Arabian Sea OMZ is not due to scarce biotic biomass and/or efficiency

2. Material and Methods

2.1 Study area

Sediment samples were taken from 2 stations on Murray Ridge, the offshore region of the Pakistan margin (Figure 1) during the PASOM cruise (64PE301) in January 2010. Station OMZ (22°32.9'N 64°02.4'E, 885 m) was located in the core of the OMZ, whereas the deeper station outOMZ (22°18.5'N 63°24.5'E, 1791 m) was below, outside the OMZ. Henceforth, these stations are referred to as STOMZ and SToutOMZ, respectively.

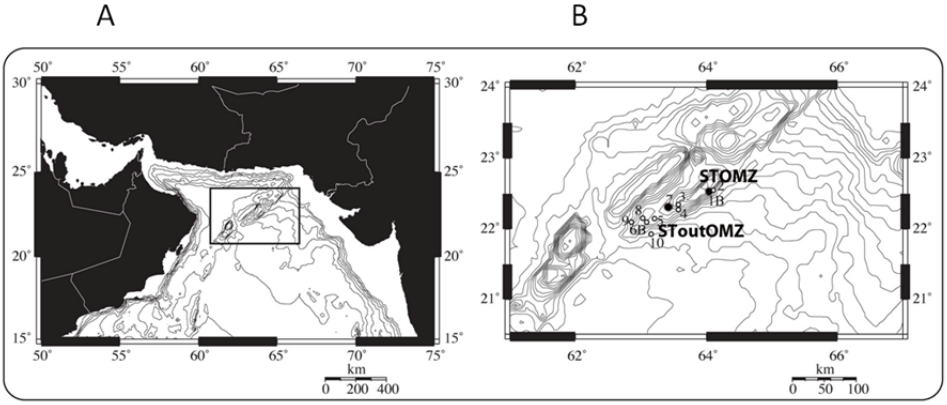

Figure 1. Sampling area A: the Northern Arabian Sea B: zoom-in of the sampling locations with the two stations investigated in this study. STB inside the OMZ, ST7 outside the OMZ.

The Murray Ridge is located south west of the Pakistan margin. It starts about 100 km from the coast, 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 deeper northern zone with water depths of 2000 m and the >4400 m deep Dalymple Trough (Gaedicke et al. 2002). The Murray Ridge divides the offshore region of Pakistan into two areas: northwest of the Ridge extends the offshore part of the Makran, south and east of the Ridge lays the Indus Fan. The sedimentary cover of the Ridge increases in thickness towards the coast, progressively widening its topographic expression. The isolated position far from shore limits the deposition of terrestrial material on the Ridge and only marine inputs reach the bottom (Shimmield et al. 1990); this characteristic makes the Ridge a perfect experimental setting, avoiding any confusing factors related to terrestrial inputs of OM.

The OMZ started approximately 150 meters below the sea surface, which was also the depth of the seasonal thermocline. CTD temperature profiles (not shown) revealed a mixed layer depth between 80 and 95 m along the Ridge, except for station SToutOMZ where the stratification started at the surface. Salinity profiles showed a distinct maximum of 36.2 at 320 m, related to the Persian Gulf outflow (Brand and Griffiths 2009). The salinity decreased below 400 m to a minimum of 34.7 at 3000 m. The Arabian Sea is a naturally highly productive system, where seasonal monsoon-driven upwelling of nutrient-rich water along the coast lead to high primary production that results in increased downward particle fluxes (Nair et al. 1989). Surface seasonal temperature variability has also been reported in this area (Aller and Mackin 1984) and is another important factor that influences the primary production and therefore the OM fluxes to the sea-bottom. Sea-surface temperature during our sampling was 25.2 °C and below the mixed layer the temperature declined to a minimum of 2.1 °C at 2600 m.
Oxygen was analyzed using three different methods during the cruise: the oxygen sensor on the CTD, Winkler titration of the water sampled from the rosette sampler and an optode fitted to the multicorer. The CTD was equipped with a sea-Bird SBE43 sensor, sensitivity 3 µmol l^{-1}, accuracy 2%. The CTD sensor was calibrated using the Winkler titration Measurements from SToutOMZ: the calibration procedure was done using a linear fit and different fit functions. One CTD cast was done per each station.

	STOMZ	SToutOMZ
Position	22°32.9'N 64°02.4'E	22°18.5'N 63°24.5'E
Depth	885 m	1791m
Bottom-water		
Temperature, °C (CTD sensor)	10	4
Salinity, (CTD sensor)	34.8	34.9
Dissolved O_2 µmol l^{-1} (CTD sensor)	2	45
Sediment		
Median grain size, µm (0-3 cm)	35.4	16.5
Water content %	82	60
% Total organic carbon (0-3 cm)	6.38	1.03
molar C:N ratio (0-3 cm)	9.75	7.87
bulk δ13C	-21.5	-20.0
bulk δ15N	8.0	8.7

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

2.2 Sample collection

Intact 10 cm internal diameter multicorer replicate cores were retrieved from STOMZ and SToutOMZ. Once on deck, the background (control) and the experimental cores were immediately transported into the 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 the same pre-made air mixtures used later during the experiment.

The first 0-3 cm of two independent cores taken at the same locations and at the same time were used to determine sediment characteristics such as: porosity, water content, sediment grain size, organic carbon and (total) nitrogen contents and natural abundance isotope ratios.

2.3 Phytodetritus preparation

Phytodetritus was used as tracer and obtained by culturing axenically the brown cosmopolitan diatom *Thalassiosira pseudonana,* previously utilized in many other studies, in a 30% ^{13}C enriched F2 medium. The

algae were harvested via centrifugation (2000 g force) and the remaining label was washed with artificial seawater and subsequently centrifuged again to remove any remaining ^{13}C-bicarbonate. The pellets were frozen overnight at -80 °C and freeze-dried. Milli-Q water was then added to burst the cells to release dissolved organic matter (inner cytoplasm and exudates), the particulate organic matter (POM) remaining was subsequently separated via centrifugation (2000 g force). POM was portioned, frozen and freeze-dried again for transport and use in the experiments. The POM consisted for 20% ^{13}C, equal to a delta value of 21500 ‰ as measured by isotope ratio mass spectrometry using a dilution procedure.

2.4 Experimental incubations

An equivalent of 400 mgC m^{-2} freeze-dried POM was added to 2 cores from station STOMZ, whereas 100 mgC m^{-2} was used for the 2 cores from SToutOMZ, to mimic the natural organic matter loads for the sites. This dose represented 0.31 and 0.19% of the organic C naturally present in the surface 3 cm of the sediment for STOMZ and SToutOMZ, respectively. We explain this choice and the possible implication in the discussion. The POM was gently delivered to the surface of each core via a long glass pipette, paying attention not to disturb the sediment.

Duplicate cores were incubated for 7 days both under oxic (54 µmol l^{-1} O_2 for STOMZ and 125 µmol l^{-1} O_2 for SToutOMZ) and suboxic conditions (6 µmol l^{-1} O_2 for STOMZ and 8 µmol l^{-1} O_2 for SToutOMZ). *In situ* oxygen concentrations were 2 µmol l^{-1} O_2 at STOMZ and 45 µmol l^{-1} O_2 at SToutOMZ. Oxygen manipulations were obtained by bubbling a pre-made mixture of air (certified O_2, CO_2 and N_2 gas mixture by Hoekloos BV, The Netherlands) in the overlying water of each core. Each core was sealed on the bottom and on the top with o-ring lids and the out-flowing air of each core entered a CO_2 trap. The oxygen measurements were carried out in the overlying core water once the incubations were stopped, using oxygen-optodes (Presens, Germany) following standard procedures of calibration (according to optode manual) and the results showed that the water oxygen concentration was indeed 6 µmol l^{-1} O_2 for the suboxic treatment and 54 µmol l^{-1} O_2 for the oxic one.

2.4.1 Sample processing

At the end of the incubations, the water overlying the sediment and the water of the bottles was gently removed. The upper 10 cm of each core was cut off when still inside the coring tube with a handsaw and frozen for later sub-sampling. This was preferred over direct slicing due to the fluffy and watery nature of the sediment, which would have caused mixing of the layers the moment the bottom lids of the cores would have been removed for slicing. The intact cores were stored in a -20 °C freezer and transported in the same freezer to the laboratory.

2.4.2 Control cores

A total of 4 control cores per station were collected. Two cores per station were used to determine water content via sediment wet weight-dry weight and sediment porosity, calculated from these values. Total nitrogen and organic carbon contents and background natural abundance isotope ratios were measured with a Thermo Electron Flash EA 1112 analyzer (EA) coupled to a Delta V isotope ratio mass spectrometer (IRMS) in grounded freeze-dried sediment samples. In particular, for total organic carbon measurements prior to analysis the samples were acidified in steps with a 30% HCl solution followed by heating block drying for ~ 1 hour. The steps were repeated until all carbonate structures in the samples were dissolved. Sediment grain-size distribution was measured with a MALVERN Mastersizer 2000 on freeze-dried sediment samples.

Two cores per station were used for fauna and bacteria ^{13}C natural abundance analysis and were pulled with the experimental cores for biomass estimations. These fauna and bacteria samples, referred to as "background", were treated exactly as explained further on for the incubation cores.

2.4.3 Respiration measurements

Respiration of the ^{13}C labeled POM was estimated from the accumulation of ^{13}C-enriched DIC (dissolved inorganic carbon). To this end, CO_2 traps were prepared in 500 ml sealed off glass bottles with a screw-on septum cap. The bottles were filled with a solution of MilliQ water and NaOH (Kristensen et al. 1992). At the end of the incubation

time, the cores were opened one by one in sequence and oxygen concentration measurements were done.
Respiration of the cores was estimated via DIC samples of the overlying core water at the end of the incubation time. Two sets of CO_2 traps were used for each core, connected to each other in succession; therefore, the CO_2 that was not absorbed by the first trap could be recovered in the second one. The traps were changed every 3.5 days. All traps were measured for DIC. Analysis of the water of the second set of traps showed values very close to zero and therefore will be considered negligible. DIC was measured in ~2 ml samples from the overlying water and from the CO_2 traps with a sterile plastic syringe. The samples were filtered on a GF/F filter attached to the syringe and injected into helium pre-flushed 5 ml glass vials that were sealed with crimp-cap with rubber septum. DIC samples were immediately acidified with 99% H_3PO_4 to inhibit biological activity stored upside down at 4 °C until analysis in the laboratory (~four weeks after sampling). CO_2 concentration and isotope ratio of the gas present in the headspace of the vials was measured in the lab on a Thermo Electron Flash EA 1112 analyzer (EA) coupled to a Delta V isotope ratio mass spectrometer (IRMS). Total ^{13}C-DIC production was determined by combining ^{13}C accumulation in overlying water and the CO_2 traps.

2.4.4 Experimental cores enrichment measurements

After arrival in the laboratory, 60 days after end of the experiment, the background and incubation cores were sliced frozen in 2 cm intervals: 0-2, 2-4, 4-6 cm with a circular electric saw. While frozen, each slice was portioned as follows: one quarter (approx. 39 ml) was freeze-dried immediately and used for analysis of bacterial fatty acids, two quarters was used for faunal extraction and the remaining quarter for archaeal biomarkers (data not presented). The fatty acid samples were freeze-dried and stored again at -20 °C until extracted and analyzed. The frozen subsample for faunal analysis was immersed in a mixture of 40% buffered formaldehyde stained with rose Bengal and allowed to thaw at room temperature. This was preferred over fixing and staining after defrosting because of better preservation of the material. The sediment was stored for 2 days at room temperature before sieving, so that the stain could penetrate animal tissues.

The bacterial tracer uptake was estimated through the enrichment of bacterial-specific phospholipid fatty acids (PLFAs) (Boschker and

Middelburg 2002). Two main biomarkers were chosen for the analysis: iC15:0 and ai15:0 because of specificity for bacteria and presence in all samples; samples were analyzed using GC-c-IRMS following Bligh and Dyer extraction (Middelburg et al. 2000). $\delta^{13}C$ values were corrected for the C addition during derivatization and also for each PLFA-specific carbon atom content differences.

Following Rose Bengal staining, the sediment was sieved on stacked 500 and 38 μm sieves to allow the separation of 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 allow better separation between the residuals of sediment and the nematode fraction of the meiofauna and an easier picking. The division between macro- and meiofauna was based on size and not on taxonomic identity. Foraminifera are usually considered to be part of meiofauna, but in our samples up to 100 specimens were retained on the 500 μm sieve. The residuals on the sieves containing the fauna and some sediment particles were then hand-picked under a binocular microscope and the fauna divided in: branched and shelled foraminifera, soft bodied protists, polychaetes and *Linopherus* sp., nematodes and Other. The fauna was transferred into pre-weighted silver cups, oven-dried (50˚C), decarbonated with 10% HCl (slow addition for foraminifera, checking under the binocular to ensure that the bubbling of the carbonate was finished, as this indicates full removal of inorganic carbonates) 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 analyzed for percentage and delta values ^{13}C, Organic Carbon and Total Nitrogen with a Thermo Electron Flash EA 1112 analyzer (EA) coupled to a Delta V isotope ratio mass spectrometer (IRMS). Fauna biomass was determined via dry weight, C content obtained from IRMS data and abundance via specimens counting.

2.4.5 Bacterial and faunal enrichment

To calculate ^{13}C enrichment in both bacterial and faunal samples we used both the incubated cores and the background (control) cores.
Relative uptake $\Delta\delta$ (the enrichment in ^{13}C stable isotope of the sample):

$$\delta^{13}C_{sample} - \delta^{13}C_{background/control}$$

reflects tracer incorporation and is used to calculate excess ^{13}C (E) over the background

$E=F_{sample}-F_{control}$, where $F = {}^{13}C/({}^{13}C+{}^{12}C)=R/(R+1)$

Here, $R = (\delta^{13}C/1000+1)$ x R_{VPDB}, where R_{VPDB} = 0.01118 and is the standard value for Vienna Pee Dee Belemnite. Total tracer uptake (I, µg tracer m^{-2}) is then calculated as the product of excess and biomass. Incorporation of ^{13}C into bacterial PLFAs was converted to incorporation into bacterial biomass by assuming that the specific PFLAs represent 11% of all bacterial PLFAs (Moodley et al. 2002) which in turn comprises 5.6% of total bacterial C. Conversion of g PLFA / g DW sediment to bacterial biomass per m^2 was done using determined sediment porosity values and sediment dry density. Therefore Bacterial biomass was calculated as

$$\text{Bacteria biomass} = \frac{\text{Conc (PLFA i-C15:0)} + \text{Conc (PLFA ai-C15:0)}}{0.056*0.11}$$

The uptake of total added algal derived C ($^{12}C+^{13}C$) for both bacteria and fauna was then calculated according to Middelburg et al. (2000) and Moodley et al. (2005b), as the quotient of total uptake (I) and the fractional abundance of ^{13}C in the tracer (0.2 for POM).

3. Results

3.1 Sediment characteristics

Differences in sediments were noted between the two study sites. Outside the OMZ the sediment was mainly composed of clay, light brown-gray and very compact. Inside the OMZ cores it was dark brown-black, watery and fluffy, poor in clay but with a high content of foraminiferan shells and diatom frustules. Visual inspection of each core indicated the presence of numerous intact burrows and showed an intact sediment surface. Total organic carbon content and the C:N ratio were higher inside the OMZ, whereas the $\delta^{13}C$ and $\delta^{15}N$ of the sediment inside the OMZ were slightly lower (Table 1).

3.2 Biomass composition of background and experimental cores.

As reported above, fauna was divided in macro and meiofauna. The macrofauna included nematodes, shelled and branched foraminifera, soft bodied protists, polychaetes, *Linopherus* sp. and other Eukaryotes; the meiofauna comprised nematodes and shelled foraminifera. Fauna biomass values are given in mgC m^{-2} and calculated using direct measurements of carbon content of 70 individuals for nematodes, 5 individuals for soft bodied protists, 100 individuals of shelled foraminifera and 3 *Linopherus* sp. and 3 polychaetes for STOMZ, 70 individuals for nematodes, 4 individuals for soft bodied protists, 50 individuals of shelled foraminifera, 3 polychaetes and 3 to 4 individual Eukaryotes for SToutOMZ. Branched foraminifera were picked *in toto* but it is not possible to give an individual number due to their arborescent shape and fragility. In our STOMZ samples the dominant genus of foraminifera in the top 2 cm was the hard-shelled *Uvigerina* sp. (approximately 70% dominance determined by visual sorting), whereas the agglutinated *Reophax* sp. became more dominant (approximately 80%) downcore. The biomass values of the cores used as controls and those of the experimental cores have been averaged to account for environmental patchiness and are reported in Table 2: a total of 6 cores per station were used to determine biomass values. Background control cores biomass in cores from STOMZ spanned between 1193 and 1201 mgC m^{-2} for bacteria, 13 to 64 mgC m^{-2} for meiofauna and 7 to 1185 mgC m^{-2} for macrofauna. Background control cores biomass in cores from SToutOMZ spanned between 1241 and 1346 mgC m^{-2} for bacteria, 16 and 76 mgC m^{-2} for meiofauna and 32 and 1893 mgC m^{-2} for macrofauna. In Figure 2 we present biomass values of the amended cores, no controls, to show that there was no treatment effect on the biomass itself. Bacterial biomass in STOMZ was 1068 mgC m^{-2}, meiofauna accounted for 63 mgC m^{-2}, macrofauna accounted for 2528 mgC m^{-2}. The highest biomass values were recorded for *Linopherus* sp. For detailed values see Table 2. Bacterial biomass in SToutOMZ was 1276 mgC m^{-2}, meiofauna accounted for 1338 mgC m^{-2} and macrofauna for 3263 mgC m^{-2}. The highest biomass values were recorded by soft bodied protists. Detailed values are presented in Table 2.

		Biomass mgC m^{-2}
STOMZ	***Bacteria***	***1068±19***
	Meiofauna	***63***
	Nematodes	16±1
	Shelled foraminifera	33±7
	Soft bodied protists	13±0.1
	Macrofauna	***2528***
	Nematodes	8±10.6
	Shelled foraminifera	30±3
	Linopherus sp.	1757±103
	Polychaetes	733±37
SToutOMZ		
	Bacteria	**1277*±13***
	Meiofauna	***1338***
	Nematodes	*16±2*
	Shelled foraminifera	*30±6*
	Soft bodied protists	*16±0*
	Macrofauna	***3264***
	Nematodes	*23±7*
	Shelled foraminifera	*395±102*
	Branched foraminifera	*752±8*
	Soft bodied protists	*1538±96*
	Polychaetes	*184±75*
	Eukarya	*372±9*

Table 2. Averaged biomass of all the cores (controls and amended cores). Cores n=2 per station for natural abundances, n=6 per station for the biomass. Samples were preserved with 40% buffered formaldehyde so values are potentially fixative-effected.

3.3 Experimental cores uptake and ^{13}C enrichment

Uptake of the ^{13}C label after 7 days was evident in both treatments although the level of enrichment differed per station and benthic group. Bacterial enrichment was lower than other groups' in the OMZ sediment, but was similar in the oxic and suboxic treatment (Table 3). In the sediment outside the OMZ the enrichment reached $\Delta\delta^{13}C$ values 5 and 10 times lower than in STOMZ for oxic and suboxic treatment respectively (Table 3). Meiofaunal enrichment in the OMZ sediments was the highest among the groups and comparable among treatments, ranging between 5 and 365 ‰ (Figure 4 A,B, Table 3). Outside the OMZ, enrichments were lower, spanning between 0 and 15.4 ‰. Macrofauna also followed the same pattern, with higher enrichment values inside the OMZ. In STOMZ $\Delta\delta^{13}C$ values ranged between 5 and 192 ‰, in SToutOMZ between 0 and 28 ‰ (Table 3). Total tracer processing was calculated from ^{13}C incorporation in the bacterial, meiofaunal and macrofaunal biomass and in dissolved CO_2 pools. On average, 5.5% of the added label was incorporated in the biomass by the benthic community over 7 days in STOMZ oxic treatment, 18.3% in the suboxic one whereas it was only 3.1% and 0.7% respectively in SToutOMZ (Table 4). Bacterial uptake of the tracer carbon was lower in the suboxic than in the oxic treatment (Table 4). Fauna uptake rates were however higher in the suboxic treatment than in the oxic (Figure 5 A,B), ranging from a total of 71302 to 19092 $\mu gC\ m^{-2}$. Also in the SToutOMZ bacteria showed higher uptake in the oxic treatment than in the suboxic, (Figure 5 C,D, Table 4). Fauna uptake was lower in the suboxic that in the oxic treatment, with total values of 102 and 1363 $\mu gC\ m^{-2}$. In total, STOMZ showed much higher uptake of the ^{13}C tracer POM offered to the cores than SToutOMZ: 22233 and 73483 $\mu gC\ m^{-2}$ (corresponding to 18 and 5.5% of the added carbon) for oxic and suboxic treatment compared to 2533 and 568 $\mu gC\ m^{-2}$ (2.6 and 0.6%) for SToutOMZ. A large proportion of the added tracer was left unprocessed in both stations and both treatments. Figure 5 shows the core-integrated tracer uptake results.

3.4 Treatment Impact index (TI)

Table 3 shows the Treatment Impact index (TI) for both stations and all the biotic groups and is calculated as

Equation 1

$$TI = \frac{(\Delta\delta\,^{13}C \text{ non natural } O_2 \text{ condition} - \Delta\delta\,^{13}C \text{ natural } O_2 \text{ condition})}{\Delta\delta\,^{13}C \text{ natural } O_2 \text{ condition}}$$

In STOMZ the natural condition is suboxic whereas in SToutOMZ the natural condition is oxic. These values give an idea of the impact that the oxygen treatment had on the organism's uptake and therefore activity, negative numbers mean that the organism's uptake was inhibited and the treatment impaired its activity, positive numbers that the organism's uptake was enhanced and that organisms thrive also in suboptimal conditions.

3.5 Respiration

The sum of ^{13}C-DIC recovered from the overlying water of each core and the connected CO_2 traps represented the total respiration of the incubated core. Respiration was the dominant fate of processed OM at both sites, accounting for up to 5-14% of the added tracer (Figure 5, Table 4). Respiration at STOMZ was ten times higher than that of SToutOMZ for both treatments. In STOMZ suboxic treatment, 1.3 µmol/core ^{13}C-DIC was present in the CO_2 traps and 3.8 µmol/core ^{13}C-DIC in the overlying core water; for the oxic treatment values were 1.4 and 3.3 µmol/core ^{13}C-DIC respectively. In SToutOMZ suboxic treatment, 0.05µmol/core ^{13}C-DIC were present in the CO_2 traps and 0.4 µmol/core ^{13}C-DIC in the overlying core water; for the oxic treatment values were 0 and 0.3 µmol/core ^{13}C-DIC respectively.

		Δδ 13C ‰		
		Oxic	**Suboxic**	**TI**
STOMZ	***Bacteria***	101.9	*79.1*	**0.29**
	Meiofauna			
	Nematodes	8.9	*42.3*	**-0.79**
	Shelled foraminiferans	365.9	*354.0*	**0,03**
	Macrofauna			
	Nematodes	32.0	*not found*	
	Shelled foraminiferans	10.0	*20.0*	**-0.50**
	Polychaetes	5.0	*220.0*	**-0.98**
	Linopherus sp.	192.0	*723.0*	**-0.73**
SToutOMZ				
	Bacteria	*20.2*	8.1	**-0.60**
	Meiofauna			
	Nematodes	*1.2*	0.00	**-1.00**
	Shelled foraminiferans	*15.4*	3.3	**-0.78**
	Macrofauna			
	Shelled foraminiferans	*28.2*	3.9	**-0.86**
	Soft bodied protists	*0.0*	0.0	
	Branched foraminiferans	*0.3*	0.2	**-0.19**
	Polychaetes	*0.0*	0.0	
	Eukarya	*0.0*	0.0	
	Nematodes	*not found*	7.1	

Table 3. $\Delta\delta^{13}C$ in ‰ and treatment impact (TI) on the uptake of the biota. Natural oxic condition values are reported in the Table in italic. Treatment Impact index (TI) gives an idea of the impact that the oxygen treatment had on the organism's uptake and therefore activity. n=4 per station, 2 per treatment

ST1Bi 400000 µgC m^{-2} tracer (^{12}C+^{13}C) addition	µgC tracer m^{-2}	% of tracer added	**ST7o** 100000 µgCm^{-2} tracer addition	µgC tracer m^{-2}	% of tracer added
BACTERIA			***BACTERIA***		
Suboxic	2181±659	0.5	Suboxic	466±78	0.5
Oxic	3141±117	0.8	Oxic	1170±113	1.2
			MEIOFAUNA		
Suboxic	1652±0	0.4	Suboxic	8.0±8	0.008
Oxic	1281±348	0.3	Oxic	66.7±16	0.06
MACROFAUNA			***MACROFAUNA***		
Suboxic	69650±21800	17.4	Suboxic	94±14	0.1
Oxic	17811±3300	4.4	Oxic	1297±1210	1.3
RESPIRATION			***RESPIRATION***		
Suboxic	54515±1000	13.6	Suboxic	6825±100	6.8
Oxic	52370±600	13.1	Oxic	4615±120	4.6
UNPROCESSED			***UNPROCESSED***		
Suboxic	272002	68.0	Suboxic	92607	92.6
Oxic	325397	81.3	Oxic	92851	92.8

Table 4. Uptake and respiration of tracer C and relative percentage of the total tracer C added. Samples n=4 per station, 2 per treatment for the uptake, n=6 per station, 3 per treatment for the respiration.

3.6 Relative Biomass turnover

Bacterial relative biomass turnover (calculated as tracer C uptake/C biomass /day) showed values very close to zero for both stations and both treatments (about 0.03% d^{-1} for STOMZ and 0.01% d^{-1} for SToutOMZ) (Figure 2). Meiofauna relative biomass turnover was higher in STOMZ than in SToutOMZ for both treatments: 0.02% d^{-1} and 0.10% d^{-1} respectively in the oxic and suboxic treatment for nematodes, and 0.87% d^{-1} and 0.84% d^{-1} respectively for shelled foraminifera in STOMZ. In SToutOMZ the relative biomass turnover was 0.005% d^{-1}, 0.08% d^{-1} for nematodes in the oxic and suboxic treatment respectively, and 0.04% d^{-1} and 0.01% d^{-1} for shelled foraminifera. Macrofaunal relative biomass turnover again showed higher values for STOMZ when compared to

StoutOMZ. The first station showed on average a 0.039% d^{-1} and 0.024% d^{-1} for shelled foraminifera, 0.07% d^{-1} and 0.26% d^{-1} for polychaetes, 0.23% d^{-1} and 0.85% d^{-1} for *Linopherus* sp. oxic and suboxic treatment respectively, and 0.012% d^{-1} for nematodes (only found in the suboxic treatment (Figure 5 A,B). The second station showed 0.03% d^{-1} and 0.004% d^{-1} turnover in the oxic and suboxic treatment in shelled foraminifera and values very close to zero for all the other groups (Figure 2 C,D).

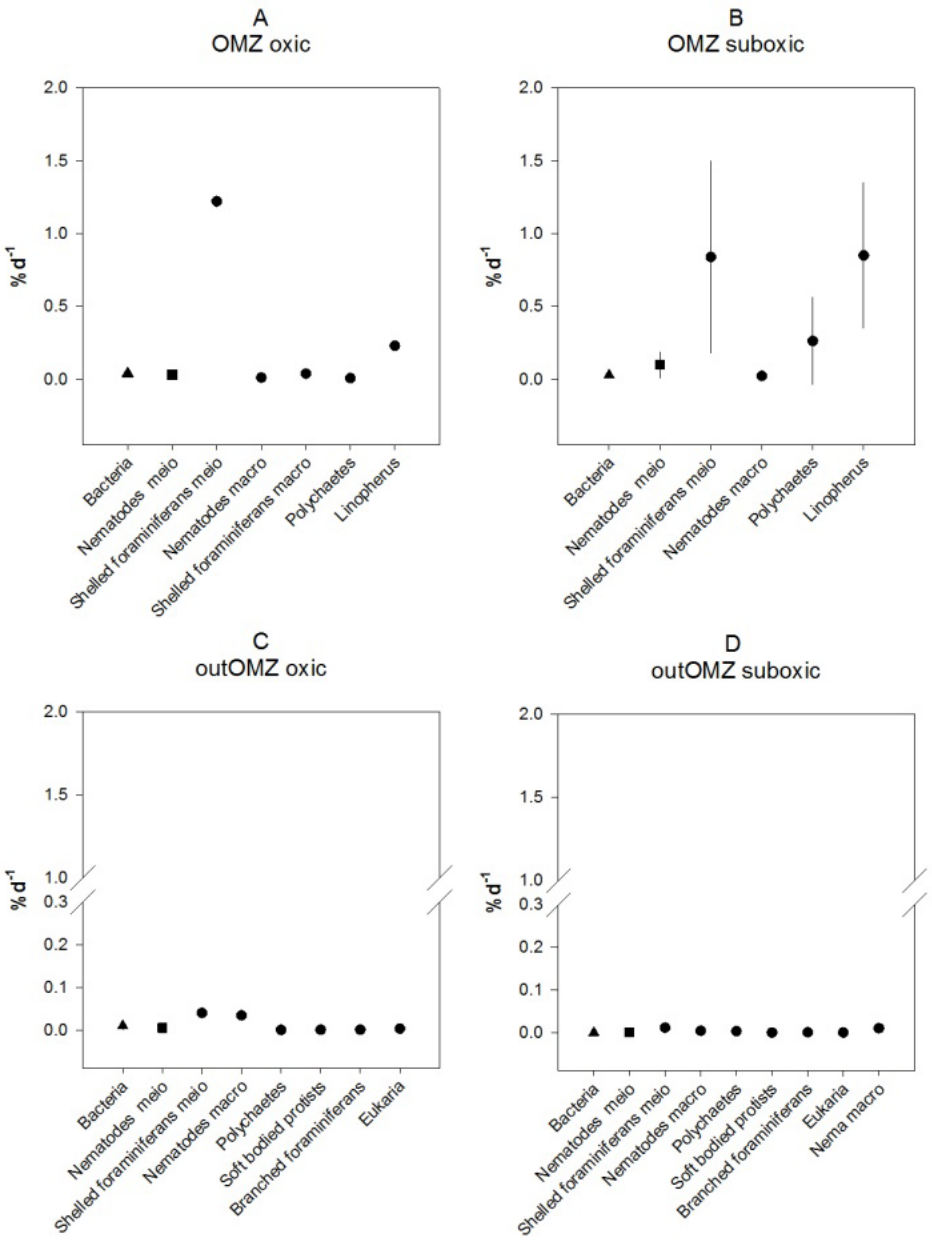

Fig. 2 Relative Biomass Turnover. Triangles represent bacteria, squares meiofauna groups and circles macrofauna groups. Lines, where present, represent the min-max values. Linopherus *is treated separately from the other polychaetes. Presented is the mean of 2 incubation cores ± the range*

4. Discussion

Sediments underlying OMZs are known to have a relatively high accumulation of OM and low diversity benthic community (Cowie et al. 1999; Hughes et al. 2009; Levin 2003; Levin et al. 2000). The extensive and permanent Arabian Sea OMZ has been widely investigated because it offers a natural laboratory to study the effect of oxygen depletion on benthic community structure, activity and OM processing and accumulation. Our approach involved on-deck incubation of sedimentary cores sampled from both inside and outside the OMZ, subjected to an input of algal derived POM and oxygen manipulation. Two observations emerged from our experiments: 1) bacterial and faunal biomass were not affected by oxygen treatment, indicating short term tolerance to variations in oxygen concentrations, 2) short-term OM processing in OMZ sediments was independent of oxygen treatment, whereas in non-OMZ sediment uptake of bacteria and fauna was reduced under suboxic conditions. The results will be discussed below but first we shortly address some limitations of our study.

4.1 Methodological issues

In order to mimic as closely as possible the natural input of organic matter to each location, we added a different dose of C (400 and 100 mgC m^{-2}) to the cores of the two stations: these additions are significantly lower than the natural maximum particle fluxes of 800 and 600 mg m^{-2} d^{-1} recorded in the western deep Arabian Sea respectively by Nair et al. (1989) and Haake et al. (1984).Given the difference in sediment organic C content among them (6.38 vs 1.03%, Table 1), the addition was equivalent to 0.31 and 0.19% of the total organic C naturally present in the surface 3 cm of the sediment. This choice might raise the concern that the different faunal and bacterial response between the two stations could be due to the diverse C additions. However, the difference in response of the community exceeded by far the 4x differences in tracer amount between stations, suggesting that results are not driven primarily by the tracer OM dose. The amount of tracer added was lower than previous studies (Sweetman and Witte 2008; Witte et al. 2003a; Witte et al. 2003b; Woulds et al. 2009). Notwithstanding this, only a small portion of the added labeled OM was processed in 7 days (7.5 to 23%) demonstrating that the addition of the

OM was sufficient to trace the bacterial and faunal uptake, as well as the respiration, and the community was never food-limited.

Due to the oxygen manipulation, it was necessary to retrieve sediment cores and work in temperature-controlled laboratories. Microorganisms (in particular bacteria) have shown to be sensitive to decompression, causing altered activity, cell lysis and ultimately death (Babu et al. 1999), in particular at water depths greater than ~2000m. This might raise the concern that biological OM processing in our experiments might have been impaired and/or altered by change in pressure. Some studies have compared *in situ* with shipboard experiments and manipulations to quantify differences and artifacts (Aberle and Witte 2003; Andersson et al. 2008). The results showed that the data produced with on deck experiments are generally reliable. Woulds et al. (2009) showed a lack of systematic differences between shipboard and lander-derived data for depths less than 2000 m, providing evidence that artifacts associated with incubations at atmospheric pressure are small. This is also supported by the fact that most studies that have reported differences between *in situ* and on deck experiment results were conducted at depths of 2000–4000 m (Woulds et al. 2007). Our study was conducted at intermediate water depth (885 and 1791 m) therefore, according to Woulds et al. (2009; 2007), there should be no decompression issue. The biomass values between background and experimental samples were very similar for both stations, which we interpret as that no significant mortality had occurred during the seven days of incubation. The difference in biomass among treatments probably reflects natural patchiness rather than experimental effects (Fig. 3).

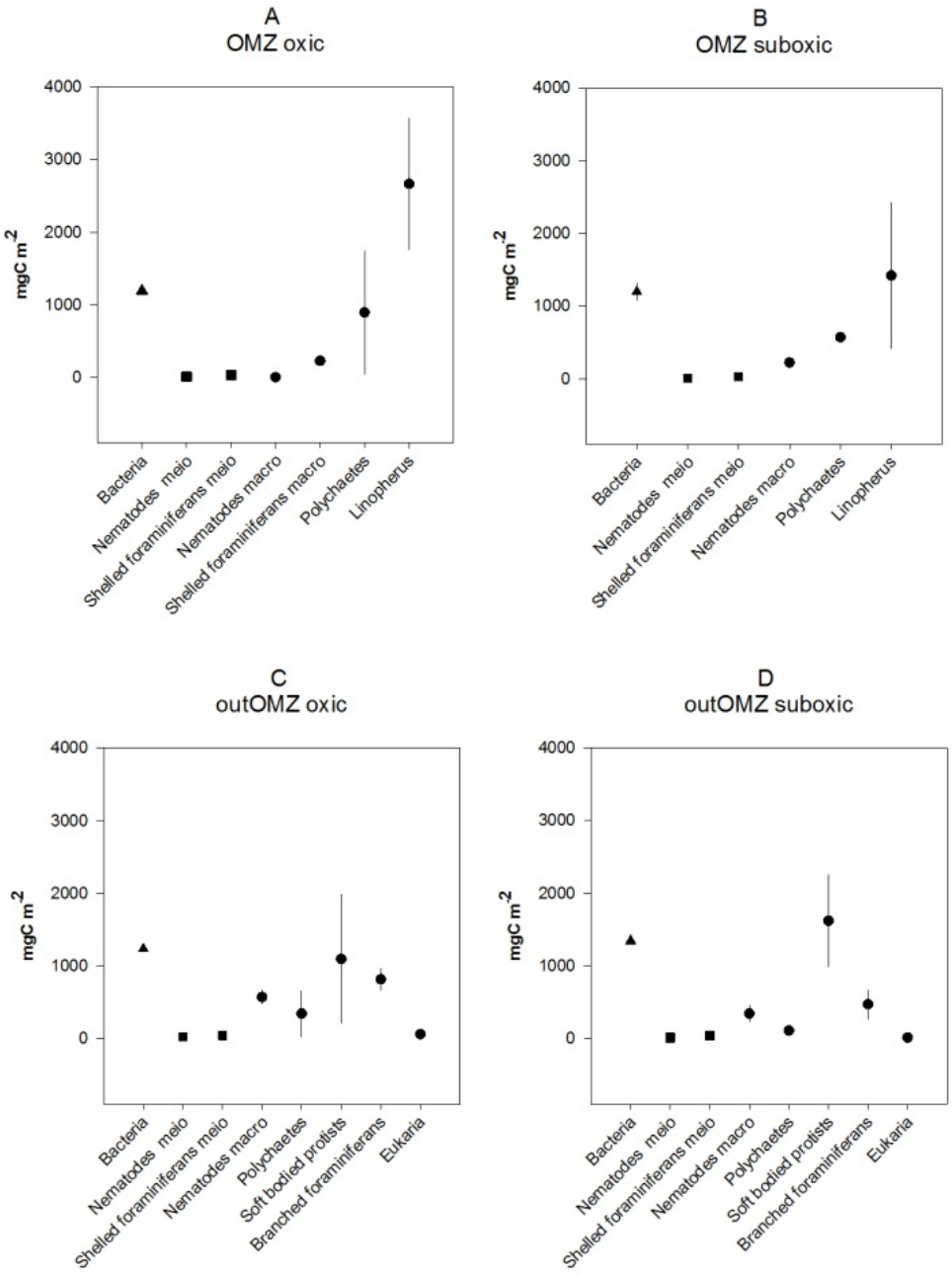

Fig. 3 Biomass values of the amended cores in the two stations. Triangles represents total bacteria, squares meiofauna groups and circles macrofauna groups. Lines, where present, represent the min-max values. The group "Soft bodied protists" includes both Gromiids and Allogromiids . "Meio" stands for meiofauna, "macro" for macrofauna. Linopherus *stands for* Linopherus *sp.*

All the cores, incubated ones and controls, used for this experiment were deep-frozen at the end of the experiment. In the laboratory, the frozen cores were sliced and portioned. The PFLA samples were removed and stored at -80°C and the rest of the slices preserved in 40% buffered Rose Bengal amended formaldehyde. We did not correct faunal isotope data for the formalin treatment, since both control and incubated samples underwent similar treatment.

4.2 Biomass values

We are aware that the amount of replicas we measured was limited (two cores per each treatment per station, two cores per station as background) and our aim was not to perform a faunal survey. We calculated biomass solely to quantify fluxes in the pulse-chase experiment. The major representatives of the meiofauna were shelled foraminifera in both stations and, in particular for STOMZ, this is in accord with what was found by Gooday et al. (2009). The high abundance of *Reophax sp.* and *Uvigerina sp.* is in line with Schumacher et al. (2007) and Woulds et al. (2007) for the Pakistan Margin. The macrofauna was dominated by foraminifera (shelled and branched and soft bodied protists) and polychaetes in both stations, but in STOMZ *Linopherus* sp. (an amphinomid polychaete) accounted for up to 84% of macrofaunal biomass, this is in agreement with the findings of Hughes et al. (2009) and Levin et al. (2009b). Our macrofauna biomass data are higher that what reported by Hunter et al. (2012a) but become comparable with those inside the OMZ when excluding *Linopherus* (that Hunter et al. do not report).

However, when we compare group level community structure, SToutOMZ appeared to be more rich and diverse in macrofauna than STOMZ, as confirmed by the presence of branched foraminifera and other eukaryotes (Table 2). The limited amount of macrofaunal groups found in STOMZ compare to SToutOMZ might be due to the high specialization required to the animals inhabiting low oxygen settings. The signs of bioturbation found in the STOMZ cores and the rapid surfacing of the macrofauna to feed on the freshly deposited OM confirm the fauna activity, showing that even if the diversity is low, the OM processing is still carried out effectively. In SToutOMZ the macrofauna, used to normoxic conditions, survived after the suboxic treatment, indicating that short-term changes in oxygen conditions can be dealt with even by

organisms that normally do not experience hypoxia, but that they have to lower their activity due to limited oxygen.

4.3 Tracer OM uptake and respiration

The $\Delta\delta^{13}C$ values of the various biotic compartments indicate the relative uptake of the labeled OM by the benthic community (Figure 4). Total tracer uptake indicates the total tracer-derived carbon incorporated by the biota. In STOMZ both treatments show a similar pattern of tracer uptake, with *Linopherus* sp. responsible for the majority of the uptake followed by other polychaetes (only for the suboxic treatment), bacteria and meiofaunal shelled foraminifera. Also in SToutOMZ the patterns are similar among treatments, but the oxic one (1170 and 1297 μgC incorporated in bacteria and macrofaunal nematodes respectively) showed almost 3 times higher uptake values than the suboxic one (466 and 94 μgC incorporated in bacteria and macrofaunal nematodes respectively). Nematode incorporation of carbon was similar to other studies (Guilini et al. 2010; Steyaert et al. 2007): even though present in high biomass in both stations (between 10 and 15 mgC m^{-2}) their total tracer uptake is negligible (Figure 5).

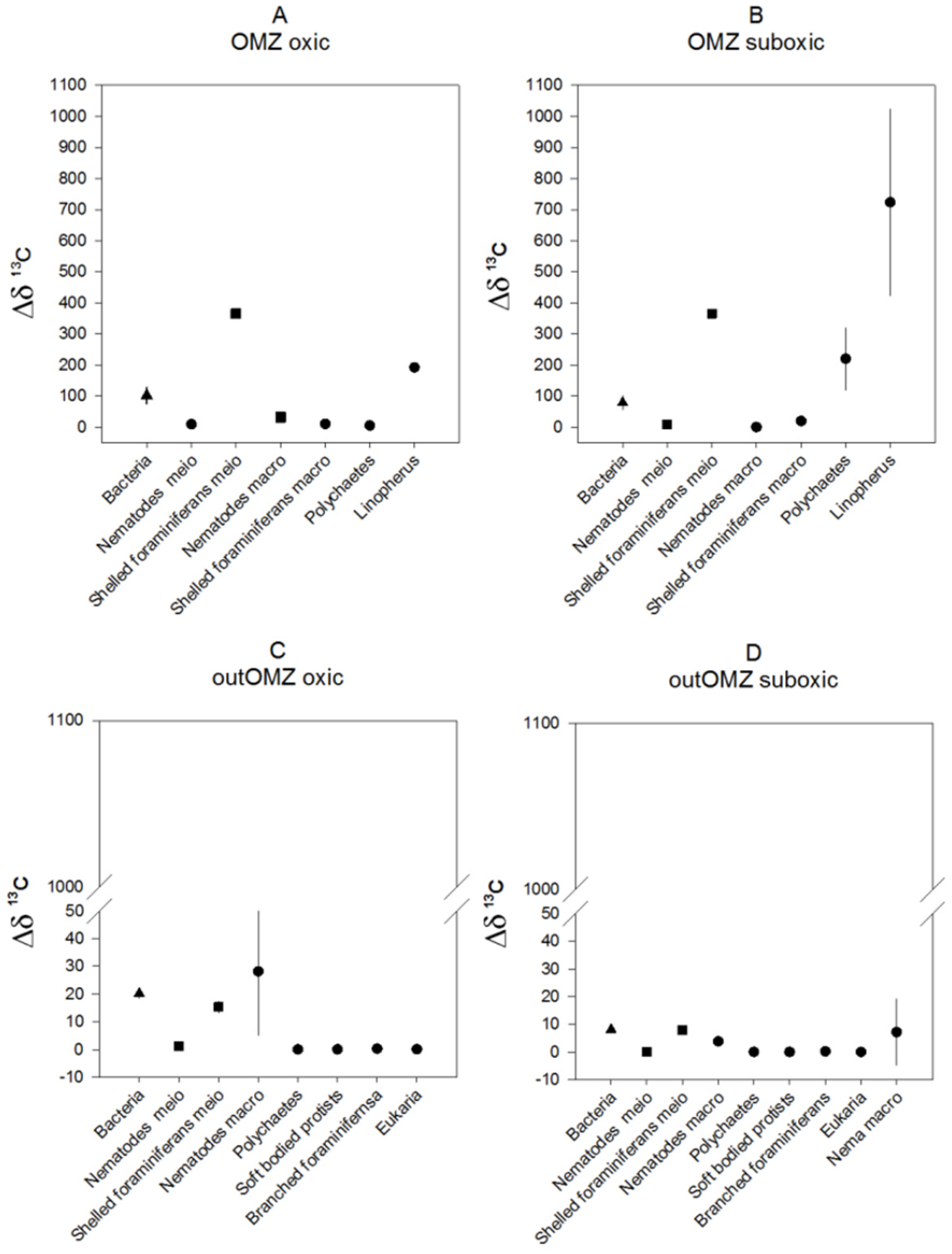

Fig. 4 Relative Uptake of tracer OM by the different biotic groups. Data are reported in Δδ values. Triangles represent the total bacteria, squares meiofauna groups and circles macrofauna groups. Lines, where present, represent the min-max values. Linopherus *is treated separately from the other polychaetes. Presented is the mean of 2 incubation cores ± the range.*

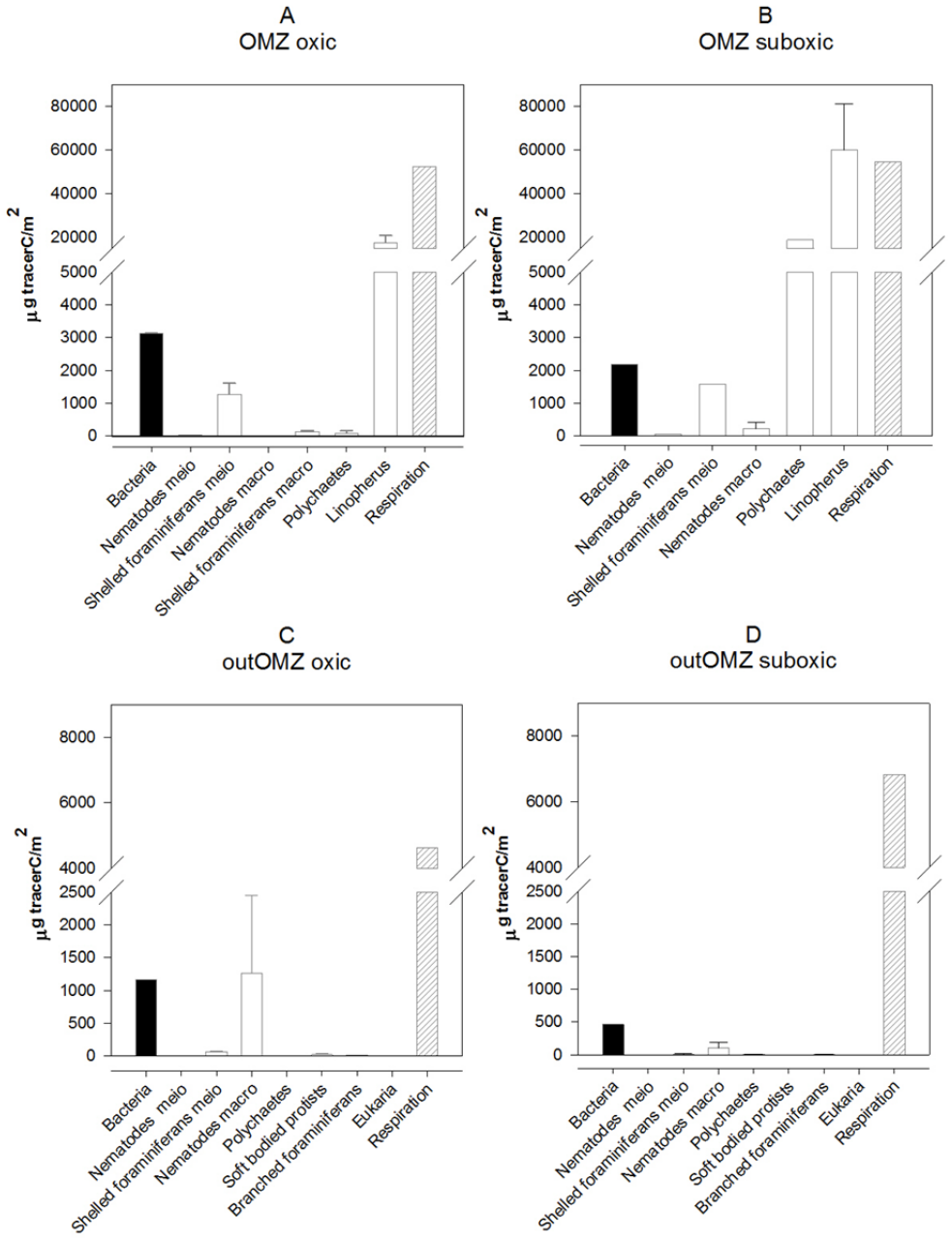

Fig. 5 Total labeled C incorporated and respired by bacteria, fauna (black = bacteria, white = fauna, hashed = respiration). Note the difference in scale between the plots. Linopherus is treated separately from the other polychaetes. Presented is the mean of 2 incubation cores ± the range.

Within the OMZ, in STOMZ, the large polychaete *Linopherus sp.* dominates the community's tracer uptake (up to 17% on total tracer added), followed by bacteria with a maximum of 0.8% and meiofauna with 0.4%. Our STOMZ data are in line with what was found by Woulds et al. (2009) in their station at 940 m depth in the Pakistan Margin, even though their bacterial uptake spanned between 0.5 and 22% of the added tracer in 2 to 5 days. The amounts of tracer being processed are several times higher than those of Andersson et al. (2008), where bacteria accounted for 0.18-0.26% of the C processing, macrofauna 3-4.38% after 5 days incubation time at 850 and 940 m depth. *Linopherus sp.*, found in a sampling trawl at 879 m depth was described as extremely abundant at 800 and 850 m in the Pakistan margin of the Arabian Sea (Pedersen 1995). Levin et al. (2009b) also reported that at 750 and 850m depth 97 to 100% of macrofauna were polychaetes, over 83% of which were *Linopherus* sp., data confirmed also by Jeffreys et al. (2012). Interestingly, *Linopherus* sp. plays an important role both in biomass and processing of OM. Woulds et al. (2009; 2007) described *Linopherus* as a voracious consumer of fresh phytodetritus but the amphinomid borrowing behavior did not seem to enhance phytodetritus subduction (Levin et al. 2009b). Our results confirm the voracity of *Linopherus* and its high incorporation of fresh OM tracer, as well as its borrowing behavior, demonstrated by the fact that we retrieved specimens down to 4-6 cm depth in the sediment. Outside the OMZ, (SToutOMZ) the macrofaunal foraminifera almost match the bacterial processing of fresh OM (in natural oxic conditions), even if values remain very low: 0.5 and 1.2% for bacteria (suboxic and oxic treatment) and 0.1 and 1.3% for macrofauna. Bacterial and foraminiferan dominance in the OM processing was probably because the remaining fauna was significantly impaired in both treatments (e.g. figure 3 C,D). In contrast, both meio- and macrofauna had very limited activity in the natural oxic treatment, and even less in the suboxic treatment. This could mean that these organisms either feed on other food sources, or that 7 days incubation time was not enough to capture their response, although in other locations they show labeling already after 2-5 days.

The Treatment Impact index (TI) data suggest that OMZ meiofaunal foraminifera, living where oxygen is depleted, can temporarily sustain their activities at higher oxygen concentration than natural conditions, while hypoxia-specialized polychaetes found in low-oxygen settings appear unable to adapt to higher oxygen conditions. The bacterial

community can adapt fast to changing oxygen conditions and thrives in every setting. Our findings are in line with what reported by Moodley et al. (2011): these authors report no evidence that biota are limited by severe oxygen depletion and that some compartments may even be favored by low oxygenation. However, in contrast with what we found, they also report high variation in uptake rates by bacteria that prevented them from solid testing of differences in oxygenation. The study by Moodley et al. (2011) concludes that overall seafloor functioning was not particularly hindered by suboxic conditions, which is in total accordance with our conclusions. Woulds et al. (2007) propose a "oxygen threshold hypothesis" to explain benthic community structure and the "feeding efficiency" values they calculated, reporting that under the oxygen concentration threshold value of 5-7 μmol l^{-1} macrofauna do not survive and therefore their processing is totally impaired, leaving foraminifera to take over. Even though our results concur with these authors in observing a different benthic community inside the OMZ compared to outside, our data are in disagreement with Woulds et al. regarding OM processing. In fact we reported higher relative uptake and incorporation rates inside the OMZ than outside, for bacteria, meio and macrofauna alike. In particular, our STOMZ has a natural O_2 concentration was only 2 μmol l^{-1} (well underneath the 5-7 μmol l^{-1} threshold) and still bacteria and fauna were present and metabolically active. For this reason, in the Murray Ridge area, we can suggest that the proposed threshold of Would's "oxygen threshold hypothesis" can be lower than the one proposed in their paper.

Respiration in SToutOMZ was lower than in STOMZ, with only 6.8 and 4.6% versus 13.6 and 13.1% of the added tracer. It is worth noticing though that in our SToutOMZ outside the OMZ respiration was higher in the manipulated suboxic treatment than in the natural oxic one, suggesting a switch in the carbon processing in the suboxic cores. In fact, following the site categorization given in Woulds et al. (2009), our respiration and incorporation data position our STOMZ suboxic treatment into the "metazoan macrofauna dominated uptake" group, in line with the authors 850 and 940m stations, whereas STOMZ oxic treatment that resembles more a "active faunal uptake" station. Our SToutOMZ oxic treatment also resembles an "active faunal uptake" station but it was situated at 1700 m depth, whereas the suboxic treatment a "respiration dominated" station, in line with Woulds et al. 1850m depth station. Our STOMZ oxic sediment was all collected at 885 m, therefore it cannot be considered shallow nor intertidal site, which are

the most commonly found by Woulds et al. to be "active faunal uptake" sites and it was extremely rich in OM, where Woulds et al. consider this site type only moderately food rich. Our SToutOMZ oxic was situated at 1791m depth and was only 4 °C in temperature, so this neither can be considered similar to shallow or intertidal sites. Finally, our SToutOMZ suboxic treatment can be appropriately described as a "respiration dominated" site because all the characteristics given by Woulds et al. are matched. For these reasons, where the categorization given by these authors is valid for our STOMZ suboxic treatment and SToutOMZ suboxic treatment, it is somehow not applicable to the STOMZ oxic treatment not to SToutOMZ oxic treatment

These results are very comparable with what was found by Moodley et al. (2002) in the Atlantic Ocean, where bacteria and foraminifera together accounted for 50% of the uptake and respiration made up for 45% of the C processed. Our results are also in line with findings of Witte et al. (2003) in their station at 1265 m in the Norwegian fjords, where bacteria and macrofauna each accounted for 0.9% of the processing of the added OM and respiration made up the 3.5%. Similar to our findings are also the data by Hunter et al. (2012b), who reported macrofauna C assimilation values ranging from 373 to 5540 μgC m^{-2} in the Indian margin of the Arabian Sea and also reported a polychaete (cirratulids) dominance in tracer assimilation at their 800 m station. Hunter and Veuger (2012a) also reported higher bacterial assimilation in their station at 800 m inside the OMZ (30-90% of the tracer) and lower incorporation in bacteria in the OMZ boundary at 1100 m (6-11% of the tracer) even though here oxygen levels were up to 42 times higher than inside the OMZ. They therefore conclude that oxygen availability did not directly influence bacterial activity, which again agrees with our findings.

Because SToutOMZ has lower temperature, lower sediment organic C content and is in deeper water compared to STOMZ, the metabolism of the organisms could be lower (Andersson et al. 2008). SToutOMZ temperature was 4 °C versus 10 °C at STOMZ: if we assume a doubling of rates with an increase of 10 °C (i.e a Q10 value of 2), and if temperature was the main factor governing fauna's low activity, then we would expect activity to be 44% less than the one recorded in STOMZ, which is far less than the observed 99% for shelled foraminifera (as evidenced in Fig 3 and 5). Therefore, temperature alone cannot explain the difference among stations. As a result, the limited food supply and Corg content of SToutOMZ sediment are to be considered the major

cause for the faunal low activity in terms of total uptake, biomass turnover and relative uptake when compared to STOMZ. This is consistent with the results of Lampadariou et al.(2009) who report that meiofaunal abundances in the Aegean Sea were positively correlated with chlorophyll *a*, phaeopigments and chloroplastic pigment equivalent, while were not correlated with any of the remaining sediment descriptors, thus indicating the dependence of meiofauna on food availability. The same authors also report that food quality was another factor that may have had accounted for the lower faunal abundances found in the Ierapetra Basin. The faunal biomass in SToutOMZ was however higher as compared to the OMZ sediments, which suggests that while fauna may have been outcompeted by bacteria with respect to the short term processing of OM (in line with what reported by Moodley et al. (2002)), over longer time periods they were not negatively affected by this limited uptake.

4.4 OMZ organic matter accumulation

The absence of a H_2S scent in the sediments from STOMZ was in line with earlier studies, implying limited sulphate reduction in Arabian Sea sediments (Law et al. 2009; Van Der Weijden et al. 1999). The high OM content of OMZ sediment and the relative high fauna biomass seem to create a mismatch, insofar one would expect that sediment OM content is high only where benthic fauna should be limited in biomass, thus allowing OM accumulation due to scarce fauna processing. One of the possible explanations for the accumulation of OM in presence of relatively high fauna biomass is the biotic "malfunctioning", as Moodley et al. (2011) already discussed. However, our results confirm Moodley et al. conclusion that fauna in hypoxic areas is present in high biomass and is capable of processing OM. Our findings however do show that, inside the Arabian Sea OMZ, the benthic community structure is simpler than outside: this is probably due to the necessity for a high level of adaptation of organisms to live in constantly oxygen depleted environment. Foraminifera offered a good example of this flexibility, showing in different studies high adaptability to many different conditions (Ciutat et al. 2007; Freitas et al. 2009; Gooday et al. 2000), sustaining their metabolism in both oxic and anoxic conditions. *Linopherus sp.* seems capable of sustaining at least 7 days of oxic

conditions, even though physiologically adapted to low-oxygen concentrations as demonstrated in the recent study by Jeffreys et al. (2012) as well as in other previous works (Gooday et al. 2009; Levin et al. 2009b; Murty et al. 2009). The few remaining polychaetes however, showed a high specialization and adaptation to the natural suboxic conditions (Lamont and Gage 2000) that lead to almost no uptake in the oxic treatment. Another theory to explain OMZ sediment high OM concentration is that even if OM is abundant, either the faunal activity inside the OMZ is limited or the fauna is incapable of feeding efficiently, and OM therefore accumulates unprocessed. None of these explanations are however supported by our data that clearly show that fresh OM deposited on the top of the experimental cores was processed and respired by the community in a very short time period (7 days). Moreover, relatively to the total amount of OM offered, the uptake and processing by the benthic community inside the OMZ was higher than outside the OMZ. Therefore, a low OM uptake of the benthos cannot be claimed as the reason for the high organic matter accumulation in STOMZ. Similar high processing rates of OM by the benthic community in the OMZ in seven days were (168 and 156 mgC m^{-2} respectively for the oxic and suboxic treatment)reported by Moodley et al. (2011) for sediment underneath the Indian margin OMZ. Moodley et al. (2011), based on the fact that in their cores subjected to high and low oxygenation community processing was not altered, already provided evidence that ecological malfunctioning due to lack of oxygen is not the factor responsible for the highly organic-rich sediment accumulating on the Indian margin of the Arabian Sea OMZ. Moodley et al. (2011) also indicated that the low bioavailability of the sedimentary organic matter rather than biomass (which was high) governed the slow remineralisation of Indian margin sediments. Consistently, Vandewiele et al. (2009), Wakeham et al. (2002) and Jeffreys et al. (2009a) reported that Arabian Sea sediment is of low quality. The very low bioavailability of Arabian OMZ sediment's organic matter might lead to the accumulation of refractory OM on the OMZ seafloor. Smallwood et al. (1999) reported that megafauna of the lower boundary layer Oman margin of the OMZ control sediment OM quality by changing the distribution of OM lipids due to metabolic reworking of the OM itself. Jeffreys et al. (2009a) present similar findings, showing that foraminifera, present in high biomass inside the OMZ, stripped the polyunsaturated fatty acids from the local OM, whereas at the lower boundary of the OMZ suspension feeders removed labile lipids from the

water column OM, lowering the quality of the material reaching the sediments. They also show that in more oxygenated settings outside the OMZ in- and epi-fauna did not alter significantly the local OM, determining the persistence of phytodetritus and therefore labile lipids in the sediment OM. Wakeham et al. (2002) already proposed the idea that lipid biomarkers were selectively degraded near the water-sediment interface relative to organic carbon and reported low water column degradation of the material raining down to the bottom. These results support the theory that OM that reaches the OMZ sediment is already of poor quality and that fauna strips it even further of labile compounds, causing an accumulation of refractory OM.

Another explanation for the OM accumulation inside the OMZ is the lower oxidative power of anaerobic degradation pathways, and as more OM escapes oxic mineralisation, this might explain the higher preservation in OMZ zones. However, our experiments showed that a higher oxygen concentration does not stimulate further OM degradation within the OMZ. Moreover, biomass turnover inside the OMZ is reduced in the oxic treatment as compared to the suboxic treatment (Figure 6). In fact, higher oxygen in STOMZ samples seems to decrease the uptake and therefore the activity in particular for meiofaunal nematodes and all the macrofauna (Table. 4). This indicates that OMZ communities are adapted to low O_2 and when a higher oxygen concentration is provided, it may impair their activity rather than stimulating it.

5. Conclusions

In conclusion, we found no difference in biomass, relative uptake, relative biomass turnover between oxic and suboxic treatments outside the OMZ in SToutOMZ. In contrast, relative and absolute uptake of phytodetritus is higher in suboxic conditions inside the OMZ than in oxic ones.
This suggests that:

1. Under both *in situ* and altered dissolved oxygen concentrations, faunal and bacterial activity can be detected and quantified.

2. Freshly deposited OM is assimilated and respired very quickly under *in situ* oxygen conditions.

3. The faunal standing stock is capable of carrying out metabolic processes and performs better inside the OMZ than outside.

4. Neither oxygen concentration nor low biomass are the responsible factors for the Arabian Sea OMZ sediment OM maximum.

5. Comparing the two stations, it appears that the benthic community in the OMZ is more efficient at processing carbon than outside the OMZ. The biota respire almost the same portion on total tracer added in the two settings but the assimilation is higher in the suboxic than the oxic condition.

The results of this study leave open several questions, among which is why the OM is so refractory upon deposition. More research is needed to close these gaps and different and more extensive experiments will be required.

6. Acknowledgements

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3 Dissolved and particulate organic matter processing by the deep-sea benthic community inside and outside the Arabian Sea oxygen Minimum zone

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In preparation for Deep-Sea Research I

Abstract. *In some areas in the oceans organic matter (OM) degradation by biota is impaired. Oxygen minimum zones (OMZs) are archetypical examples of such areas and have in common the characteristic accumulation of OM in their sediment. In this study we focused on the Arabian Sea OMZ and investigated OM degradation by different benthic groups. We incubated intact sediment cores under suboxic conditions and amended them with two different tracers: isotopically labeled dissolved organic matter (DOM) and particulate organic matter (POM). This approach allowed us to quantify benthic incorporation and respiration of the added ^{13}C, isolating each group's participation to the processes and discriminating between DOM and POM degradation pathways. DOM and POM were assimilated at both stations, accounting for up to 13.6% and 6.7% of the added tracer on average inside the OMZ and 6.8% and 2.1% outside. Inside the OMZ, the upper sediment layer, 0-2 cm, accounted for a small part (only 12%) of the POM uptake and in the deeper layer, at 2-4 cm, almost all the POM incorporation took place. In contrast, DOM uptake mainly took place in the upper layer, 87% of the total, dominated by bacteria. In the deeper layer, bacteria accounted for the 13% of the total uptake. Outside the OMZ, the upper layer POM incorporation was dominated by bacteria and in the deeper layer only 0.6% of the total*

POM uptake was recorded. The DOM incorporation was also dominated by bacteria. We were able to elucidate food-web interactions such as absence of predation on bacteria by meio and macrofauna and to provide an indication of biotic response to the possible spreading of suboxic conditions to normoxic areas.

1. Introduction

Suboxic and anoxic areas of different morphology, from coastal regions to estuaries and bays to enclosed seas, continental shelves, margins are increasing in number (Levin et al. 2009a). When they develop in open settings such as margins or continental shelves they are commonly referred to as Oxygen Minimum Zones (OMZ). These settings are archetypical examples of areas where OM degradation is impaired, which results in an accumulation of OM in the sediment (Middelburg and Levin 2009). Because of their OM accumulation these are interesting areas to study control factors on OM degradation, preservation and burial (Calvert et al. 1995; Henrichs 1992).

Several explanations have been given for the OM accumulation. One is believed to be the oxygen depletion of the water body that overlies the sediment: this limits the presence of benthic fauna thus impairing degradation (Demaison and Moore 1980; Jeffreys et al. 2009a). Lee (1992) reports that anoxic sediments may sequester organic matter as bacterial biomass, or as bacterially derived products, in the absence of bacterial grazers. Thus, differences in the numbers and diversity of organisms that graze upon bacteria between oxic and anoxic sediments may explain part of the difference in carbon preservation rates that have been observed between the two types of systems. Another explanation is that faunal biomass is comparable to other deep-sea locations but their activity is reduced due to lack of oxygen (Woulds et al. 2007).

Hedges and Kiel (1995b) proposed that OM quality and its interaction with the inorganic material are another possible explanation for OM enrichment in OMZ sediment. Based on pigment analysis, Woulds et al. (2009) concluded that Arabian Sea OM has been degraded and is of low quality compared to other settings. Vandewiele et al. (2009) reach the same conclusion based on amino acids analysis. Henrichs (1992) suggested that the preservation of organic matter in sediments could be

due to the incorporation of organic compounds into refractory geomacromolecules or humic substances or that much of the organic matter preserved in sediments is refractory when deposited and survives diagenesis with little change. Resolving these questions is beyond the aim of this study but we hypothesize that if sediment OM is of low quality, then it requires higher energy investment by biota to be processed and fewer organisms would find it palatable. Less biotic degradation would cause further OM accumulation but the primary cause would then not be oxygen limitation on the benthos but incoming OM of bad quality or low availability as Henrichs (1992) and Vandewiele (2009) already suggested.

To evaluate the role of OM type on benthic OM processing and separate it from oxygen concentration impacts, we subjected sediment from inside and outside the Arabian sea OMZ to suboxic conditions for 7 days, amending the cores with tracer OM to follow how the benthic community reacts to the treatments. To clarify if indeed OMZ fauna has very low processing rates and, if so, whether it is because of low biomass and limited activity or because the OM is not palatable, we used well defined fresh labile OM amended to intact Arabian Sea OMZ sediment cores. The mid-water OMZ in the Arabian Sea is permanent, well defined and the largest on Earth (Helly and Levin 2004). These characteristics make the Arabian Sea OMZ a perfect setting to study the OM processing by an OMZ benthic community. Previous studies confirmed OM accumulation in the Arabian Sea OMZ sediment (Cowie et al. 1999; Hughes et al. 2009). Based on pigment analysis Woulds et al. (2009), lipid biomarkers Wakeham et al. (2002) and Jeffreys et al. (2009a), molecular characterization Smallwood et al. (2000) and amino acids Vanderwiele et al. (2009) concluded that Arabian Sea OM is degraded or of low quality compared to other settings. The role of benthic fauna in controlling the OM that is deposited within OMZ sediments has been also investigated and documented previously (Smallwood et al., 1999; Jeffreys et al., 2009). Wakeham and his co-workers discussed the role of zooplankton in the quality of OM deposited in OMZ sediments in the Arabian Sea and Cowie (2005) in his review analyzed the biogeochemistry of Arabian Sea Surface sediments and the benthic-pelagic coupling effect on OM. Tracer experiments have been conducted in various settings to assess the uptake and processing of labile OM by the benthic community (Witte et al. (2003a; 2003b), Woulds et al. (2009), Andersson et al. (2008) Moodley et al. (2002; 2005b)). In these earlier studies, focus has been on the identification of the key players in the processing of labile OM after a

phytodetritus pulse. In this study however, we use the isotope tracing method to tease apart the effects of OM quality from those of low oxygen availability in OMZ sediments. To be able to thoroughly investigate OM quality we used two different OM traces: particulate organic matter (POM) and dissolved organic matter (DOM). This also allowed us to follow two main routes of OM degradation: POM ingested by metazoans and degraded by bacteria (Chrost 1991a; Chrost 1991b; Mayer 1989), and DOM taken up by bacteria. With this approach we can trace the C transfer along the food chain and assess the different contribution of faunal groups to OM degradation. Bacterial transformation of the OM also accounts for the upward transfer of C towards the fauna via bacterial predation and separate DOM incubations allow us to clearly trace it, without the mixed effect of direct metazoan OM ingestion.

This study can therefore offer clear and complete information on benthic ecosystem functioning in the Arabian Sea OMZ. If, on the one hand, the response we measure is comparable to that of other settings, then the community degradation potential is not impaired by hypoxia. If, on the other hand, we measure a reduction in OM processing even in presence of biomass comparable to other settings, then oxygen depletion becomes the most probable cause of the OMZ OM accumulation.

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 (see Fig. 1). Station STOMZ inside the OMZ, was shallower (22°32.9' 64°02.4', 885 m) and in the core of the OMZ, the deeper station SToutOMZ (22°18.5' 63°24.5', 1791 m) was located at the lower boundary of the OMZ.

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 in width and 750 km in length. The Ridge is composed of three different parts: the southern crest at water depth <1000 m, the northern zone fairly 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 purely marine OM processing because it is isolated and far away from shore (Shimmield et al. 1990). These characteristics prevent any terrestrial input of OM to this site, avoiding any interpretation problems of the experimental results due to mixed OM sources.

The area occupied by the OMZ starts approximately 150 meters below the sea surface and coincides with the seasonal thermocline. The mixed layer depth, as reported by CTD temperature profiles, is found at STOMZ between 80 and 95 m; at station SToutOMZ the water column was completely stratified up to the surface without mixed layer. Sea surface temperature was 25.2 °C and below the mixed layer the temperature declined with 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 decreases below 400 m to a minimum of 34.7 at 3000 m.

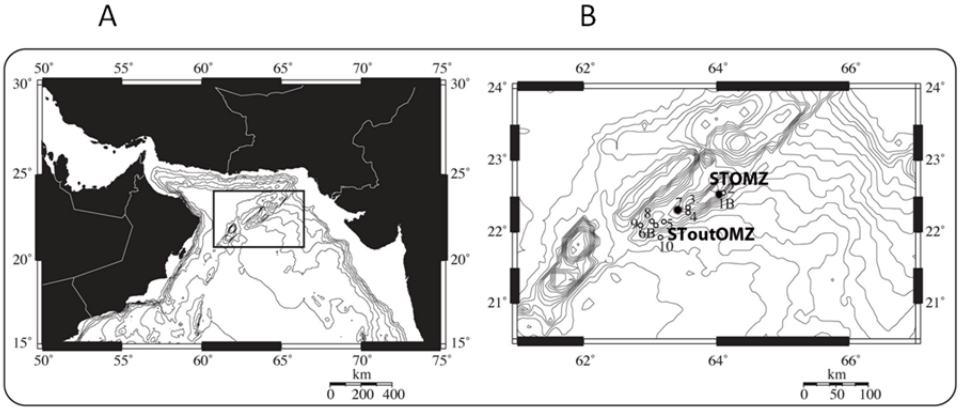

Figure 1 Sampling area. In panel A a general view of the Northern Arabian Sea and in panel B a zoom-in of the sampling area on the Murray Ridge.

	STOMZ	STOutOMZ
Position	22°32.9' 64°02.4'	22°18.5' 63°24.5'
Depth	885 m	1791m
Bottom water		
Temperature °C (CTD sensor)	10	4
Salinity (CTD sensor)	34.8	34.9
Dissolved O_2 µmol/L (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
$\delta^{15}N$ ‰	8.04	8.68

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

2.2 Phytodetritus POM and algal derived DOM preparation

POM and DOM were prepared in the laboratory by culturing axenically the brown diatom *Thalassiosira pseudonana* in a 30% ^{13}C-enriched F2 medium. Algae were harvested via centrifugation (2000 Gforce), the pellet washed of any residual label with artificial seawater and subsequently centrifuged again. The pellets were frozen overnight at -80˚C and freeze-dried. We harvested ~ 0.8 g dry algae from 9L of axenically cultured *Thalassiosira pseudonana*. Sterilized Milli-Q water was then added to the freeze-dried pallets to burst the still intact algal cells and release the inner cytoplasm and exudates from the algal frustules. DOM was separated from POM by centrifugation (2000 Gforce). This procedure was repeated three times. The supernatant was then filtered through a GF/F pre-combusted filter and subsequently 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 to be used for 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 Thermo Electron Flash EA 1112 analyzer (EA) coupled to a Delta V isotope ratio mass spectrometer (IRMS).

2.3 Sample collection

Intact 10 cm i.d. multicorer cores were retrieved from both stations. Upon arrival on deck, the cores were transferred into 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 mixtures of air (certified O_2, CO_2 and N_2 gas mixture by Hoekloos BV, The Netherlands) with oxygen concentrations of 8 µmol l^{-1} O_2 for SToutOMZ and 6 µmol l^{-1} O_2 for STOMZ. The first 0-3 cm of separate cores were used to determine sediment characteristics (see details below).

2.4 Sediment characteristics

Two cores per station were sampled together with the incubation cores and used to determine sediment characteristics. The upper 3 cm of each core was sliced off, homogenized and 10 cm^3 of each slice sub-sampled and used for analysis. Sediment water content and porosity were calculated on sediment wet/dry measurements using an average sediment density of 2.55 g cm^{-3}. Grounded freeze-dried sediment samples were measured to determine 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) in. Sediment grain size distribution was assessed with a MALVERN Mastersizer 2000 on freeze-dried sediment samples.

2.5 Experimental incubations

8 cores were incubated in total: from STOMZ, 2 with the equivalent of 400 mgC m^{-2} in POM 2 with the equivalent of 400 mgC m^{-2} in DOM, from SToutOMZ, 2 with the equivalent of 100 mgC m^{-2} in POM and 2 with the equivalent 100 mgC m^{-2} in DOM. The selected quantities of the tracer additions represents 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 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 injected down to approximately 4 cm into the sediment with a micro glass syringe via numerous injections of 10 μl each, equally distributed over the surface of the core. Duplicate cores from both stations were sealed on the bottom and on the top with O-ring lids and incubated for 7 days under suboxic conditions (6 μmol l^{-1} O_2 for STOMZ and 8 μmol l^{-1} O_2 for SToutOMZ,), bubbling pre-made mixtures of air (certified O_2, CO_2 and N_2 gas mixture by Hoekloos BV, The Netherlands) in the overlying water of each core. To ascertain 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 before and after the experiment. The out-flowing air of each core entered into two successively placed CO_2 traps, connected to each other and changed every 3.5 days to prevent oversaturation. The CO_2 traps were prepared in 500 ml sealed off glass bottles with a screw on septum cap. The bottles were filled with a solution of MilliQ water and NaOH (Kristensen et al. 1992).

The same treatments were repeated in 1L glass bottles containing only bottom water, to quantify tracer degradation in the water column. At the end of the incubation time, the cores and the water bottles were opened one by one in sequence, oxygen concentration measurements done in the cores and samples of the water were taken.

2.6 Incubation sample processing

At the end of the incubation, the overlying water of the cores and the content of the CO_2 traps was gently removed and filtered on GF/F pre-combusted filters. The filters were then analyzed for phospholipid fatty acids (PLFA) to estimate the water column bacterial contribution to OM degradation. The sediment cores where then frozen in their coring tube. The upper 10 cm of each core was subsequently cut off when still inside the coring tube with a hand saw for later sub-sampling. This procedure was preferred over normal slicing due to the extremely soft nature of the sediment and to the in-core bubbles, which would have caused mixing during regular slicing. The intact core sections were stored in a -20 °C freezer and transported to the laboratory in Yerseke.

After arrival in the laboratory, four weeks later, the background and incubation cores were sliced frozen in 2 cm intervals: 0-2, 2-4, 4-6 cm with a circular electric saw. While frozen, each slice was portioned as follows: one quarter (approx. 39 ml) was freeze-dried immediately and used for analysis of bacterial PLFA, two quarters were used for faunal extraction. The fatty acid subsample was freeze-dried and stored at -20 °C until further processing. The frozen subsample for faunal analysis was immersed in a mixture of 40% buffered formaldehyde stained with rose Bengal and allowed to thaw at room temperature. This was preferred over fixing and staining after defrosting because of the better preservation of the fauna. The sediment was stored for 2 days at room temperature before sieving, to give the stain sufficient time to color the animals.

2.7 $\delta^{13}C$ isotope measurements

2.7.1 Bacteria

The bacterial tracer incorporation was estimated through the enrichment of bacterial-specific PLFAs (Boschker and Middelburg 2002). Two main biomarkers were chosen for the analysis: iC15:0 and ai15:0 because of specificity for bacteria and presence in all samples. Fatty acids were extracted using the Bligh and Dyer extraction protocol and analyzed using GC-c-RIMS (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 bacterial PLFAs was converted to incorporation into bacterial biomass by assuming that the specific PFLAs represent 11% of all bacterial PLFAs (following Moodley et al. 2002), which in turn comprise 5.6% of total bacterial carbon. Conversion of 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 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 divided in: branched and shelled Foraminifera, soft-bodied protists (both Gromiids and Allogromiids), Polychaetes, *Linopherus* sp., nematodes and other. The fauna was transferred into pre-weighted 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 weighted to establish the dry-weight of the fauna and pinched closed. The samples were then analyzed for carbon and nitrogen concentration and isotopic signature with a Thermo Electron Flash EA 1112 analyzer (EA) coupled to a Delta V. Fauna biomass was determined via dry weight and C content based on IRMS, abundance via specimens counting.

2.8 Uptake calculations

Relative uptake $\Delta\delta$ (the enrichment in ^{13}C stable isotope of the sample), calculated as

$$\Delta\delta = (\delta^{13}C_{sample} - \delta^{13}C_{background/control})$$

reflects tracer incorporation and is used to calculate excess ^{13}C (E) over the background $E=F_{sample}-F_{control}$,

where $F = {}^{13}C/({}^{13}C+{}^{12}C)=R/(R+1)$.

$R = (\delta^{13}C/1000+1) \times R_{REF}$, where $R_{REF} = 0.01118$. Total uptake (I, μg tracer ^{13}C) is then calculated as the product of excess and biomass. The uptake of total added algal derived C ($^{12}C+^{13}C$) for both bacteria and fauna was then calculated according to Moodley et al. (2005b), as the quotient of total uptake (I) and the fractional abundance of ^{13}C in the tracers (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. Relative biomass turnover due

to tracer uptake was calculated as (total tracer uptake/incubation time)/biomass per each core separately.

2.9 Respiration measurements

Respiration of the isotope tracers was estimated via ^{13}C analysis of the DIC (dissolved inorganic carbon) samples of the traps (changed every 3 days), the overlying core water at the end of the incubation time and the water column only incubations. The second traps in succession showed values very closed to zero and therefore will here be considered negligible. Water samples of ~2 ml 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 that 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 that accumulated then 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 ^{13}C-DIC was determined by summing the production in overlying water and volume of the CO_2 trap.

3. Results

3.1 Sediment

The sediment was very different between the 2 stations: in SToutOMZ it was mainly composed of light brown-gray and very compact clay whereas in STOMZ it was dark brown-black, watery and fluffy, poor in clay but with a high content of foraminiferan shells and diatom frustules. Freshly retrieved cores from STOMZ contained bubbles down to 30 cm and it is unclear to us what might have caused them. The cores showed intact layering and burrows, which indicated that no alteration of the sediment occurred during sampling and retrieval; therefore we believe that the bubbles must have been present before sampling. Total organic

carbon content and the 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 Biota biomass and natural ^{13}C abundance

Bacterial biomass ranged between 1000 and 1600 mgC m^{-2} respectively in SToutOMZ and STOMZ, meiofaunal nematodes between 19 and 13 mgC m^{-2}, meiofaunal-sized shelled foraminiferan biomass ranged between 42 and 38 mgC m^{-2}. Macrofaunal-sized shelled foraminiferal biomass spanned between 118 and 183 mgC m^{-2}, polychaetes ranged between 87 and 897 mgC m^{-2} with *Linopherus* sp. the most abundant species in STOMZ. Macrofaunal nematodes accounted for 17 mgC m^{-2} in SToutOMZ but were found only in background cores in STOMZ, macrofaunal branched foraminifera and soft bodied protists were found only in SToutOMZ and accounted for 208 and 497 mgC m^{-2} respectively. Other eukaryotes accounted for 0.2 mgC m^{-2} and were found only in SToutOMZ. Table 2 presents the data per layer and station.

Natural abundance $\delta^{13}C$ values (i.e. background) are comparatively similar among groups and between stations (see table 2 for details). The average is -20‰, lowest values were determined for PLFA-derived bacteria signature that ranged between -21.8 and -25.4‰ (due to the fact that PLFA values are usually lighter than the total cell signature), the highest signature was determined for SToutOMZ Eukarya with a value equal to -15‰. Meiofaunal shelled foraminifera of STOMZ showed a -23.7‰ and macrofaunal nematodes of SToutOMZ a -17.4 ‰. It is important to notice that the samples used to run natural abundances (the background or control cores) were formaldehyde preserved. This was done to be able to compare values to the experimental samples, also preserved, and to avoid correcting the uptake values for extra C atom input due to the preservative.

		Natural Abundance δ13C ‰	Biomass mgC/m^{-2}
STOMZ	***Bacteria***		
	0-2	-25,4 ±3.1	872,7 ±10
	2-4	-24,4 ±2.2	745,4 ±100
	Meiofauna		
	Nematodes		
	0-2	-22,3 ±1.0	8,9 ±1
	2-4	-22,2 ±1.1	4,6 ±0.8
	Shelled foraminifera		
	0-2	-23,7 ±0.1	26,9 ±5
	2-4	-11,1 ±13.0	11,2 ±3
	Soft bodied foraminifera		
	0-2	-5,7 ±0.0	12,9 ±0.0
	2-4	n.f	n.f
	Macrofauna		
	Shelled foraminifera		
	0-2	-21,5 ±0.1	128,7 ±80
	2-4	-20,8 ±0.7	55,0 ±20
	Linopherus sp.		
	0-2	-20,0 ±0.1	230,1 ±102
	2-4	-20,0 ±0.0	667,3 ±197
	Polychaetes		
	0-2	-16,9 ±0.0	319,7 ±0.0
	2-4	n.f	n.f
SToutOM	***Bacteria***		
	0-2	-21,8 ±0.8	677,6 ±50
	2-4	-23,2 ±1.1	332,9 ±70
	Meiofauna		
	Nematodes		
	0-2	-23,2 ±0.0	10,8 ±1
	2-4	-23,3 ±0.1	8,1 ±0
	Shelled foraminifera		
	0-2	-24,2 ±0.0	22,4 ±7
	2-4	-24,7 ±2.1	20,2 ±5
	Macrofauna		
	Nematodes		
	0-2	-17,4 ±2.1	7,5 ±0.0
	2-4	-17,2 ±0.0	9,8 ±0.0
	Shelled foraminifera		
	0-2	-20,3 ±0.2	64,6 ±36
	2-4	-20,0 ±0.1	53,7 ±22
	Branched foraminifera		
	0-2	-21,6 ±0.2	208,2 ±75
	2-4	n.f	n.f
	Soft bodied protists		
	0-2	-20,6 ±1.0	394,5 ±105
	2-4	-19,5 ±0.0	103,7 ±33
	Polychaetes		
	0-2	-19,4 ±0.1	31,8 ±7
	2-4	-18,5 ±0.0	55,4 ±12
	Eukaria		
	0-2	-15 ±2	0,2 ±0.0
	2-4	n.f	n.f

Table 2. Vertical profiles of natural $\delta^{13}C$ signatures and average biomass. "n.f." stands for not found. The terms "Soft bodied protists" regroup both Gromiids and Allogromiids.

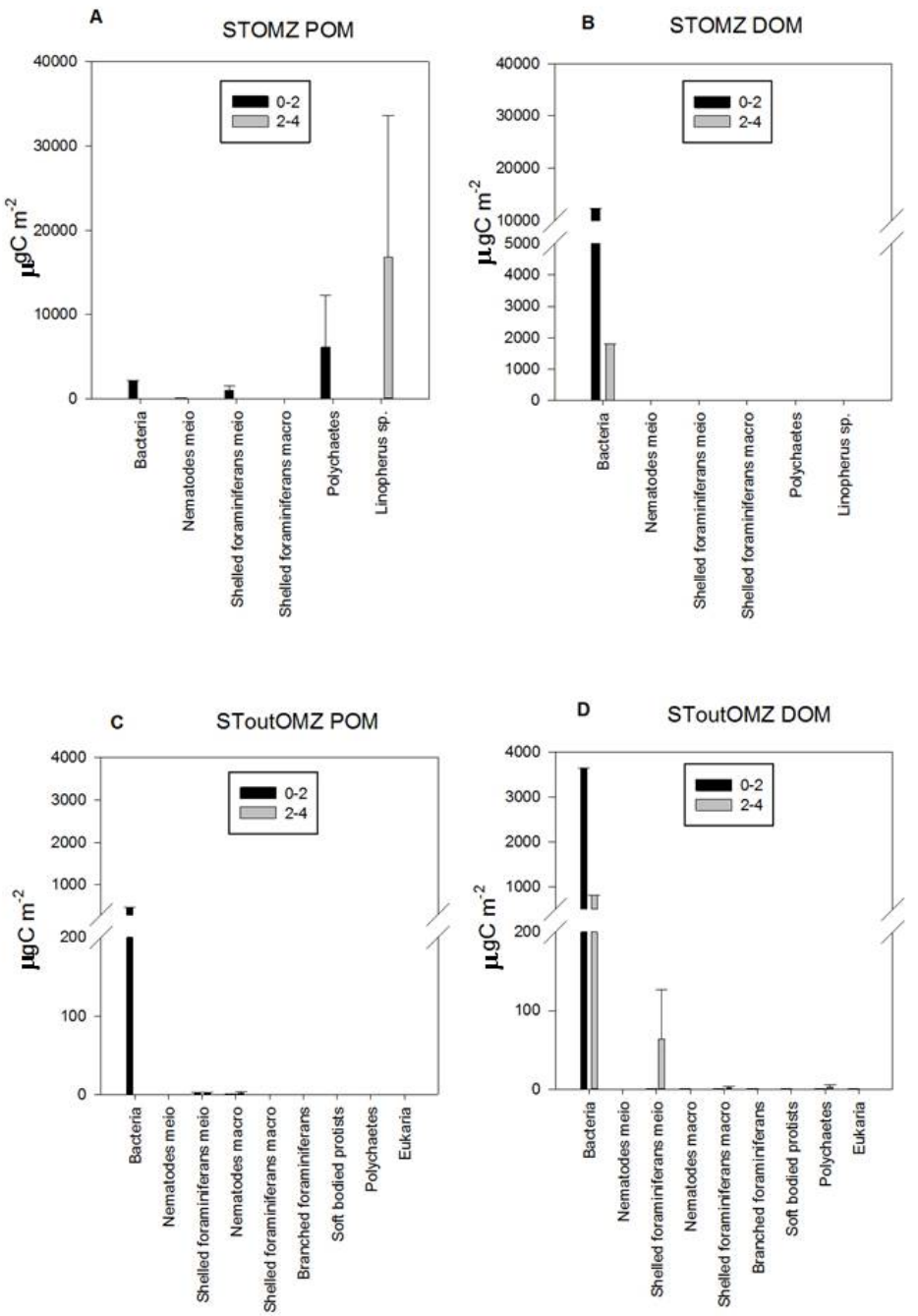

Figure 2 Tracer incorporation. Panel A and B for STOMZ, POM and DOM treatment; panel C and D for SToutOMZ POM and DOM treatment. The terms "Soft bodied protists" regroup both Gromiids and Allogromiids.

3.3 OM processing

Incorporation into biota of the two ^{13}C label OM types was evident in both treatments, although the level of enrichment differed per station, benthic group and depth layer (Fig. 2).

STOMZ. The upper layer, 0-2 cm, accounted for a small part (only 3247 μgC m^{-2} or 12% of the total uptake) of the POM uptake: bacteria incorporated 2181 μgC m^{-2}, meiofaunal nematodes 36.8 and shelled foraminifera 1028 μgC m^{-2}, macrofaunal shelled foraminifera incorporated 0.13 μgC m^{-2} and *Linopherus sp.* accounted for 1.08 μgC m^{-2}. In the deeper layer, 2-4 cm, almost all the POM incorporation took place (23000 μgC m^{-2} or 87% of the total uptake). Meiofaunal nematodes accounted for 2.66 μgC m^{-2} and shelled foraminifera for 6.55 μgC m^{-2}, macrofaunal shelled foraminifera accounted for 4.83 μgC m^{-2}, polychaetes for 6133 and *Linopherus* sp. for 16807 μgC m^{-2}. The bacterial contribution to POM uptake was negligible.

The DOM processing pattern was different from that of POM with clear bacterial dominance. In the upper layer, bacteria did not incorporate all the tracer that was added but only 12312 μgC m^{-2} (equal to 87% of the total uptake), followed by meiofaunal nematodes with 2.33 μgC m^{-2}; macrofaunal shelled foraminiferans incorporated 0.02 μgC $m^{-2}$and *Linopherus* sp. 0.05 μgC m^{-2}. Meiofaunal shelled foraminiferan contribution was negligible. In the deeper layer, 2-4 cm, bacteria clearly dominated uptake, incorporating 1803 μgC m^{-2} (equal to 13% of the total uptake), while meiofaunal shelled foraminifera accounted only for 0.01 μgC m^{-2}, meiofaunal and macrofaunal nematodes as well as all macrofauna were negligible in their contribution to total tracer uptake.

SToutOMZ. The largest part of the POM uptake was carried out in the upper layer, 0-2 cm. Bacteria incorporated 466 μgC m^{-2}, meiofaunal shelled foraminifera 2.06 μgC m^{-2}, macrofaunal nematodes 0.42 μgC m^{-2}, with negligible uptake by the remaining groups. In the deeper layer, 2-4 cm, only 0.6% of the total POM uptake was recorded by meiofaunal shelled foraminifera (1.47 μgC m^{-2}) and macrofaunal nematodes accounted (1.80 μgC m^{-2}).

DOM uptake in the upper layer was dominated by bacteria (3637 μgC m^{-2}), whereas uptake by the other groups was negligible with 0.01 μgC m^{-2} for meiofaunal shelled foraminifera and macrofaunal polychaetes and it was absent for the other groups. The deeper layer, 2-4 cm, showed a different picture: bacteria incorporating 805 μgC m^{-2}, meiofaunal shelled forams 63.7 μgC m^{-2}, macrofaunal shelled foraminifera 1.94 μgC m^{-2} and polychaetes 2.63 μgC m^{-2}.

3.4 Respiration

Most tracer OM was not processed within the 7 days incubation time (Fig. 3) and respiration was the dominant fate of processed DOM and POM at both stations, accounting for up to 13.6% and 6.73% of the added tracer on average for STOMZ POM and DOM treatment and 6.80% and 2.07% for SToutOMZ (Fig. 3). STOMZ POM treatment showed values for respiration twice as high when compared to the DOM treatment, average of 54.5 versus 26.9 mgC m^{-2}; the same pattern was also found for SToutOMZ, with the POM treatment accounting for an average of 6.82 mgC m^{-2} versus 2.1 mgC m^{-2} of the DOM treatment. Water samples of the water column only incubations from STOMZ showed values of 0.06 mg ^{13}C DIC for the POM treatment and 0.07 mg ^{13}C DIC for the DOM, compared to the 0.005 mg ^{13}C DIC and the 0.01 mg ^{13}C DIC respectively for the samples from SToutOMZ. These results confirm that sediment bacteria and fauna were the major groups responsible for the processing of the labelled OM and that the core overlying water community contribution was negligible.

3.5 Relative Biomass turnover

In STOMZ POM samples, relative biomass turnover values ranged between 0.81 and 0.00% d^{-1}, the highest for meiofaunal shelled foraminifera in the upper 0-2 cm layer (Table 3). SToutOMZ showed negligible relative biomass turnover for all components, but for bacteria and meiofaunal nematodes for the upper layer, with 0.10 and 0.01% respectively. In both stations in the DOM treatment samples, biomass turnover values were lower than for the POM treatment (Table 4). Relative biomass turnover values ranged between 0.00 and 0.16% in STOMZ, the highest for 0-2 cm layer bacteria and between 0.00 and 0.6% in SToutOMZ, the highest again for 0-2 cm layer bacteria.

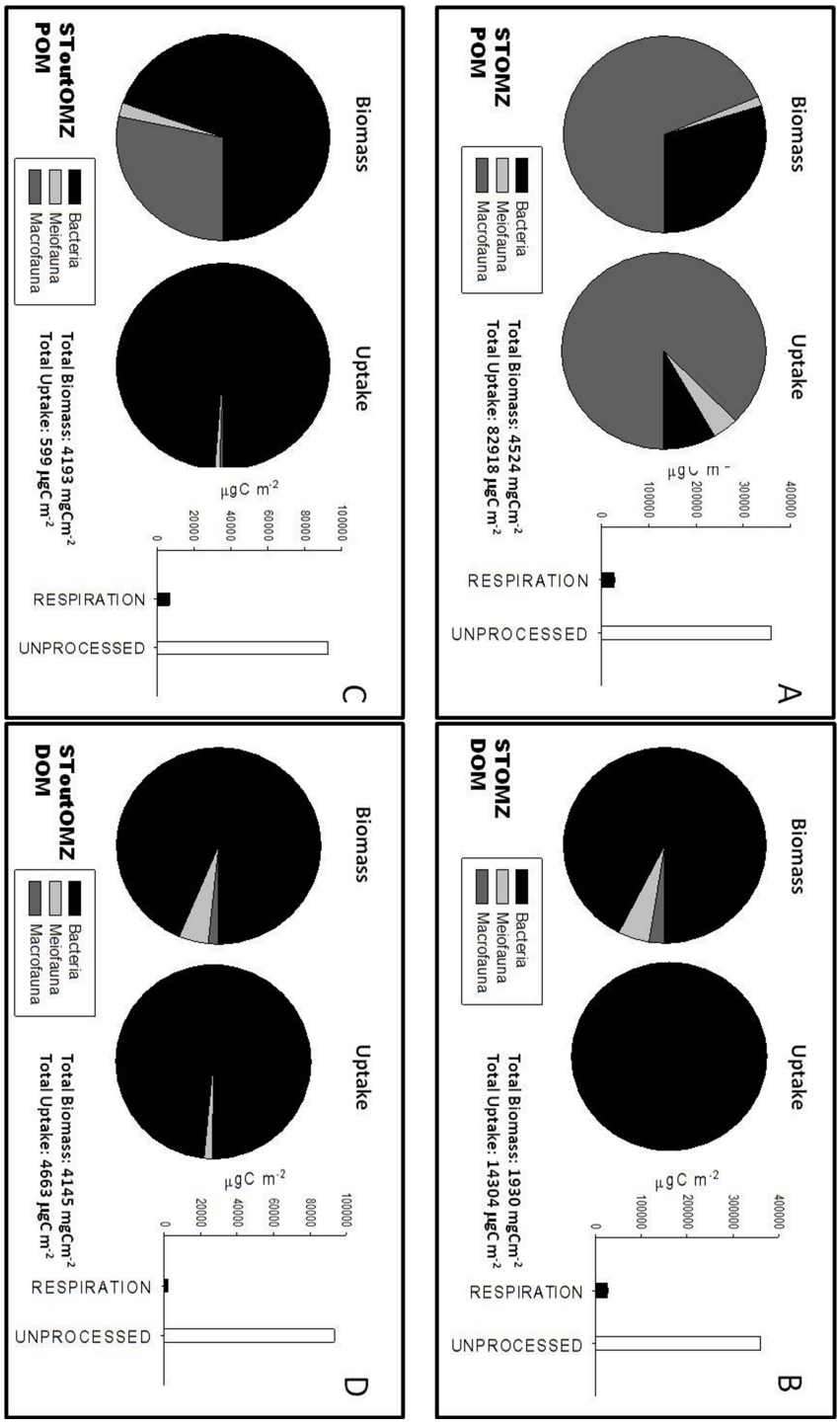

Figure 3 Carbon budget. Panel A and B for STOMZ, POM and DOM treatment; panel C and D for SToutOMZ POM and DOM treatment. Each panel represents a synthesis of the Carbon budget in respect to biomass. Uptake and biomass are directly comparable and given in the circles, total tracer carbon processed and respired are given in the plots.

		Relative Uptake Δδ13C ‰	Relative Biomass Turnover %/d^{-1}
STOMZ	***Bacteria***		
POM	0-2	79,1±22,8	0.06±0.00
	2-4	0±0.0	0.00±0.00
	Meiofauna		
	Nematodes		
	0-2	69±46	0.08±0.25
	2-4	16±5.6	0.02±0.02
	Shelled foraminifera		
	0-2	685±404	0.81±2.18
	2-4	23±6.6	0.03±0.04
	Soft bodied foraminifera		
	0-2	n.f	n.f
	2-4	n.f	n.f
	Macrofauna		
	Shelled foraminifera		
	0-2	18±17	0,00
	2-4	1.1±0.3	0,00
	Linopherus sp.		
	0-2	44±44	0.00±0.00
	2-4	136±116	0.01±0.00
	Polycahetes		
	0-2	185±167	0.01±0.00
STOutOM	***Bacteria***		
POM	0-2	13±2.0	0.10±0.00
	2-4	0.0±0.0	0.00±0.00
	Meiofauna		
	Nematodes		
	0-2	0.0±0.0	0.01±0.00
	2-4	0.0±0.0	0.00±0.00
	Shelled foraminifera		
	0-2	3.9±1.8	0.00±0.01
	2-4	0.6±0.6	0.00±0.00
	Soft bodied foraminifera		
	0-2	n.f	n.f
	2-4	n.f	n.f
	Macrofauna		
	Nematodes		
	0-2	0.8±0.2	0.00±0.00
	2-4	n.f	n.f
	Shelled foraminifera		
	0-2	0.2±0.0	0.00±0.00
	2-4	n.f	n.f
	Branched foraminifera		
	0-2	0	0.00±0.00
	2-4	n.f	n.f
	Soft bodied foraminifera		
	0-2	1.0±1.0	0.00±0.00
	2-4	n.f	n.f
	Polychaetes		
	0-2	1.0±1.0	0. 00±0.00
	2-4	7.3±7.3	0.00±0.00

Table 3. Relative uptake and % relative biomass turnover for POM treatment. "n.f." stands for not found. The terms "Soft bodied protists" regroup both Gromiids and Allogromiids

		Relative Uptake Δδ13C ‰	Relative Biomass Turnover %/d^{-1}
STOMZ	***Bacteria***		
DOM	0-2	210±46	0.16±0.00
	2-4	65±26	0.05±0.00
	Meiofauna		
	Nematodes		
	0-2	5.9±10	0.01±0.25
	2-4	0.4±0.9	0.00±0.03
	Shelled foraminifera		
	0-2	3.6±3.4	0.00±2.18
	2-4	9.3±0.8	0.01±0.04
	Soft bodied foraminifera		
	0-2	n.f	n.f
	2-4	n.f	n.f
	Macrofauna		
	Shelled foraminifera		
	0-2	5.2±2.7	0.00±0.09
	2-4	11±10	0.00±0.00
	Linopherus sp.		
	0-2	1.5±1.5	0.00±0.00
	2-4	11±9.6	0.00±0.00
	Polycahetes		
	0-2	n.f	n.f
STOutOM	***Bacteria***		
DOM	0-2	40±3.3	0.06±0.00
	2-4	26±26	0.03±0.00
	Meiofauna		
	Nematodes		
	0-2	1.8±0.0	0.00±0.00
	2-4	0.0±0.0	0.00±0.00
	Shelled foraminifera		
	0-2	24±20	0.03±0.01
	2-4	40±39	0.05±0.00
	Soft bodied foraminifera		
	0-2	n.f	n.f
	2-4	n.f	n.f
	Macrofauna		
	Nematodes		
	0-2	2.2±2.0	0.01±0.00
	2-4	2.5±1.6	0
	Shelled foraminifera		
	0-2	0.4±0.2	0.00±0.00
	2-4	0.5±0.5	0.00±0.00
	Branched foraminifera		
	0-2	0.8±0.8	0.00±0.00
	2-4	n.f	n.f
	Soft bodied foraminifera		
	0-2	0.3±0.2	0.00±0.00
	2-4	6.0±6.0	0.00±0.00
	Polychaetes		
	0-2	0.3±1.0	0.00±0.00
	2-4	0.5±0.4	0.00±0.00

Table 4. Relative uptake and % biomass turnover for DOM treatment. "n.f." stands for not found. The terms "Soft bodied protists" regroup both Gromiids and Allogromiids.

4. Discussion

In most environmental settings, the majority of the OM that reaches the bottom of the ocean is processed by the benthic community, which uses it for biomass production and respiration (Burdige 2006). This processing occurs basically along two pathways. The POM pathway involves metazoans ingestion and processing the organic matter in particulate form while consortia of bacteria degrade POM to DOM first before it is taken up. The DOM pathway involves the microbial community that incorporates the DOM directly or after hydrolysis. OM accumulates in oxygen minimum zones, indicating that these two lanes are somewhat impaired. We specifically traced both pathways to assess if and where the malfunction happens, to investigate which group(s) might be responsible for such lack of OM degradation and what are the causes that inhibit it.

The use of two different tracer loads in the two stations might raise critics. Such decision came from the intention of being consistent with a natural OM input. The different dose of C added (400 and 100 mgC m^{-2}) reflected the difference in sediment organic C content in the two stations (6.38 vs 1.03%, table 1), making the addition equivalent to 0.31 and 0.19% of the total organic C naturally present in the surface 3 cm of the sediment. The possibility that such diverse OM load caused the difference in response of the biota between stations cannot be ruled out, but it is unlikely to be the main cause. Furthermore, even though our tracer additions were lower than the 1g m^{-2}, 1.2g m^{-2} and 700 mg C m^{-2} (360 mg C m^{-2} for lander experiments) used in previous studies (Sweetman and Witte 2008; Witte et al. 2003a; Witte et al. 2003b; Woulds et al. 2009), only a small portion of the added labelled OM was processed in 7 days (8.55% in the DOM and 13.7% in the POM, values averaged among stations) leaving the majority of the tracer available to the biota.

Bacterial, protist and faunal biomass in both stations was comparable to other deep-sea settings: the ~1.5 mgC m^{-2} bacterial biomass, up to 16 mgC m^{-2} foraminiferal biomass, up to 22 mgC m^{-2} meiofaunal biomass and ~40 mgC m^{-2} macrofauna reported by Moodley et al. (2002) in the North East Atlantic, the 2500 mgC m^{-2} bacterial biomass, 15 mgC m^{-2} foraminifera, 5 mgC m^{-2} nematoda and 120 mgC m^{-2} macrofauna reported by Witte et al. (2003b) in the Porcupine Abyssal Plain (PAP). Conversions of the original data were made according to Rowe (1983). Such presence of

prokaryotic and metazoan biomass in the OMZ implies that the absence or scarcity of benthos cannot be claimed to be main cause for OM accumulation. In both POM and DOM treatments, fauna and bacterial PLFAs contained tracer OM. These results confirm that biota in the OMZ core were active even at suboxic conditions. Our results also indicate that during a relatively short period (seven days incubation) both the tracers were fed upon, indicating that the OMZ fauna was not impaired by to lack of oxygen. Our results also show that DOM and POM in the Arabian Sea initially follow two different and separate processing pathways.

4.1 DOM

DOM was introduced in injection wells at high resolution, but heterogeneous label distribution in the sediment is inevitable. However, injection is the preferred method to introduce label while minimizing sediment disturbance (Dobbs et al. 1989; Van Oevelen et al. 2006b). This implies a different delivery of the tracers compared to POM which was distributed on the core surface but this has been taken into consideration during the interpretation of the results. In STOMZ, bacteria are the main group in terms of uptake in the DOM treatment and all the other groups showed negligible uptake. The DOM pathway therefore seems to be a dead end in the food web, as demonstrated by the high bacterial uptake, which is not followed by ^{13}C transfer to meio and macrofauna. This finding confirms that DOM and POM pathways are sometimes uncoupled.

4.2 POM

The POM was incorporated by all benthic groups as already been shown by other studies carried out in the Indian and Pakistan margin of the OMZ using labeled phytodetritus. In fact, Andersson et al. (2008) reported bacteria and macrofauna accounting for 11% of the total processing and foraminifera followed shortly after, and Woulds et al. (2009) reported bacteria responsible for 0 to 32% of total processing, foraminifera 1 to 68% and macrofauna 0 to 83%, depending in the station and the season. These results together with ours show that the POM consumption chain works and all the groups can take advantage of phytodetritus inputs. In our POM treatment though, *Linopherus* sp.

incorporation dominated over all the other groups, followed by the other polychaetes. This confirms that in the OMZ macrofauna, when present, is capable of degrading even more OM than bacteria.

Our results confirmed that DOM as food source could be used only by bacteria and the other groups could not take any direct advantage from it, whereas POM was more widely processed and incorporated. When comparing the values among treatments, our data suggest that the different biotic compartments relied on different food resources, POM for macro and meiofauna and bacteria, DOM only for bacteria that were not grazed upon. This finding therefore shows that the POM incorporation that we saw in the different benthic groups reflected direct uptake of the tracer rather than food web transfers. Other studies (Hoste et al. 2007; Iken et al. 2001; Vanreusel et al. 1995) pointed to the possibility of a transfer of OM from bacteria to nematodes via direct predation, observing positive correlations between bacterial density and nematodes abundance in the deep sea and speculating that bacterivory might be one of the feeding strategies applied by nematodes in the Porcupine Abyssal Plain. Our results did not confirm such hypothesis. Moodley et al. (2002) showed that nematode uptake of phytodetritus was limited in the short term and Nomaki et al. (2005), Sweetman and Witte (2008) reported it as absent. Therefore, in our DOM treatment, the only way for nematodes and other metazoans to show ^{13}C enrichment would have been if they actively ingested labeled bacteria. This did not happen. The DOM incorporation data presented here show that OM transfer along the food chain, as fresh supplied DOM ingested by bacteria, in turn grazed upon by fauna, might not have been as important as the direct feeding on OM, be it POM or DOM, and that bacteria were not a principal food source at least in the short term. Our results are in line with what found by Guilini et al. (2010) in the Hausgarten site in the Greenland sea showing that, even when offered a more complex dissolved source of OM (compared to the glucose, acetate, bicarbonate or amino acids used by Guilini et al. 2010), only bacteria seemed to utilize it and no direct uptake by fauna occurred. If bacteria in the OMZ were not grazed upon by nematodes nor by other metazoans and, if as reported by Rex et al. (2006), their standing stock is rather constant in marine sediments, then viral lysis seems to be the main factor controlling bacterial biomass in the sediment, abating 80% of prokaryotic heterotrophic production (Danovaro et al. 2008). The only other group that showed ^{13}C enrichment in the DOM treatment was the meiofauna shelled foraminifera of the deeper layer (2-4 cm) in

StoutOMZ. This could be due to either direct feeding of the foraminifera upon DOM or grazing on bacteria that previously incorporated the DOM. Few studies (Delaca 1982; Delaca et al. 1981; Lee et al. 1966) reported foraminiferan feeding directly on dissolved organic carbon. Recent research conducted by Nomaki et al. (2006) on different foraminiferan species, clearly showed how algal carbon was better assimilated than bacterial carbon even though the protists still incorporated bacteria, probably via indirect selective ingestion. Our experiment was not designed to define feeding preferences of foraminifera, but we cannot exclude that the ^{13}C uptake we saw in our StoutOMZ DOM treatment was the result of bacterial grazing and due instead to direct DOM ingestion by the meiofaunal shelled foraminifera.

From the above it appears that the biota inside the OMZ are not limited by the hypoxia, not in biomass, nor in activity, dismissing the possibility that OM accumulation in this setting is due to biotic malfunctioning. The clear effect of OM quality on its processing though, confirms that quality might be the main cause that allows OM accumulation in the Arabian Sea, supporting the hypothesis of Henrichs (1992), who suggested the accumulation is caused by the refractory nature of the detritus that arrives to the bottom.

The situation changes when StoutOMZ oxic sediment is artificially exposed to hypoxia. Our data on StoutOMZ hypoxic OM processing show that only surface bacteria incorporated the POM tracer and only bacteria and deep dwelling shelled foramimifera incorporated the DOM tracer. The lack of labeling into the other faunal groups might have been due to low adaptation of these to process OM in lower oxygen concentration. This confirmed that bacteria can survive (at least for a short time) suboxic conditions and suggested that the more sensitive macrofauna had difficulties in resisting and would therefore be severely negatively impacted by a quick and sudden expansion of the OMZ. These findings are in line with the 0 to 66 gWW m^{-2} of macrofauna reported by Hughes (2009) and the 0 to 14 gWW m^{-2} macrofauna shown by Gooday (2009), that present drastically diminished meio- and macrofaunal biomass values for suboxic areas of the Arabian Sea OMZ. In the case of an expansion of the OMZ, according to what reported by Roosenberg et al. (2001), a change in species and abundance is to be expected, which may lead to a community that becomes more similar to STOMZ. Previous

results and reviews reported some general trends in community response to hypoxia: Diaz (2001) in his review across systems reports fauna large-scale migrations and/or mass mortality, change in composition and reduced growth and biomass. This resembles well the conditions depicted by our study and the data regarding STOMZ, where biomass and diversity were lower than in SToutOMZ. The experimental sudden drop in oxygen concentration caused the activity of the fauna in SToutOMZ to be impaired, suggesting a mortality process of the non-adapted species. This might not happen though, if the suboxic conditions are established gradually in time as exemplified in the Gulf of Mexico, where hypoxia gradually increased in size and duration from its inception and therefore the ecosystem's response seems to have been gradual and not catastrophic (Diaz 2001). The polychaete *Methanoaricia dendrobranchiata* (Blake) is such an example of adaptation: it survives at null or almost undetectable oxygen concentration and developed physiological mechanisms to thrive in anoxic environments (Hourdez et al. 2002). The worms regulate their rate of oxygen consumption, developed a large gill surface area, a small diffusion distance from sea water to blood and a high hemoglobin-oxygen affinity. Similar adaptations are found in the Arabian Sea OMZ polychaete *Linopherus* sp., endemic of this area and found only in the OMZ core station (Hughes et al. 2009; Levin et al. 2000; Levin et al. 2009b; Woulds et al. 2009; Woulds et al. 2007). This species reaches high biomasses and is responsible for a large portion of the metazoan OM processing in the OMZ (see Fig. 3 as well as literature mentioned above).

5. Conclusions

In conclusion, four observations emerged from our experiments: 1) fauna, protists and bacteria were present in both stations and short term OM processing, in terms of uptake and respiration was recorded, with processing rates by the OMZ biota being comparable to oxygenated open slope and abyssal sediment, 2) there is an evident separation in DOM and POM processing pathways, 3) in 7 days no direct DOM derived-C transfer up the food-chain due to predation occurred and bacteria seem

not to be a main food source for metazoans, 4) faunal activity in oxic sediment subjected to suboxic conditions is very low. If biota are active inside the OMZ as demonstrated by our data, if short term processing of both POM and DOM is carried out in quantity that are similar to those of other settings as our comparisons pointed out, then biota oxygen-induced inactivity cannot be claimed as the main cause for the OMZ organic matter accumulation. This leads us to conclude that the probable cause for the OMZ OM accumulation must be the highly refractory nature of the OM standing stock.
Further investigation is needed to unravel dissolved organic matter assimilation pathways in the deep sea and future studies should consider a double approach and utilize both POM and DOM in stable isotope pulse chase experiments.

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4 Organic matter processing in the deep sea: a comparison between North Atlantic and Mediterranean sediments

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Abstract. *Previous short-term tracer pulse-chase experiments suggest that incoming organic matter (OM) may be rapidly respired, with limited impact of seafloor community structure yet significantly dependent on bottom-water temperature. Here we report on manipulation experiments in two contrasting deep-sea sediments from 1900 m water depth: cold, fauna dominated sediment from the northeast Atlantic (NEA) and warm and bacteria dominated sediment from the western Mediterranean (WM). We utilized diatom-derived ^{13}C-labelled particulate organic matter (POM) and dissolved organic matter (DOM) as tracers in 7 days on-deck core incubation experiments. The tracer's fate was followed under oxic and suboxic bottom water conditions at in-situ temperature. A clear benthic community response was recorded and CO_2 was the major processing product after 7 days POM incubations (95% and 99% in the NEA oxic and suboxic treatment, 90% and 92% respectively in the WM). When amended as POM, significantly more tracer was processed by sediment biota (8 to 9% of POM versus 5 to 7% of DOM additions). Despite absolute processing values being higher for POM than for DOM, assimilation efficiency values followed the opposite trend (5% and 10% for POM respectively in the NEA and in the WM versus 19 to 52% for DOM respectively in the NEA and in the WM). This confirms that the nature of the tracer clearly modifies its benthic fate. This study also supports*

reports on the flexibility of bacteria to cope with changing redox conditions: assimilation was 273 and 65 µgC m^{-2} for the NEA and 828 and 695 µgC m^{-2} for the WM, for POM oxic and suboxic treatment respectively. Our results also revealed that previous studies may have overestimated OM processing due to utilizing relatively large quantities of highly degradable whole diatoms as tracer.

1. Introduction

The seafloor is the largest habitat on the planet and one of its key processes is organic matter transformation. Organic matter (OM) that escapes water-column remineralization, fuels the life of the seafloor community, which in turns transforms it into living biomass, carbon dioxide and nutrients. The biomass formed may transfer C and N to higher trophic levels and becomes the power to perform basic ecosystem functions such as bioturbation and respiration. Understanding the relationship between sediment community structure and seafloor ecological processes is a central focus in current marine ecology. This, and the growing concern about marine biodiversity loss (often attributed to anthropogenic environmental changes), has provided an extra impetus for experimental work, aimed at elucidating the role of different benthic compartments in basic ecosystem functioning (Austen et al. 2002; Raffaelli et al. 2003; Solan et al. 2006). One powerful approach involves utilization of ^{13}C-enriched algal material as a tracer, to follow the organic carbon flow pathways (community respiration versus assimilation by microbes and fauna) (Blair et al. 1996; Buhring et al. 2006; Gontikaki et al. 2011; Moodley et al. 2002).

In short-term (1 day) incubation experiments, it was observed that incoming OM may be rapidly respired (~ 15% of added carbon within 24 hours) by the benthic community both in shallow-water and deep-sea sediment cores, with limited impact of seafloor community structure and at significantly lower rates under low temperature (Moodley et al. 2005b). Their processing rates may however have been overestimated, due to the use of large amounts of highly degradable diatom organic

matter that are used in these tracer studies, typically equivalent to the total annual input of OM.

Several studies have employed specific isotopically enriched pools or manipulated the quality of OM (Enge et al. 2011; Guilini et al. 2010; Moodley et al. 2011; Nomaki et al. 2006) to investigate its fate. In reality, OM arriving at the seafloor is mostly of poor quality, as by its degradation during its slow descent to the bottom, it is already stripped of the rapidly degradable components such as dissolved organic matter (DOM). DOM is a major pool of OM in the marine environment but seems not to be utilized directly by most sediment fauna (Guilini et al. 2010; Van Oevelen et al. 2006a), except for some epifauna (De Goeij et al. (2008) and references therein. Here we report on manipulation experiments, in two deep-sea sediments from 1900 m water depth, utilizing particulate and dissolved organic matter (POM, DOM) as tracers in on-deck incubation experiments. This allowed us to investigate the role of temperature in OM processing, how community structure influences OM degradation and to characterize the processing pathways of POM and DOM. We hypothesize that the two sites will show differences in quantity of OM processed due to temperature and that the structures of the *in-situ* communities will determine the fate of the tracer amended (i.e. bacterial vs fauna assimilation). We also expect quality of OM to be one of the main factors influencing its degradation.

Redox conditions can also influence OM processing in the sediments and need to be considered: suboxia/hypoxia may affect ecological processes directly or indirectly by altering community structure (differential sensitivity of benthic compartments, e.g. Middelburg & Levin (2009) and references therein). Seafloor hypoxia is one of the most drastic impacts of global warming on its own, or in combination with eutrophication and is increasing in occurrence, duration and intensity. Deep-sea floor communities commonly exposed to oxygen depletion are adapted to low oxygenation (e.g. sediment underlying the eastern Arabian Sea oxygen minimum zone, (Levin et al. 2009a; Moodley et al. 2011)) but it is difficult to foresee the reaction of non-adapted fauna to hypoxic events and if and how organisms' OM processing change. Previous work done in different sub-anoxic settings already reported about bacterial and faunal responses to diminished oxygen concentrations. Field studies have demonstrated meiobenthos' sensitivity to bottom-water low oxygenation. Moodley (1997) reported that the decreasing biological interactions during anoxia were beneficial to some foraminiferal species, resulting in

a shift in faunal patterns and a strong reduction in foraminiferan diversity. Gooday (2003) in his review reported that foraminiferan abundance and species composition is strongly influenced by organic flux to the bottom and that oxygen concentration shows effect only below 0.5ml l^{-1} (~ 22.3 μM O_2). Murrell (1989) reported how meiofaunal benthic biomasses drastically declined during hypoxia in the Gulf of Mexico but the effect on nematodes was not as dramatic as for the other groups. It is in fact generally agreed that nematodes are the less sensitive among meiofauna and that food quality is the major predictor of their abundance (Cook 2000; Neira 2001). Macro and megafauna are by far the more sensitive to hypoxia, which causes physiological adaptations (Diaz and Rosenberg 1995) and community changes due to mass mortality and migrations (Rosenberg 2001). These findings suggest that among fauna, nematode and foraminiferan biomasses should be the highest in oxygen depleted settings and therefore these groups should be the major players in OM degradation. As these authors report though, often the effects on OM processing in hypoxic conditions are mixed with the biota response to OM quality and quantity; hence, distinguishing their separate influences might be difficult. To investigate the role of oxygen concentration on the benthic community processing of OM and to assess whether oxygen depletion causes drops in OM processing, a parallel set of intact sediment core incubations were run under suboxic conditions (6 - 15 μM O_2).

2. Material and methods

2.1 Study area and sampling

Sediment and bottom-water samples from two contrasting deep-sea sites (1900 m water depth) were collected during two cruises with the RV Pelagia conducted as part of the EURODEEP-BIOFUN project. One station in the Northeast Atlantic (NEA) was sampled in September-October 2008 and the other in the Western Mediterranean (WM) was sampled in November 2009 (Fig. 1 and Table 1). These sites differ in temperature, 4 °C for the NEA sediment and 13 °C for WM sediment. Also, the NEA station is exposed to high currents (see Hernandez-Molina et. al. (2008) and references therein) and is fauna-dominated, whereas the WM station is more sheltered and bacteria-dominated.

Figure 1. Sampling areas. NEA= North-East Atlantic, Galicia Bank, WM= Western Mediterranean, South-East Balearic Sea

	Northeast Atlantic (NEA)	Western Mediterranean (WM)
Station Position	42°27, 64'N, 10°39, 28'W	39°25,2'N, 4°16,2'E
Bottom water temperature	4°C	13°C
Bottom water salinity	35	38
Surface Sediment		
median grain size (µm)	15.9	15.1
C_{org} content (g $C.m^{-2}$)	47.4	64.7
C/N ratio	6.98	5.45
$\delta^{13}C_{org}$ (‰)	-20.7	-20.7
$\delta^{15}N_{tot}$ (‰)	4.9	5.47

Table 1. Location and setting of the 2 stations (1900 m water depth) and their surface sediment (0-2 cm) characteristics.

2.2 Intact sediment-core incubations

For the experiments addressing sediment community functioning, intact sediment cores with overlying bottom water were sampled using multicorer polycarbonate tubes (9.5 cm inner diameter, 30 cm long) and directly stored on board in the dark in an *in-situ* temperature-regulated container (4 °C for the NEA sediment and 13 °C for WM sediment). Bottom water was collected with Niskin bottles and identically stored.
In the different replicate cores pre-designated for either normoxic (equal to *in situ* concentration) or suboxic conditions (2 replicates per treatment), the overlying water was gently bubbled with either air from an aquarium pump (oxic, 290-300 µM O_2) or from a certified gas mixture (Hoekloos BV, The Netherlands) to obtain suboxic conditions (from a 50 liter gas cylinder holding a mixture of 0.5% O_2, 300 ppmv CO_2 and the remainder N_2; CO_2 was added at atmospheric concentration levels to prevent drastic changes in water pH). This gas mixture at the given salinity and temperature resulted in overlying water having an oxygen content of 6 - 15 µM O_2, as determined with oxygen-optodes (Presens, Germany) following standard procedures of calibration.

In the laboratory, the two diatom derived substrates, diatom-DOM and diatom-POM, were extracted from axenically cultured diatoms (^{13}C-enriched *Thalassiosira pseudonana*), labelled and concentrated as described in Moodley et al. (2002). Axenic diatom cells were thoroughly rinsed to remove residual ^{13}C-enriched bicarbonate, concentrated by centrifugation and then freeze dried. First, dissolved organic matter was isolated: freeze-dried diatom cells were mixed with MilliQ, vortexed, centrifuged and the supernatant collected. Following 3 extractions, the collected supernatant was passed through a 0.2 µm polycarbonate filter (Millipore) and the obtained DOM stored in a glass tube. A subsample was taken to determine carbon content and isotope labeling (atomic % ^{13}C) in order to prepare aliquots of required carbon content. Thereafter, a fixed volume of DOM was transferred to 10 ml glass bottles, freeze-dried and stored frozen until initiation of experiments onboard ship. Just prior to the experiment 0.2 µm filtered seawater was added to re-dissolve the DOM.

The residue pellet, made of diatom cell walls, frustules and other rigid structures, was freeze-dried and used as diatom-particulate organic

matter (POM) substrate. As in the case of DOM, aliquots with required amounts of carbon were prepared in 10 ml glass bottles and just prior to the experiments, 0.2 µm filtered seawater was added to the tracer to facilitate amendment in the experiments.

The core incubations were conducted for 7 days, in the dark at *in situ* temperature after amending them with the diatom derived POM or DOM. POM was added using a long glass pipette delivering it directly onto the sediment (Moodley et al. 2005b). DOM was injected at regular spaced horizontal intervals at approximately 1cm depth in the sediment with a glass syringe mounted on an extension rod. Both treatments consisted in amending the equivalent of 100 mgC m^{-2} per core. Each core was then connected to a CO_2 trap (replaced once after 3.5 days) to collect the exiting CO_2 produced by the cores, in order to quantify total respiration of the tracer OM (sum of overlying water and CO_2 trap).

At the end of the 7 days, prior to processing, the cores were visually examined for traces of fauna activity, sediment texture, color and odor. After sampling for ΣCO_2 (4 ml water transferred through a GF/F filter into a helium pre-flushed vial sealed with a shrimp-cap with rubber septum), the overlying water was carefully removed and volume determined. The sediment of each core was extruded, sliced and placed into plastic buckets (0-2, 2-4 cm intervals). After gentle homogenization, the bucket sediment was sub-sampled with a cut-off syringe as follows: 10 ml for PLFA (polar lipid derived fatty acids) and the rest for fauna. Fauna samples were preserved in Bengal-rose buffered formaldehyde and PLFA samples were directly frozen for later freeze-drying and extraction (e.g. Middelburg et al., 2000). Fauna samples were sieved on a 38 µm mesh and the residuals retained were used for determination. Sample processing, including fauna and bacteria tracer assimilation as well as respiration, was done as described in Moodley et al. (2002). Finally, the amount of tracer carbon in a specific pool or benthic compartment was determined as the product of excess ^{13}C and carbon biomass or concentration, divided by fraction ^{13}C in the tracer OM. Fauna biomass, as organic carbon content, was measured directly (Moodley et al. 2005b) and bacterial biomass estimated from concentration of bacteria specific PLFA (Middelburg et al. 2000).

Community assimilation efficiency was calculated as

Ass.eff. = (Assimilation/(Assimilation+Respiration)*100),

percent of amended tracer carbon processed as

(Assimilation+Respiration)/(Tracer added).

Background sediment characteristics of the upper 2 cm layer were determined in sediment from separate replicate cores: granulometry (sediment not acidified) using a Malvern Particle Analyzer, sediment C_{org} content and isotope signatures as described in Moodley et al. (2005b).

The contribution of the overlying water to tracer respiration was also examined: the same tracers were incubated in 1000 ml glass bottles filled with bottom water without sediment. The bottles had caps fitted with a rubber septum allowing a plastic tube in (for supply of air or gas-mixture) and a tube out into the CO_2 trap. Diatom- POM and diatom-DOM was pipetted onto the bottom of the bottle and water samples processed as described for sediment core incubations.

All results are presented as averages with standard deviation; statistical significance of differences was tested with analysis of variance (ANOVA) of log-transformed or arcsine transformation data followed by pairwise comparisons (Tukey HSD test) using SYSTAT (Systat Inc.).

3. Results

3.1 Seafloor and sediment community characteristics.

Bottom-water temperature was higher in the western Mediterranean (WM) than at the northeastern Atlantic (NEA) station (Table 1). Surface sediment (upper 2 cm) median grain size was similar at both stations (~15 μm) and bulk sediment organic matter characteristics such as C/N ratio (5 -7), $\delta^{13}C_{org}$ (-20.7 ‰) and $\delta^{15}N_{tot}$ (~ 5 ‰) suggest that the OM reaching both sediment communities is primarily of marine origin (Table 1). However, C_{org} content (upper 2 cm seafloor) was higher at the WM station (65 $gC.m^{-2}$ vs 47 $gC.m^{-2}$ in the NEA station). There were significant differences (ANOVA, $p < 0.05$) in the size and structure of the

seafloor communities (upper 4 cm) from the 2 stations (Table 2): total biomass standing stock (sum bacteria and fauna including foraminifera) was significantly higher in WM sediment (on average, 989 ± 180 mgC m^{-2} versus 696 ± 285 mgC m^{-2} in NEA sediment). Bacteria constituted a significantly higher fraction of total biomass in WM sediment (on average 97% in WM and 59% in NEA sediment). In contrast, fauna biomass was almost one order of magnitude higher in NEA sediment as compared to the WM (Table 2).

Biotic Compartment	Northeast Atlantic (NEA)			Western Mediterranean (WM)		
	POM-OX	POM-SUBOX	DOM-OX	POM-OX	POM-SUBOX	DOM-OX
Bacteria	470 ± 18	366 ± 172	337 ± 18	1151 ± 98	788 ± 87	929 ± 64
Fauna	488 ± 418	210 ± 25	218 ± 27	25 ± 11	27 ± 4	46 ± 25
% Bacteria	54.2 ± 24.5	62.1 ± 8.6	60.8 ± 1.7	97.9 ± 0.8	96.6 ± 0.9	95.2 ± 2.8

Table 2. The biomass (mgC m^{-2} in the 0-4 cm seafloor) of bacteria and fauna (> 38 µm macro-and meio-fauna including foraminifera) at the two stations in the cores of the different treatments (average ± sd, n=2). DOM is diatom derived dissolved organic matter injected 1 cm deep into the sediment, POM-OX is diatom particulate organic matter amended in incubations with oxic bottom water (290 - 300 µM O_2) and POM-SUBOX is diatom particulate organic matter amended in incubations with suboxic bottom water (6 - 15 µM O_2).

3.2 Intact sediment-core incubations

The total amount of tracer processed (sum of respiration and assimilation, Table 3) was equivalent to 5% to 9% of the tracer organic carbon amended and was not significantly different between stations or oxygenation treatments (2-way ANOVA, $p > 0.05$).

In the POM tracer amendments, respiration was the major fate of the tracer (95% and 99% for the NEA oxic and suboxic treatment, 90% and 92% respectively for the WM, Table 3), and the values did not significantly differ between stations (ANOVA, $p > 0.05$). However, community tracer OM assimilation efficiency (AE) was significantly different between sites: AE was higher in WM sediment, spanning

between 7% and 52% vs. values ranging from 0.9% to 18% for the NEA (Table 3).

At both stations, bacteria and fauna contributed to tracer POM assimilation but the contribution varied as a function of site. In the WM assimilation was primarily bacterial (on average 93% - 98% of the total assimilation under oxic and suboxic conditions, see Fig. 2 for data calculated on total processing), due to the relatively low fauna biomass standing stock (Table 2). In NEA, fauna accounted for a larger fraction (between 7% and 34% of the total assimilation).

The DOM extracted from the diatoms, otherwise commonly included in organic matter tracer studies, was incubated separately as previously described. Respiration was the major fate for the processed DOM in the NEA (81%) but in the WM respiration accounted for only 47%. The assimilation efficiencies were higher compared to the POM treatment (19% for NEA and 52 % for WM, Table 3) and uptake by bacteria was strongly promoted when tracer was offered as DOM (97% of total assimilation in NEA, 99% in WM). In Fig. 2 we present the benthic community incorporation of tracer. DOM did enter the pool of fauna carbon, albeit in relatively low amounts (3 to 20 μgC m^{-2} compared to 86 to 127 μgC m^{-2} when offered as POM under oxic bottom waters, Table 3).

The severe oxygen depletion of the suboxic treatments led to lower assimilation efficiency at both sites (Table 3, 2-way ANOVA, $p < 0.05$). The fauna in NEA sediment assimilated significantly lower amounts in the POM during suboxic conditions (on average 8% of total assimilation) than in oxic conditions (35%). WM fauna showed a large variation in uptake rates among replicates and the treatments were not significally different, which prevented solid testing (ranging from 0.4% to 13% of the OM processed for the oxic treatment and 0.6 and 3.9% for the suboxic). Both stations showed high bacteria flexibility to changing redox conditions, as reflected in the % of trace processed in the different oxygen treatments (Table 3).

	Northeast Atlantic (NEA)			Western Mediterranean (WM)		
	POM-OX	POM-SUBOX	DOM-OX	POM-OX	POM-SUBOX	DOM-OX
Bacteria Ass	272 ± 156	65 ± 11	801 ± 402	828 ± 438	695 ± 119	3179 ± 723
Fauna Ass	127 ± 2	6 ± 4	20 ± 3	86 ± 118	15 ± 14	3 ± 3
Respiration	8105 ± 562	8046 ± 4	3470 ± 418	7880 ± 2624	9210 ± 1596	2901 ± 608
Ass.eff. %	4.6 ± 1.4	0.9 ± 0.0	18.6 ± 5.8	9.9 ± 2.7	7.2 ± 0.2	52.2 ± 10.9
% processed	8.5 ± 0.7	8.1 ± 1.7	5.2 ± 1.0	8.1 ± 2.3	9.2 ± 1.6	7.4 ± 0.1
½ Life-cores	59.8 ± 4.1	61.4 ± 12.7	115.7 ± 14.0	65.0 ± 21.6	53.3 ± 9.2	140.7 ± 29.5
½ Life-bottles	54.1 ± 10.0	47.3 ± 3.4	27.5 ± 2.3	49.7 ± 2.6	73.6 ± 5.4	32.0 ± 2.7

Table 3. Deep-sea floor ecological functioning under different oxygenation in intact sediment-core incubations (average ± sd, n = 2). Fate of tracer C_{org} after 7 days incubation under oxic versus suboxic conditions in the overlying water and upper 4 cm of the sediment. POM is particulate algal material and DOM is diatom derived dissolved organic matter. Assimilation (Ass) is split into bacteria and fauna (incl. foraminifera) tracer uptake and community respiration (R, recorded in overlying water) in µgC m^{-2}

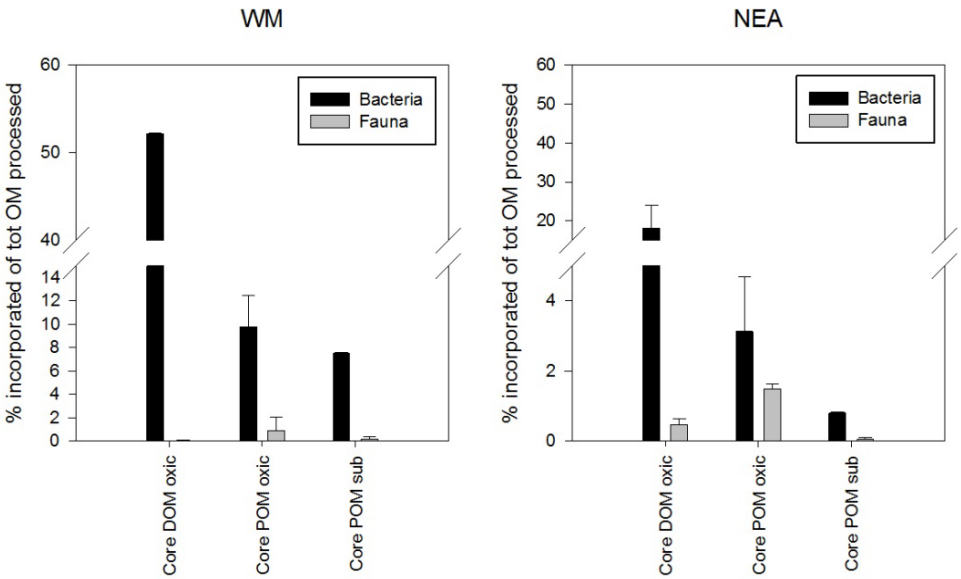

Figure 2. Percentage of added tracer OM, incorporated after 7 days incubation among the different biotic compartments (in the upper 4 cm after 7 days incubation) by the community from the western Mediterranean (WM) and NE Atlantic (NEA). Values are averages, bars are the ranges

3.3 Water-bottle incubations

A significantly larger fraction of added carbon was respired in the water oxic incubations DOM treatment (5% for NEA and 5.5% for WM) t, ANOVA, $p < 0.05$, Fig. 3) compared to the POM water oxic incubations treatment (3% NEA and 2.5% WM). Bottle DOM oxic incubation respiration at both stations was also higher than core DOM oxic incubation in the NEA (5.5% for WM and 5% for NEA vs 3.5% core POM NEA and 6% core POM WM).

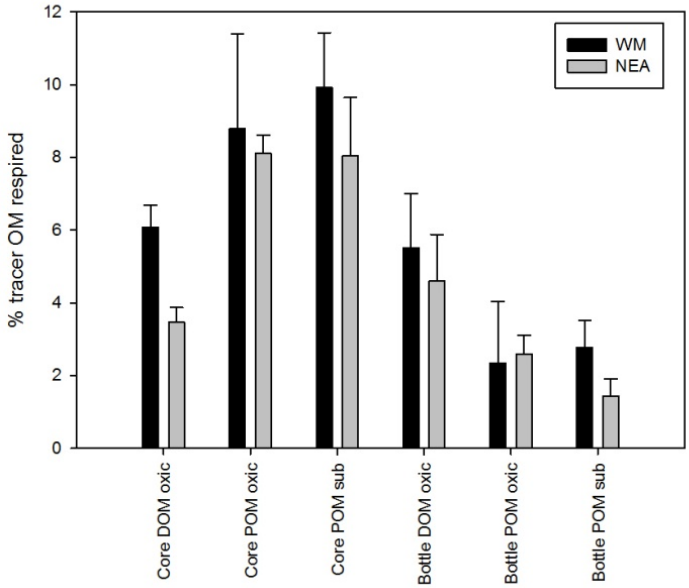

Figure 3. Percentage of added tracer OM, respired after 7 days incubation. Core is intact sediment cores with overlying water under oxic versus suboxic conditions. POM, diatom derived particulate OM. Bottle+DOM is the diatom derived dissolved organic matter incubated in 1-liter bottles with bottom water under oxic conditions. Values are averages; NEA is the NE Atlantic station and WM is western Mediterranean station

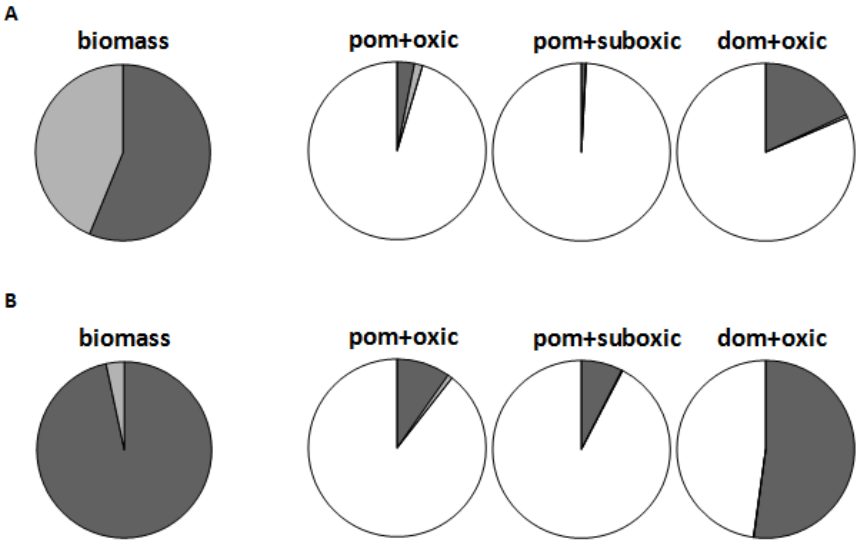

Figure 4. Average proportional division of biomass and processed carbon in sediment cores after 7 days in the different benthic compartments: dark gray is bacteria, light gray is fauna (macro- and meio-fauna including foraminifera) and white is community respiration. (A) is sediment community from NE Atlantic and (B) is sediment community from the western Mediterranean. Values are averages.

4. Discussion

Long-term studies on North Pacific and North Atlantic deep-sea settings have documented community changes and related them to variations in the phytodetritus input (Ruhl et al. 2008; Smith 1992; Smith et al. 2008; Smith et al. 2009; Soetaert et al. 1996). Studies have also been carried out on composition and structure of Mediterranean benthic communities below 1000 m depth (Laubier and Emig 1993; Sarda et al. 2004). Despite this, the effects on the deep Mediterranean fauna of homeothermia (Sarda et al. 2004; Tyler 1988; Tyler 2003) and of the oligotrophy of these waters are mostly unknown. Similarities and differences between deep Mediterranean and Atlantic fauna processing are also yet to be established and comparative studies such as ours can be valuable. Our approach involved on-deck incubation of sedimentary cores and bottom water sampled from the NE Atlantic and the Western

Mediterranean, subjected to an input of algal derived POM and DOM and oxygen manipulations, to investigate the response of the biota to different kinds of OM inputs under varying oxygen concentration. Five main observations emerged from our experiments.

First of all, there was no significant difference between the two sites (NEA and WM) with respect to the total amount of POM processed. This indicates that, on a 7 day time scale, OM recycling is not limited by low temperature, contrasting with earlier findings. Pomeroy et al. (2001), Rivkin et al. (1996) and White et al. (1991) reported limited heterotrophic bacterial activity and growth rate at low temperature at a variety of time scales, depths and habitats. Our results also disagree with the findings of Moodley et al. (2005b) who reported low respiration of 7.2 mgC m^{-2} at a slope station in the NE Atlantic (2170 m, 4°C) compared to 60 mg C m^{-2} in sediments from the deep-sea sites in the Eastern Mediterranean (1552 and 3859 m, 14 °C) in 24-hr incubation experiments. Although our experiments were performed at similar temperatures to those of Moodley, results clearly show that a similar quantity of carbon was processed in our 2 different sites in 7 days (see Table 3). This suggests that the results obtained in very short-term incubation experiments might have been biased by experimental constraints (Andersson et al., 2008). Maybe the biota did not have enough time to recover from the sampling and readjust, even when kept at *in situ* conditions.

Secondly, the community structure did not drastically alter the fate of the tracer POM, with respiration being the major component. Pfannkuche et al. (1993a) reported a different reaction to POM deposition events depending on which biotic group was examined, being it bacteria meio or macrofauna. Again Pfannkuche et al. (1993b), as well as Gooday et al. (2002) reported a strong correlation between seasonal OM input and benthic standing stock, correlating higher activity of the benthos (in particular the smaller size groups) to enhanced OM availability. These previous studies led us to expect that NEA and WM, having different benthic community would have shown differences in OM processing. While bacteria and fauna biomasses are similar at the NEA site, the biomass at the WM site is dominated by bacteria (98% of the total). Nevertheless, in both stations the majority of the OM assimilation was carried out by bacteria, with a preference for DOM over POM (Fig. 3). This suggests that in the NEA and in the WM, metazoans are less

efficient than bacteria in utilizing the OM and their contribution to the total benthic remineralisation is low when compared to bacteria, as already found by Moodley et al. (2002). Heip et al.(2001) reported >90% in biomass and about 80% in respiration dominance of microbiota in the Goban Spur and van Oevelen et al. (2011) showed a 93% respiration by prokaryotes in the Hausgarten site. Our findings are therefore in line with these studies but in contrast with what found by Witte et al. (2003a; 2003b) who demonstrated macrofaunal initial domination on OM processing in short-term (three days) experiments.

Thirdly, in our POM incubations a maximum of only 9% OM was processed (sum of respiration 8.9 mgC m^{-2} and biotic assimilation 6.1 mgC m^{-2}), in line with what was found by Woulds et al. (2009) (between 2% and 31% in 2 to 5 days), Andersson et al. (2008) (between 2 and 22% in 3.5 days) and Witte et al. (2003a) (5% in 3 days). Significantly more OM was however processed or respired when OM was amended as DOM. These results show that the quality of OM added to core incubations determines its processing. The scarcity of POM processed may also be attributed to the relatively lower reactivity of the tracer and/or to the biota's low physical accessibility to the tracer OM, as a consequence of delivering it to the surface only.

Fourth, bottom-water oxygen depletion did not significantly affect the total amount of POM processed (sum of respiration and assimilation, 8.1 – 9.2 % of the added carbon) during 7 days. However, total sediment community assimilation efficiency was significantly lower under oxygen-depleted conditions i.e. less OM was transformed into biomass in the sediment collected at these two sites. This seems to be mainly caused by a reduction in fauna assimilation (see Table 3 and Fig. 4), which in turn causes a shift towards bacterial dominated processing. This is in line with findings in previous studies (see Moodley (2005a) and references therein) conducted in naturally oxic environments. In contrast, Woulds et al. (2007) reported a strong control of oxygen concentration on OM processing in the Pakistan margin of the Arabian Sea, at sites that are naturally exposed to bottom hypoxia. It should be noted that, whereas in the short-term (up to 7 days) the benthic community seems to endure oxygen depletion, albeit showing a reduced efficiency, in the long term, suboxic conditions might result in a change in benthic structure, towards species adapting to the new situation.

Finally, the high values of tracer respiration (Fig. 3) found in the bottle DOM incubations where no sediment was present suggests that

both POM and DOM, when delivered on the sediment surface, can be utilized in the so called benthic boundary layer (BBL) by both surface sediment and bottom-water communities. Thomsen et al. (2002) confirmed that organic carbon passing the BBL during normal flow conditions is trapped, accumulated or remineralised and showed that benthic interface feeding foraminifera can scavenge the organic-rich aggregates transported in the BBL. Unless metazoan fauna surfaces to take advantage of the newly deposited food pulse and burrows it immediately in the sediment, part of it may be used by boundary-layer bacteria or resuspended under natural conditions. Our study demonstrates that water-column bacteria are similarly or even more active than sediment bacteria. Our results therefore indicate that interpretations of tracer studies need to be done with utmost care when the labeled material is delivered on top of the sediment: part of the response recorded, at least in terms of respiration, may be due to the biota living in the BBL. These findings complete and refine what was stated by Moodley et al. (2005b) who considered the sediment-water interface as "an integral part of the benthic environment and the ^{13}C-bacterial enrichment as a "true" benthic response".

In conclusion, while confirming the rapid benthic response of deep-sea sediment communities to an input of fresh OM, the outcome of our study also reveals that POM that resembles *in situ* incoming OM of low quality, may not be so rapidly recycled in deep-sea sediments (only 9%). The observed limited OM processing may be due to its low accessibility, both physical and biological. Our results corroborate the flexibility of the bacteria to changing redox conditions and highlight the altered benthic fate of tracer OM as a function of redox and tracer OM characteristics (degradability and complexity).

5. Acknowledgements

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5 Food assimilation from a mixed diatom/flagellate diet by *Cerastoderma edule* : you are what you eat…… or not?

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In preparation for Limnology and Oceanography

Abstract. *The famous quote "You are what you eat" is the working hypothesis in studies that use biomarkers to reconstruct diets. This study used feeding experiments and isotope tracing techniques to quantify carbon incorporation in fatty acids (FA) and amino acids in muscle and gut tissues from the cockle,* Cerastoderma edule*, to identify selective feeding and retention of diatom- and flagellate-derived compounds. To assess the contribution of each algal species to the cockle metabolism we used a mixture of isotopically labelled* Chaetoceros muelleri *and* Pyraminonas parkeae*, in which either of the algal species was labelled with* ^{13}C*. This technique allowed us to follow carbon assimilation of each source. Bulk tissue isotopic data showed that the animals selectively retained more diatom-derived than flagellate-derived carbon, consistent with the fatty acids incorporation and with the clearance rate data. D/L-alanine ratio enrichment showed higher labeling in muscle tissue from the diatoms than from flagellates but the gut tissues D/L-alanine incorporation was fairly similar among treatments. The analysis of individual hydrolysable amino acids showed clear differences in composition between the algae and the faunal tissues, where the faunal tissues were richer in glycine, L-glutamine, lysine and D-alanine, and depleted in L-alanine, aspartic acid and the aromatic amino acids phenylalanine and tyrosine relative to the diet. The cockle's relatively high D-alanine content is interesting, since high D/L*

alanine ratio is commonly used as a proxy for bacteria and bacterially-derived organic matter, and it proves cockle D-alanine synthesis. Analysis of individual fatty acids showed that the FA C20:5ω3, 22:6ω3, C:18, C16s present in the diet were reflected in the cockle tissues, whereas others like the C18:1ω7c, C18:3ω3 and C18:3ω4 were not present. Some fatty acids were not present in the diet (among which C13:3methyl, C22:2a, C22:2b, C22:3a and C22:3b) but were found in the animals, indicating de novo synthesis. FA labeling showed similar profiles among tissues but differences among treatments: i.e. higher values for the diatom treatment than for the flagellate treatment. In particular, high ^{13}C incorporation in C20:5ω3 indicated that, despite being found in both algae, the animal selectively incorporated the ^{13}C enriched version mainly from the diatom. The FA C22:6ω3, also present in both algae, seems instead to be retained equally from both species. Based on these findings, we conclude that cockles synthesize de novo some FA using diet-derived carbon and that different animal tissues store different compounds. The "you are what you eat" quote therefore only partly applies to cockles. Cockle metabolism produced FA and amino acids compound-selective accumulation, losses and synthesis and C. edule *were in particular capable of FA and D-alanine de novo synthesis.*

1. Introduction

Studies of animal energy budgets and growth are needed to better understand population dynamics and to optimize aquaculture profits. These studies have focused on particle concentration and feeding rates (Navarro et al. 1992), as well as on absorption and digestion efficiencies (Bochdansky et al. 1999; Cowie and Hedges 1996). The traditional way to investigate food absorption and digestion involves providing animals with a well-defined food source such as a single algal species (e.g. Cowie and Hedges, 1996). This approach has generated much of our knowledge regarding food preference and differences in assimilation efficiencies. However, in nature consumers are exposed to mixed diets and various studies have shown that mono-specific feeding experiments may give a false impression of the nutritional quality of

phytoplankton species (Schindler 1971; Schmidt and Jonasdottir 1997; Whyte and Nagata 1990).

Recent studies (Reis Batista et al., in prep) showed that a particular diet, a mixture of the diatom *Chaetoceros muelleri* and the flagellate *Pyramimonas parkeae* could be a good food combination for growth of the bivalve *Cerastoderma edule*, both in terms of their nutritional value and animal-tissue weight. It is unclear, though, why such a diet would be optimal and why the cockle seems to need both species of algae for its growth. The bivalve *Cerastoderma edule*, i.e. the common cockle, is a widespread bivalve living in the intertidal along the European Atlantic coast and a commercially exploited species for human consumption. It is ecologically important because of its abundance, its sediment reworking (i.e. bioturbation) activity, and its effects on sediment stability (Eriksson et al. 2010). Moreover, as a filter feeder, it clears the water from particles and is therefore pivotal to ecosystem dynamics including attenuation of algal blooms (Herman et al., 1999). It has been used as a model species for many studies, in particular for long and short-term rhythms of shell growth (Bourget and Brock 1990).

Much work has been done on *C. edule*'s clearance rates (CR) (Hawkins et al. 1998), feeding efficiency (Ibarrola et al. 1998), ingestion-rejection of particles and pseudofaeces production (Navarro and Widdows 1997; Urrutia et al. 1997). Cockles are known for their capabilities of food selection, discriminating between organic-rich particles (algal cells) and detritus, which normally is egested via pseudofaeces (Foster-Smith 1975; Iglesias et al. 1992; Iglesias et al. 1996; Navarro et al. 1992; Urrutia et al. 2001). Very little is known however, on the biochemical composition of their different tissues (Ibarrola et al. 2000) and what pathways dietary C and N follow once ingested and absorbed by the animal digestive gland (Ibarrola et al. 1996; Mchenery and Birkbeck 1985). To answer the first important question "why is a mixed diet needed by the cockle for its growth?" we need first to know what's the fate of the carbon ingested, what it is used for and in which tissues it is stored. We hypothesize that the bivalve uses the two algal species (*C. muelleri* and *P. parkeae*) in different metabolic pathways and transforms them into different biochemical compounds depending on storage, respiration and other needs. We also hypothesize that the necessity of this mixed diet arises from the fact that *C. edule* synthesizes *de novo* macromolecules (in particular phospholipid fatty acids -PLFAs) using carbon derived from the diet compounds.

To verify our hypotheses, feeding experiments with isotopically labelled food sources are a great tool, because they allow us to follow the labelled carbon molecules during their passage through gills and guts and their retention into organs and tissues, providing information on metabolic pathways, biochemical synthesis and carbon storage. To investigate the role of the *C. muelleri* and *P. parkeae*-derived carbon in *C. edule* and the synthesis of new biochemical compounds, we used therefore laboratory feeding experiments with a mixed diet of the isotopically labelled and unlabelled algae, analysing the biochemical composition (amino acids and PLFAs) of different cockle tissues.

2. Material and methods

2.1 *Cerastoderma edule*

C. edule is an obligatory suspension-feeding bivalve belonging to the Cardidae family. It is an edible clam commonly found in muddy and sandy tidal areas and estuaries. *C. edule* is a bivalve with a carbonate shell that encloses the animal completely. The cockle is a subsurface dweller, burrowing into the sediment and protrudes its two siphons to connect to the overlying water. A muscular foot allows the animal to move and burrow, gills protected by the mantle perform gas exchange ensuring O_2 supply and select food particles. Two separate siphons are used to pump water and food inside the gills system and pump out faeces and pseudofaees and CO_2-rich water (see Fig.1).

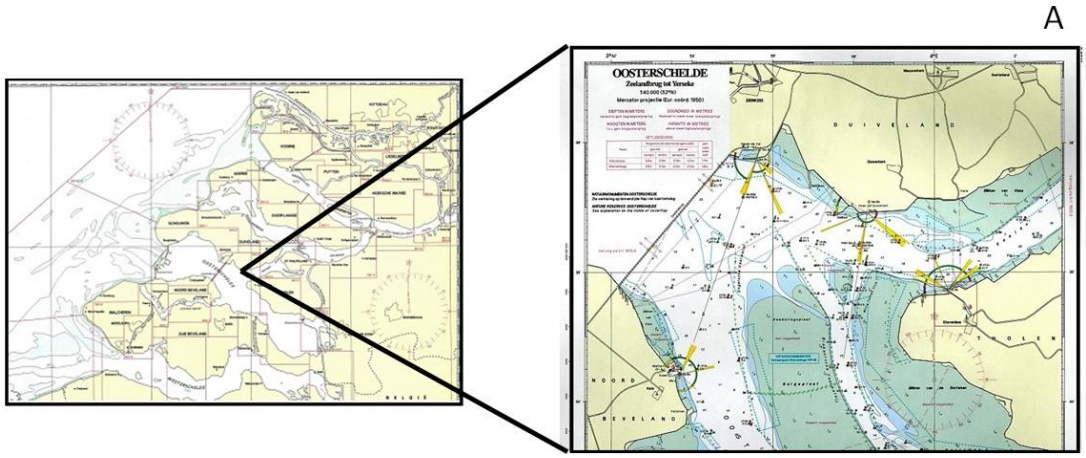

Figure 1. Location of cockle harvest (A),

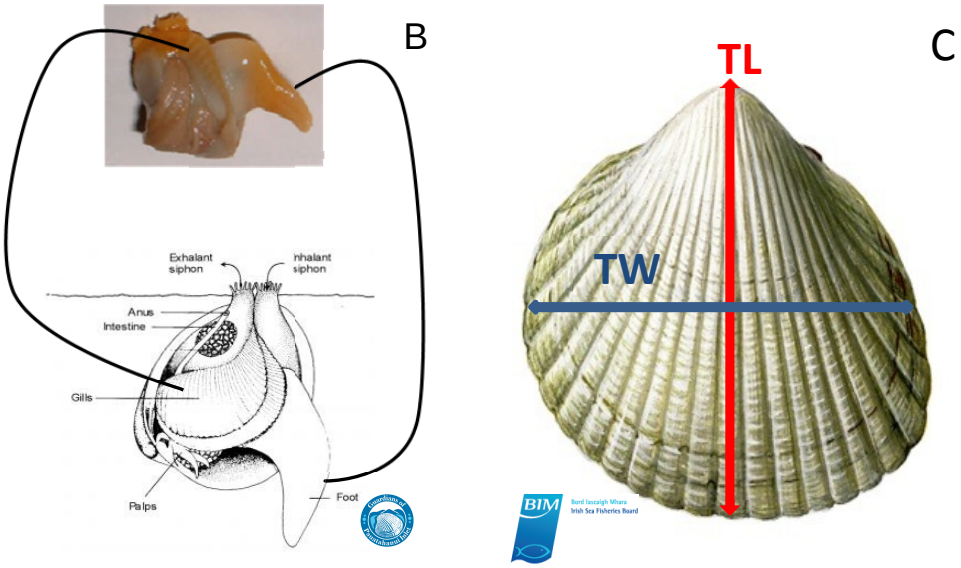

Figure 1. General schematic of the animal (B), representation of the main dimensional axis: TL= Total length, TW= Total width (C). Credits for the pictures are embedded in each panel.

Cockles were collected from the Oosterschelde estuary, the Netherlands, in the proximity of Ouwekerk at the end of June 2010 (Fig. 1). Average shell length was 27.4±2.6 mm, average total width was 33.3±3.0 mm, indicating an age of >1 year Genelt-Yanovskiy et al. (2010).

2.2 Diet

The two algal species were chosen after careful literature review (Iglesias et al. 1996; Pettersen et al. 2010) and consultation of the work done by Reis Batista et al. (in preparation).

A pilot study and literature survey determined a threshold of 10 mg/L as the critical particle concentration to induce pseudofaeces production. We therefore adopted this concentration as total amount of suspended particulate matter (SPM) to provide to the animals during the experiment, to make sure the animals were sufficiently fed. In the attempt to mimic natural conditions, the 10 mg/L SPM was composed of 5 mg/L of algae and 5 mg/L of hashed sediment that was collected from the same location as the animals.

The algal part of the diet was cultured in two different ways: ^{13}C labeled cultured algae and aquaculture-style batch cultured algae. ^{13}C *C. muelleri* and *P. parkeae* were cultured in 1.2L flasks (Erlenmeyer and Fernbach), in F2 and T+ mediums amended with ^{13}C-$NaHCO_3$ to a final enrichment of 23% and 33% ^{13}C. Cultures were kept at 16 °C with an 8 hrs dark-16 hrs light cycle. All the flasks were found clean of bacteria. Aquaculture-style batch cultured *C. muelleri* and *P. parkeae* were cultured in 10L Nalgene flasks, in Walne's medium constantly aerated with 0.2 µm filtered air in 24 hours light at 19 °C. The diatom medium was amended with 4ml/L-medium $NaSiO_3^-$ solution. Bacteria are unavoidable in these large-scale cultures.

The labeled and unlabeled algae were combined for the two mixed-diets treatments. Treatment where *C. muelleri* was labeled CM received 2.5 mg/L of aquaculture-style *P. parkeae*, 1.25 mg/L of aquaculture-style *C. muelleri* and 1.25 mg/L of isotopically enriched *C. muelleri.* Treatment where *P. parkeae* was labelled PP received 2.5 mg/L of aquaculture-style *C. muelleri*, 1.25 mg/L of aquaculture-style *P. parkeae*, and 1.25 mg/L of enriched axenic *P. parkeae.* This mixed solution was necessary due to the amount of algae needed to feed the animals which was simply too large to use a completely ^{13}C labeled diet.

2.3 Experimental setup

A total of 250 animals was manually collected during low tide, and divided among four experimental tanks with running 0.2 μm filtered Oosterschelde water. The animals were allowed to acclimatize for 2 days in the tanks and fed once a day with the unlabelled experimental diet. Twelve animals were selected randomly from the 4 tanks, and used to determined flesh wet-dry weight. The experiment was carried out in a temperature-controlled room to ensure stable conditions; the light was on only during sampling time and cleaning procedures to minimize algal and bacterial growth. The experimental tanks held 98 L during the flow-through period (21 hours) and 24 L during the feeding period. The system was connected with transparent PVC tubing and PET valves. We used two Masterflex peristaltic pumps to guarantee a fixed constant inflow of cooled, filtered, and oxygenated water (from a 120 L storage tank kept at constant 20 °C with a Lauda cooler) to the experimental tanks, at a rate of 25L/hr. Hence, the turnover time of the water in the tank is ~4 hours, in order to avoid self-poisoning of the animals. The tanks were covered only during the feeding period with fitted transparent Plexiglas lids but the system was not air tight. We decided to use 0.2 μm filtered water to reduce bacteria and algal fouling in the tanks. Each experimental tank held 50 cockles randomly selected from a total of 250 animals. Animals were fed every day at the same time (10.30 am) with two diets: tanks 1 and 2 received the CM treatment and tanks 3 and 4 received the PP treatment.

Before feeding the water level of each tank was lowered, food was delivered by hand to guarantee precision and repeatability. During the feeding period the water inlet and outlet were closed to retain food in the tanks. Animals were allowed to feed for 3 hours. Every day at the end of the feeding and after sample collection, each tank floor was siphoned to remove faeces. After faeces collection the regular flow-through system was re-established.

The experiment lasted 28 days, during which 5 samplings were carried out: T0 sampling in the first day right after entering food in the tanks, T1 after 1 week, T2 after 2 weeks, T3 after 3 weeks and T4 on the 28th day, all at the end of the feeding time. Six animals were sampled per tank each time, 3 for tissue analysis and 3 for bulk animal analysis. We chose to pool several individuals together (according to sample type) before measurement to account for animal heterogeneity.

2.4 Biochemical and isotope analysis

The three samples per tank for bulk tissue analysis were immediately separated from the shells and frozen at -20 °C. The three animals per tank for specific tissue analysis were dissected and divided, immediately upon collection, in muscle tissue (foot, mantle, no gills) and gut tissue (internal tissue contained in the sac minus the intestines) and then frozen separately at -20 °C. All samples were stored in glass vials in the freezer till the end of the experiment and then freeze-dried under vacuum. Bulk animal, animal tissues and diet samples were measured for total organic carbon (Corg) and total N, bulk ^{13}C stable isotope signatures, and the concentration and isotopic composition of phospho-lipid derived fatty acids (PLFAs) and hydrolysable amino acids. Bulk total organic carbon contents and isotope ratios were measured with a Thermo Electron Flash EA 1112 analyzer (EA) coupled to a Delta V isotope ratio mass spectrometer (IRMS) samples for both diet and cockle tissues samples.

The tracer incorporation was estimated in bulk animals and in all identifiable PLFAs. Samples were analyzed using GC-c-IRMS (Thermo GC coupled to a Delta plus isotope ratio mass spectrometer via a GC III interface) following Bligh and Dyer extraction (Middelburg et al. 2000) and $\delta^{13}C$ values were corrected for the C addition during derivatization. PLFA concentration was directly measured via GC-c-IRMS and is presented per tissue dry weight.

Tracer incorporation was also estimated for 13 individual amino acids: D and L Alanine, Threonine+Valine, Glycine, Isoleucine, Serine, Leucine, Proline, Asparagine, L-Glutamine, Phenylalanine, Tyrosine and Lysine. Samples were processed and analyzed according to Veuger et al. (2005; 2007). In summary, samples were suspended in 6 mL 2 M HCl in 10 mL glass test tubes and centrifuged (10 min at 600G). This was repeated and supernatants (containing the HAAs) were pooled and purified by cation exchange chromatography. After this, the purified samples were derivatized via evaporation at 50 °C under a gentle flow of N_2. The amino acids were then esterified by adding 500 µL isopropanol that had been freshly acidified with acetylchloride in a 4:1 ratio and heated for 90 min in a block heater at 110 °C. After esterification, the samples were acylated, cooled and further purified by solvent extraction. ^{15}N and ^{13}C incorporation into amino acids was analyzed by gas chromatography-combustion-isotope ratio mass spectrometry (GC-c-IRMS with a Thermo GC coupled to a Delta plus isotope ratio mass spectrometer via a GC III interface).

2.5 Clearance and respiration rate measurements.

Filtration or clearance rates (CR) are commonly used to indirectly determine the volume of water pumped by a filter-feeding animal (Coughlan 1969). Water samples were taken before feeding and after feeding, and analyzed directly upon collection with a Beckman coulter counter Multisizer3™ to determine particle quantity and size distribution. The use of the Multisizer3™ also allowed us to distinguish between uptake of algae and other suspended particles based on size spectra. Calculation of CR followed Coughlan (1969) and is expressed on number of cockles and on mg dry weight.

Oxygen consumption measurements were made at the end of the experiment, outside the experimental tanks in sealed perspex cylinders, 0.5L volume, using a PreSens Oxygen Dipping probe coupled to an Oxy-4 transmitter, after standard calibration. These measurements continued for a maximum of two hours and each cylinder contained 3 cockles per measurement, randomly selected from the remaining experimental population. Blanks were run as well. Oxygen concentration was measured automatically every 30 seconds and oxygen consumption rates were then calculated as the difference from initial and final concentrations. Total μmol O_2 min^{-1} and cockle O_2 min^{-1} consumption were then calculated dividing the total consumption per minute and per cockle.

2. Results

2.1 Clearance rates

The size spectrum of the two algal species clearly differed: the diameter of *C. muelleri* ranged from ~ 4 to 9 μm and *P. parkeae* from ~ 9 to 18 μm, while detritus and background noise are for the most part smaller than 2 μm (Fig. 2). Panels E and F (Fig. 2) show the particle size distribution for the two diets, while Fig.2A-D show the temporal succession of measurements. These confirm that both before feeding (Fig. 2A) and 90 minutes after feeding (Fig. 2D) no food particles were found in the water and the algal cell counts started to decrease from the moment of the food addition. These data also confirm that the 3-hours feeding time was more than sufficient to allow the animals to ingest all the food provided (Fig. 2D). CR increased with decreasing animal

number, as expected when keeping constant the particle concentration in the water (Table 1). When 50 animals were present, we find a CR of 0.4 L cockle^{-1} hr^{-1} and the CR increases to 9.1 L cockle^{-1} hr^{-1} with 12 individuals present.

	Tank 1	Tank 2	Tank 3	Tank 4	Tank 1	Tank 2	Tank 3	Tank 4
	Cockle dry weights mg				Cockle nr			
T0	2566	2363	2032	2009	50	50	50	50
T1	2989	2968	2288	2750	41	39	43	43
T2	2420	2099	1742	2418	33	16	35	36
T3	1830	1582	2024	2021	26	7	28	29
T4	771	771	897	897	6	4	10	12
	CR l mgdw^{-1} hr^{-1}				CR l cockle^{-1} hr^{-1}			
T0	0.009	0.009	0.010	0.011	0.467	0.406	0.419	0.437
T1	0.008	0.007	0.009	0.008	1.337	0.520	0.487	0.508
T2	0.010	0.010	0.012	0.009	1.661	1.268	0.599	0.606
T3	0.013	0.013	0.010	0.011	2.108	2.899	0.749	0.753
T4	0.030	0.026	0.023	0.024	9.136	5.073	2.096	1.819

Table. 1 Clearance Rates (CR) expressed per mg dry weight and per cockle per hour. T0 to T4 are the samplings. In gray treatment CM (diatom), in white treatment PP (flagellate). Dry weights are the sum of each individual weight, numbers are the sum of animals in each tank and each sampling time

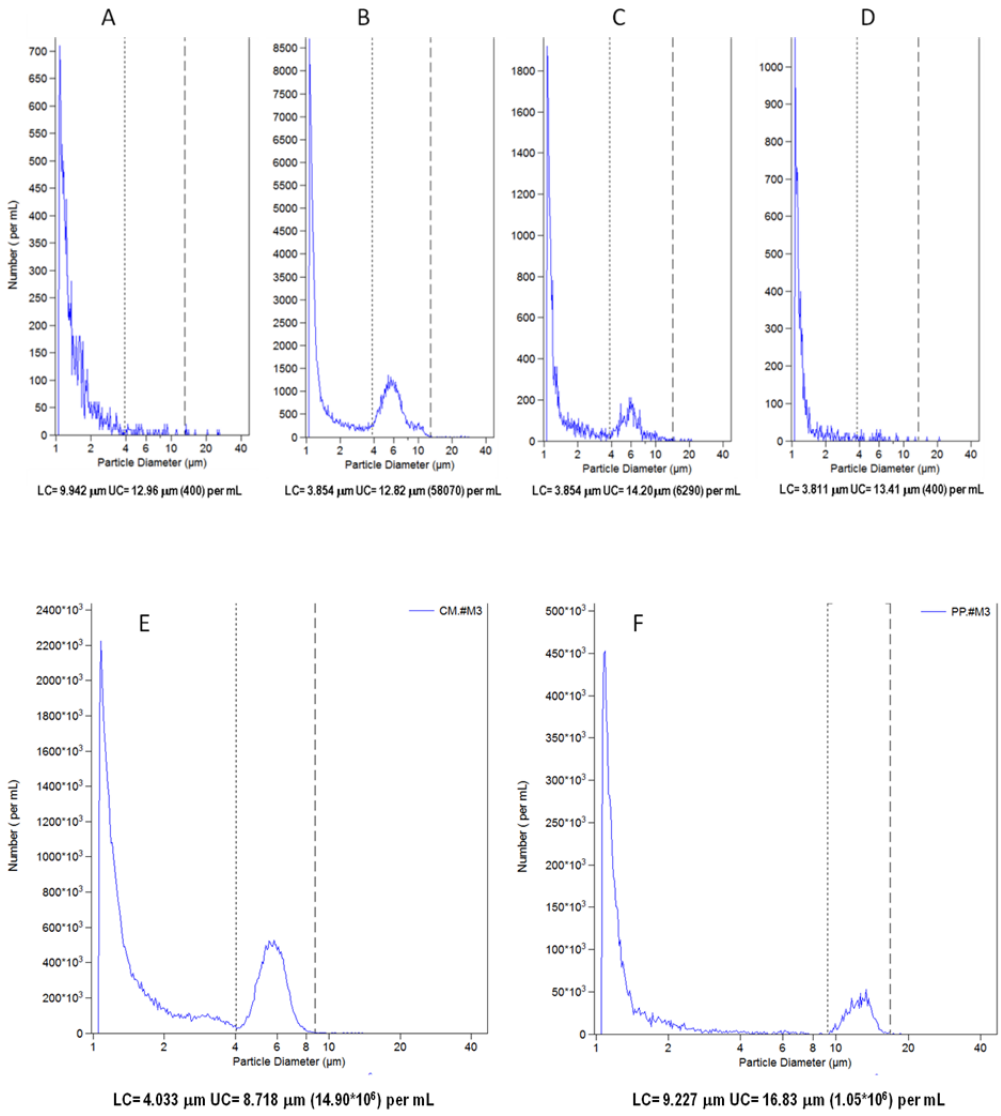

Figure 2. Particle size distribution in water during feeding (A=T0, B= 10 min after feeding, C= 45 min after feeding D=90 after into feeding) and algal cells size (E= C. muelleri and F= P. parkeae). Dashed lines mark the relevant part of the spectra, LC= lower size range UC= upper size range; in brackets particle number counted in the delimited part of the spectra. Note differences in scale among panels.

2.2 Respiration

The vitality of the cockles in our experiment was further checked with respiration rates (Table 2). Our average of 0.3 mg O_2 gdw h^{-1} is lower than the 1.8 mg O_2 gdw h^{-1} maximum value estimated by Jansen et al. (2009) for *Mytilus galloprovincialis* but within the range of the 0.2 to 1 mg O_2 gdw h^{-1} for *M. baltica*. When applying the CO_2:O_2=1:1 ratio, our values for oxygen consumption also match the respiration data of 0.2-0.9- mgC g^{-1} hr^{-1} reported by Lejart et al. (2012) for *C. gigas*.

	Total µmol O_2 core^{-1} hr^{-1}	**Total µmol O_2 cockle^{-1} hr^{-1}**	**mg O_2 per gdw cockle^{-1} hr^{-1}**
TANK 1	18.7	6.2	0.36
TANK 2	18.8	6.2	0.36
AVERAGE Treatment CM	**18.7**	**6.2**	**0.3**
TANK 3	17. 6	5.8	0.3
TANK 4	18.5	6. 2	0.3
AVERAGE Treatment PP	**18.0**	**6.0**	**0.3**

Table 2. Oxygen consumption of the cockles, measured on 3 animals per tank. Tanks one and two had treatment CM and tanks three and four treatment PP.

2.3 Phospholipid fatty acids (PLFA)

There are clear differences in PLFA profiles between the two algal species making up the diet (Fig. 3). Diatom PLFA spectra were rich in C14:0, C16:0, C16:2ω7, C16:2ω4, C16:3ω4, and C22:6ω3, and in particular in C16:1ω7c and C20:5ω3. The PLFA spectrum of the flagellate showed high abundances of C16:0, C16:1ω7c, C16:4ω3, C18:3ω3, C18:4ω3, and C22:6ω3. Moreover, the PLFAs C15:0, C16:2ω4, C18:1ω9t, C20:4ω6 were present only in the diatom whereas C16:1ω7t, C16:4ω3?, C16:3ω3?, C18:2ω6c, C18:3ω3, C18:4ω3, 18:5(ω-3,6,9,12,16) andC22:5ω3 were found only in the flagellate.

PLFA spectra of cockle tissues were dominated by C16:0, C18:0, C20:5ω3 and C22:6ω3 (average among tissues of 300 μgC gdw^{-1}, 275 μgC gdw^{-1}, 440 μgC gdw^{-1}, 250 μgC gdw^{-1} respectively), but also contained substantial quantities of C14:0 and C16:1ω7c (found also in both algae), and PLFA C13:3methyl, C22:2a, C22:2b, C22:3a and C22:3b which are unique to cockle tissue (Fig. 3).

Interestingly only gut tissues showed the presence of the marker C13:3methyl, and it is basically absent in muscle tissue. Comparing tissue and diet profiles, it appears that more than half of the markers are found only in the cockle tissues and not in the algae, while some algal biomarkers (e.g. the poly-unsaturated C18 series 18:3w3 and 18:3w2 of the flagellate and 18:2w6c and 18:3w6 of diatoms) are not present in the cockle samples. In particular, only C14:0, C15:0, C16:0, C16:1ωtc, i-C17:0, C16:2ωt, C18:0, C18:1ω9c, C20:4ω6, C21:5ω3, C22:5ω3, and C22:6ω3 were present in all the profiles.

Bulk cockle ^{13}C incorporation values were 600 μg^{13}C gdw^{-1} for CM and 380 μg^{13}C gdw^{-1} for PP. ^{13}C incorporation into PLFAs was higher in guts tissue than in muscles (maximum value of 71 vs 40 μg^{13}C-PLFA gdw^{-1}, respectively). CM treatment resulted in more ^{13}C incorporation into the muscles (40 μg^{13}C-PLFA gdw^{-1}) and guts (71 μg^{13}C-PLFA gdw^{-1}) than the PP treatment (6 and 14 μg13C-PLFA gdw^{-1} respectively in muscle and guts) (Fig. 4) consistently with the higher bulk labelling. The PLFA labelling pattern clearly differed between the two treatments: the CM treatment showed high incorporation into the saturated PLFAs C14:0, C16:0 and C18:0, mono-unsaturated PLFAs C16:1w7c, C18:1w7c and the polyunsaturated PLFAs C20:4w6, C20:5w3,

C22:5w3 and C22:6w3, as well as the C22:2s and C22:3s series. The PP treatment also resulted in enrichment of the very same saturated and mono-unsaturated PLFA (Fig. 4), but spectra were different with more C16:0, less C20:5w3 and in particular large quantities of polyunsaturated C22:5w3 and C22:6w3. There were various labelled PLFA in the cockle tissues that were not present in the diets: e.g. PLFA C13:3methyl in the guts of both treatments and PLFA C22:2a, C22:2b, C22:3a and C22:3b in muscle and guts tissues. Moreover, PLFA C22:5w3 incorporated label in all tissues and both treatment although it was not labelled in the diatoms added in the CM treatment indicating that cockle use food-derived carbon to synthesize it.

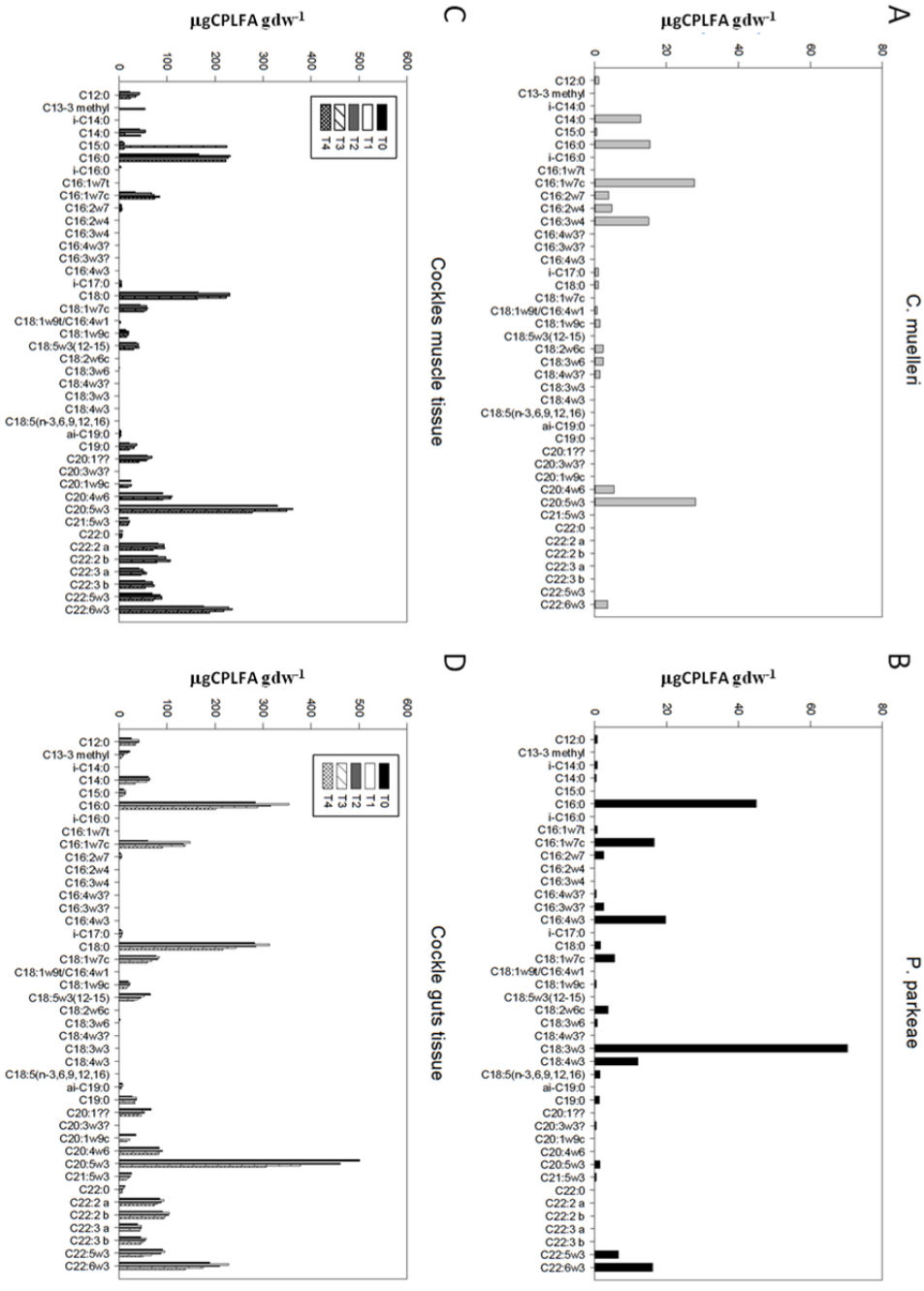

Figure 3. PLFA carbon concentrations in cockle (averaged among the two different treatments, CM and PP) tissues (c= muscle, D= guts) and in the diet (A= C. muelleri, B= P. parkeae). Numbers are related to sample dry weight. T0, T1, T2, T3, T4= sampling time steps, 1 week apart from each other. Note difference of scale between upper and lower panels. Panels A and B do not report any time-steps, representing diet composition which was fixed throughout the

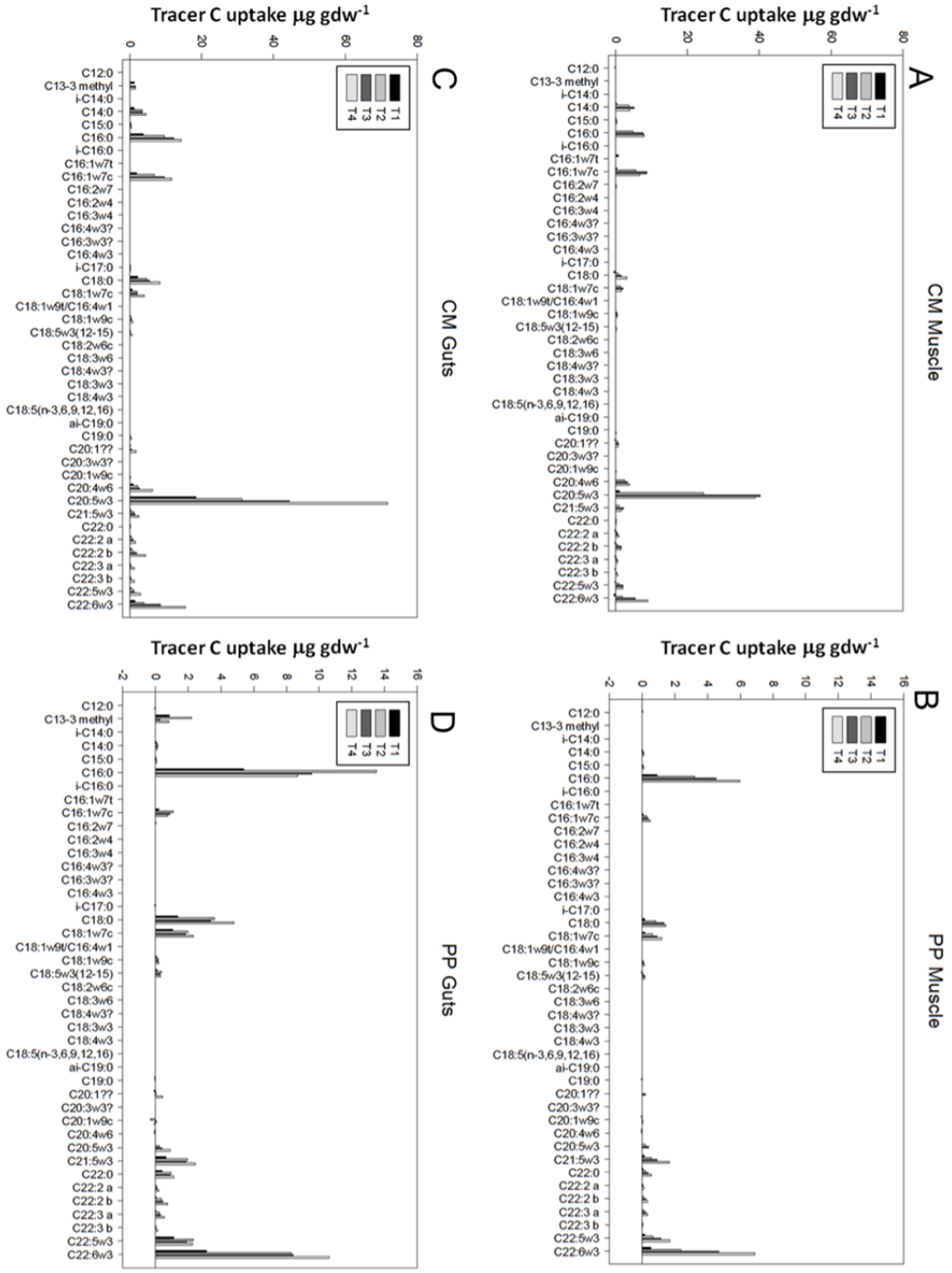

Figure 4. Tracer carbon incorporation into cockle PLFAs. Data are divided per treatment (A, C=CM and B,D= PP) and per tissue (muscle and guts). T1, T2, T3, T4= sampling time steps, 1 week apart from each other. Note difference of scale between left and right panels. Panels A and B do not report any time-steps, representing diet composition which was fixed throughout the experiment.

2.6 Amino Acids

The flagellate was richer in glycine, leucine and serine and poorer in aspartic acid and phenylalanine relative to the diatom (Fig. 5). The composition of muscle and guts tissues were very similar, but differed from that of the algae because the cockle tissues were enriched in glycine, L-glutamine, lysine and D-alanine, while they were depleted in L-alanine, aspartic acid and the aromatic amino acids phenylalanine and tyrosine.

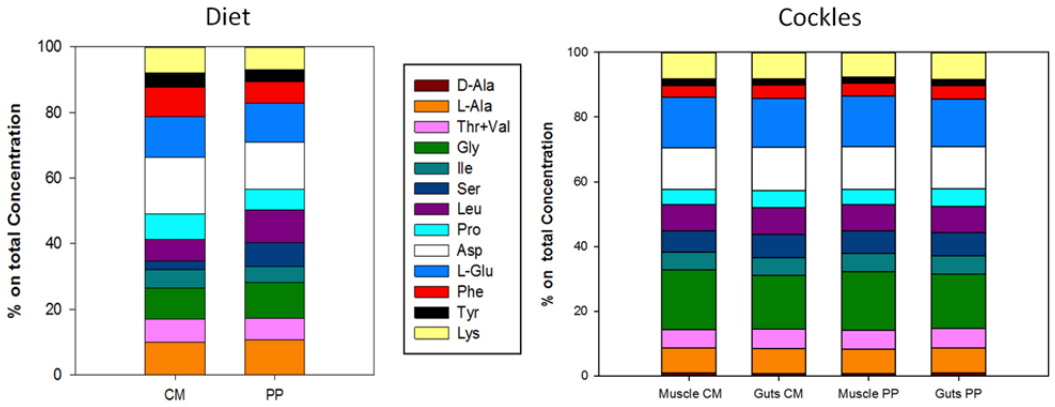

Figure 5. Amino acids profile in diet (A) and cockles (B). Coloured bands represents the concentration of the different amino acids in percentage of total hydrolysable amino acids extracted. Data are divided per treatment (CM and PP) and tissue (muscle and guts).

The relatively high D-alanine content of cockles is interesting, since the D/L alanine ratio is a commonly used tool as a proxy for bacteria and bacterially-derived organic matter. Both the algal species of the diet were devoid of the D-alanine stereoisomer with the consequence that their D/L ratio is close to the racemization background of 0.017 (Fig. 6; Veuger et al. 2007). In contrast, muscle and gut tissue samples were about 0.1, close to the highest ratio reported for bacterial cultures (Veuger et al. 2005). Isotope labelling results of amino acids showed incorporation of ^{13}C in all amino acids, with no clear difference between tissues and treatment (Fig. 6), but for D-alanine.

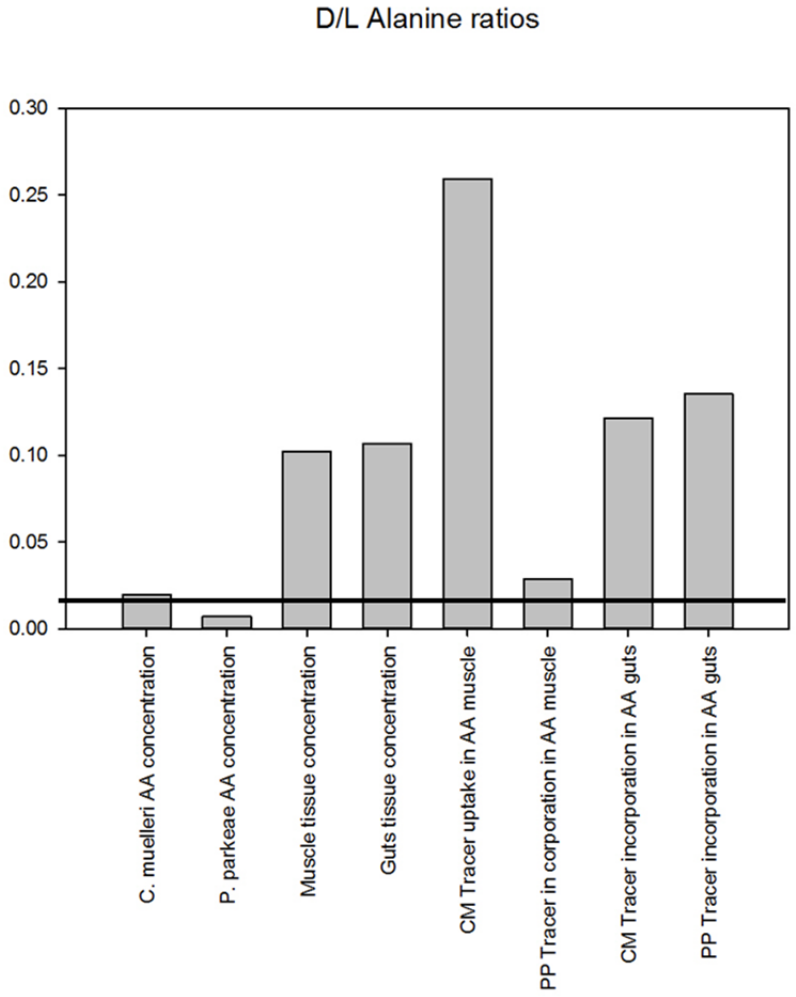

Figure 6 Diet and cockle D/L- Alanine ratios calculated on amino acids concentrations and tracer uptake. The black line represents the amino acid racemisation background value.

The D/L-alanine ratio of tracer uptake was highest for muscle tissue of the CM treatment and lowest in the muscle tissue of the PP treatment. Tracer incorporation D/L-alanine ratios of cockle's guts were similar to the D/L-alanine ratios of the amino acids tissues concentrations (Fig. 7).

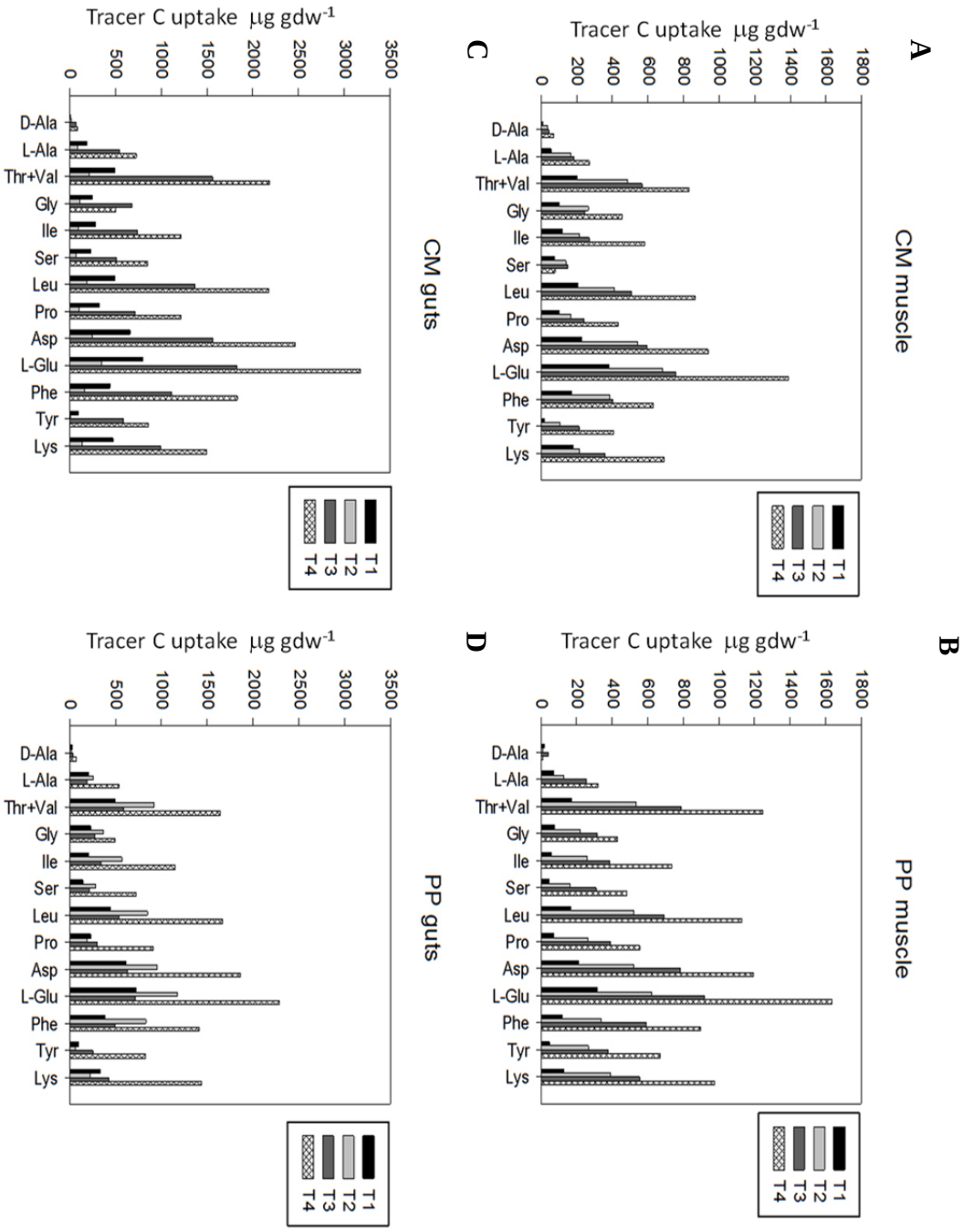

Figure 7 Tracer carbon uptake into cockle amino acids. Data are divided per treatment (CM and PP) and per tissue (muscle= A,B and guts= C,D). T1, T2, T3, T4= sampling time steps, 1 week apart from each other. Note difference of scale between upper and lower panels.

5. Discussion

The added value of our study, done using stable isotope tracers, is that we were able to trace the source of the carbon that constitutes tissue molecules. The labelling could in fact offer information on how much of the added algal-derived C enters the amino acids and PLFAs of cockles and how much carbon is instead derived from pre-existing storages. In addition, our study indicates food assimilation preferences.

Clearance and oxygen consumption rates have been measured to assess the overall fitness of the animals during the experiment and to compare our results with other studies. The data reported show that the cockles were acclimatized to the experimental setup, vital, normally active when compared with other species and other studies, and that they indeed ingested the food particles provided. Overall, the CR values are in line with the range of 0.3 to 2.7 L cockle^{-1} hr^{-1} found by Iglesias et al. (1992), the 1.8 L cockle^{-1} hr^{-1} found by Urrutia et al. (2001) and the 1.7 to 2.2 L cockle^{-1} hr^{-1} found by Widdows and Navarro (2007). This indicates that during the experiment the animals were not stressed and were feeding as in natural conditions. In our experiment no mortality was recorded in 28 days, the valves were always open to allow siphons pumping and no decrease in amino acid concentration was found. Therefore, we can assume that for the whole period the animals were never subjected to hypoxic conditions (Long et al. 2008; Soldatov et al. 2009).

5.1 Amino acids.

The amino acid composition of muscles and guts were very similar but different from the diet (Fig. 5). Spectra of ^{13}C incorporation into amino acids were also rather similar (Fig 7). Cockle tissues were depleted in L-alanine, aspartic acid and the aromatic amino acids phenylalanine and tyrosine and enriched in glycine, L-glutamine, Lysine and D-alanine. Woulds et al. (2012) studied the processing of amino acids from ^{13}C-labelled diatoms by two polychaetes and observed that the worms were enriched in glycine, valine, proline and phenyaline and depleted in L-alanine, aspartic and glutamic acids. Our results for cockles are therefore in partial agreement with results for deposit-feeding worms of Woulds et al. (2012). Valine, phenylalanine, leucine, iso-leucine and threonine are considered essential amino acids for

metazoans that need to be acquired with the diet. An accumulation of such compounds is therefore to be expected in animal tissue and such accumulation can be achieved in two different ways: via selective feeding and/or via selective assimilation or retention. Langebuch and Portner (2002) showed that amino acid (of essential or non-essential) metabolism covers almost all cellular energy demands in annelids. We expected that the non-essential amino acids were used in the animal's metabolism (Gomez-Requeni et al. 2004) whereas the essential AA were incorporated in tissues. The cockle tissues had D-Alanine concentrations up to 101 μg gdw^{-1}. D-amino acids, including D-alanine, are conventionally thought to be produced by bacteria only (Fujii 2002). D/L-alanine ratios in bacteria range between 0.04 and 0.1 (e.g. Veuger et al., 2005, 2007). The D/L-alanine ratio for cockle tissue is ~0.1, which indicates that bacterial D-alanine cannot explain our observations. Moreover, the D/L-ratios of ^{13}C-incorporation were up to 0.25 for muscle tissue, although this differed with treatment (Fig. 7), indicating that D-alanine was produced by the animals. Few studies have been conducted on metazoan D-amino acids but they have been found in annelids, mammals, insects and marine bivalves (Corrigan 1969; Felbeck and Wiley 1987; Preston 1987). Abe et al. (2005) reported that aquatic crustaceans and some bivalves have high concentrations of free D-alanine in their tissues, in particular when subjected to salinity fluctuation stress, suggesting that D-alanine is one of the major osmolytes used by the animals for intracellular isosmotic regulation. Given the intertidal habitat from which we collected the cockles, they may experience salinity fluctuations and thereby need relatively high quantities of osmolytes. The high D/L-alanine ratio fits with this reasoning. Our data clearly show that the total concentration of the D-alanine isomer is similar in both tissues but the D-alanine in the muscle tissue shows a higher concentration of diatom-derived carbon than flagellate-derived, whereas D-alanine in the guts tissue was assimilated from both algae similarly, suggesting differential and preferential use of one food source for muscle storage.

5.2 Fatty acids

Fatty acid composition of cockle muscle and guts were fairly similar among tissues but very different from the diet profiles (Fig. 3) and ^{13}C incorporation into PLFAs was similar among tissues but different among treatments (Fig. 4). Few other studies reported data on the distribution of lipids in bivalves (Jarzebski et al. 1986; Klingensmith and Stillway 1982; Kluytmans et al.

1985; Swift et al. 1980) but limited fatty acids information is available for *C. edule* (Vale 2010), therefore comparison is difficult.

In our results few PLFAs were present only in the cockles and not in the diet, in particular: PLFAs C13-3methyl, C22:2a, C22:2b, C22:3a and C22:3b and these were all ^{13}C-enriched. This confirms that at least part of the carbon used by *C. edule* to create such compounds came from the diet and that the new PLFAs were biosynthesized *de novo*. Kawashima and Ohnishi (2004) and Pond et al. (2002) reported that marine bivalves and warms can convert 18-PUFA to 20-PUFA and subsequently to 22-PUFA. C13:3methyl, C22:2a, C22:2b, C22:3a and C22:3b were present also in adult cockles of both sexes sampled directly from the field (in the same location where the experimental animals were taken) and immediately analysed, and in juvenile specimens subjected to different diets (data not shown). Therefore, the presence of these markers is diet-independent and not limited to certain age classes or sex. Moreover, our data showed that C13:3methyl presence was limited to gut tissue, while the C22:2s and C22:3s were also found in muscle tissue. This is in partial agreement with what found by Braeckman et al. (2012) for marine polychaetes, where no differences in composition between entire organisms and gutless samples was found and shows how cockles tissue composition differs from each other and from the whole organism.

Fatty acids are often used as trophic markers to unravel food-web relationships and feeding habits of heterotrophs (Budge et al. 2006; Dalsgaard et al. 2003), based on the concept "you are what you eat". Two of the most often used markers are EPA (20:5ω3), considered exclusive for diatoms, and DHA (22:6ω3) for flagellates (Braeckman et al. 2012; Dalsgaard et al. 2003). In our diet C18:0, C16:2ω7, C20:5ω3 are present in both the diatom and the flagellate albeit in different concentrations, C18:1ω7c and C22:5ω3 only in the flagellate and C16:2ω6 is absent in both algae (Fig. 3). This is in contrast with previous studies (Dijkman and Kromkamp 2006) who reported C18:0 absent from diatoms, C18:1ω7c present in both, C16:2ω7 absent in flagellates, C16:2ω6 present in flagellates and identified C20:5ω3 as exclusive for diatoms and C22:5ω3 exclusive for flagellates. This difference likely reflects differences in culture conditions, because identical analytical procedures were used.

It is believed that fatty acid composition of bivalve tissues is diet-dependent (Pettersen et al. 2010). According to Voogt (1983), PLFAs 20:5ω3 and 22:6ω3 are the predominant ones in bivalves and, together with other ω3 polyunsaturated fatty acids (Whyte 1988), have an essential role in the diet.

We cannot confirm that these two markers were directly incorporated as such from the food and then stored in cockle tissue nor that they were synthesized *de novo* using diet-derived C but the former possibility is unlikely, given that is energetically more efficient to incorporate already made compounds than producing them from scratch. Our data though show that where some FA like C20:5*ω*3, 22:6*ω*3, C18:0, C16's are present in the diet and in the cockle tissues, some others like the C18:1*ω*7c, C18:3*ω*3 and C18:3*ω*4 are not found in the consumer, while other compounds not present in the diet (such as the C20:a,b and C22:a,b) are instead found in the animals. Furthermore, when considering relative abundances of single FA, diet composition is not reflected proportionally (i.e. in a 1:1 ratio) in the tissues. The data we report in Fig. 3 show a higher concentration of C20:5ω3 in the diatom than in the flagellate and the ^{13}C incorporation reported in Fig. 4 shows different labelling of the compound among treatments, spiking higher in the CM than in the PP. This indicates that despite finding the same compound in both algae, the animal selectively incorporates the ^{13}C enriched version from the diatom and confirms its use as trophic transfer marker, with the caution that it is not only a diatom marker. C22:5*ω*3 enrichment values are fairly equal among treatments which is unexpected because the marker is present only in the flagellate.

Based on our findings, we can conclude that some PLFAs are produced *de novo* by *C. edule*, created using carbon molecules obtained preferentially from one food source or another. This corroborates our hypothesis that cockles use carbon derived from different diet sources to build different biochemical compounds that are stored preferentially in certain tissues. Therefore we can say that the "you are what you eat" quote is not entirely applicable to cockles, but that, in a rather flexible manner, they modify their food in order to remain what they are. They can create the compounds they need starting from a range of molecules. The enrichment profiles show that independently from which carbon source they used, cockles do build the same PLFAs, but in different concentrations. This means that as flexible as they might be, cockles have a limit in what they can metabolically achieve starting from their food sources, and that there are compounds they necessarily have to synthesize. In the formulation of the diet, three orders or magnitude more diatom cells than flagellate were required to match the carbon requirement of cockles, because the diatoms were smaller than the flagellates. This suggests a preferential incorporation of diatom-derived than flagellate-derived carbon, which is also consistent with the CR data, reporting that the diatoms were cleared faster than the flagellates.

Our study shows some limitations to the use of FA as trophic markers and our results are in agreement with what reported by Braeckman et al. (2012), who say that the applicability of biomarkers to assess the diet of higher trophic levels is constrained by the *de novo* biosynthesis and breakdown that marine animals are capable of.

6. Acknowledgements

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6 DISCUSSION

This thesis contributes to the understanding of benthic processes and carbon cycling by focusing on 3 main topics:

1. studying the effect of oxygen availability on organic matter degradation, transformation, preservation and composition.

2. using ^{13}C labelled algae to trace the involvement of bacteria, protists and metazoans in phytodetritus and dissolved organic matter processing, assimilation and transformation.

3. investigating bivalve use and transformation of algal diet.

Tracers and Biomarkers

This thesis offers an overview on what kind of data can be obtained by the combination of tracers and biomarkers and how these tools can broaden our understanding of carbon processing, storage and respiration.

The research presented here used algal and algal-derived tracers, obtained by laboratory cultured strains grown in ^{13}C enriched medium, according to Moodley et al. (2002; 2005b; 2011). Whereas in the first three studies we used diatom-derived DOM (dissolved organic matter) and POM (particulate organic matter), in the last one we used living diatoms and flagellates. This shows the broad application of stable isotopes and their suitability to be used in a variety of situations, adapting to the experimental necessities, together with natural ^{13}C abundances (Calvert et al. 1995; Cowie et al. 2009; Kurten 2010). Despite analysis costs and the fact that they need to be incorporated into a carrier (in this case food particles), stable isotopes are still the best tool to study this type of processes, both qualitatively and quantitatively (Fry 2006; Peterson and Fry 1987). Selecting the right carrier is usually straightforward, more

complicated is finding the right incorporation protocol. Once these issues have been solved though, ^{13}C can be traced back into single biochemical compounds, bulk tissue samples, faecal material, respired CO_2 and carbonate structures, making stable isotopes a universally applicable tool (Middelburg et al. 2000; Moodley et al. 2005b; Van Oevelen et al. 2006a; Van Oevelen et al. 2006b).

The biomarkers used in this thesis are mainly Phospholipid-derived fatty acids (PLFA) but D/L-alanine and to a certain extent amino acids can also be considered biomarkers (Veuger et al. 2005; Veuger et al. 2007; Woulds et al. 2012) . The strength of PLFAs is that they are membrane lipids and therefore they are a constitutive part of each cell. This makes it so that once the cell dies and bursts, PLFAs are degraded, so they cannot be present long after cell death (Boschker and Middelburg 2002). This also implies that they are part of the organism and not just attached to the tissue or coming from gut material. PLFAs results are therefore very useful in tracing metabolism, carbon assimilation and biomass (Braeckman et al. 2012; Van Oevelen et al. 2011; Van Oevelen et al. 2006b; Veuger et al. 2012). These studies also show that they are extremely useful in quantitative research, providing reliable data for carbon budget quantifications and food processing (Van Oevelen et al. 2006a). PLFAs can also offer information of food preferences and biochemical synthesis, as shown in chapter 5.
D-alanine is commonly used as bacterial biomarker in the sediments (Veuger et al. 2005; Veuger et al. 2007) and the D/L-alanine ratio as a diagenetic proxy for bacterial-derived molecules (Vandewiele et al. 2009). Amino acids have been largely used as tools to quantify organic matter degradation in the sediment (Dauwe et al. 1999; Vandewiele et al. 2009) and also to determine alteration of organic matter (OM) during macrofaunal guts passage (Cowie and Hedges 1996; Woulds et al. 2012).

Effect of oxygen and temperature on sedimentary metabolism

Bottom-water dissolved oxygen concentrations can exert a strong control over metazoan biomass (Levin et al. 2009a; 2009b; 2003; 2000; Stramma et al. 2010) and OM processing (Andersson et al. 2008; Cowie and Levin 2009; Woulds et al. 2007). Gooday et al. (2009) and Jeffreys et al. (2009a) also reported how oxygen levels strongly influenced the taxonomic

composition of all faunal groups and therefore played an indirect role in controlling OM quality, both in the water column and at the seafloor. Moodley et al. (2011) discussed these findings: their 7 days on-deck incubations performed with intact sediment cores from the Arabian Sea OMZ sediment amended with ^{13}C labeled phytodetritus and oxygen manipulation showed evidence that tracer organic matter assimilation (by bacteria and fauna) and respiration was evident and similar under both oxic and suboxic treatment, and demonstrated that the benthic response was not hindered by severe oxygen depletion. This was true for the Arabian Sea setting as well as for the NE Atlantic and Western Mediterranean.

The results presented in this thesis are in agreement with Moodley et al. 2011 findings and provide further evidence that, whereas biodiversity inside the OMZ is lower than outside, biomass values of the different groups are similar but most importantly carbon processing rates are higher inside the OMZ than outside. In agreement with previous studies (Levin et al. 2009b; Pedersen 1995; Woulds et al. 2009), locally adapted fauna such as *Linopherus* sp. thrived in oxygen depleted settings and, together with foraminifera and bacteria, was the major group in biomass and organic carbon processing

Temperature has also been found to be an important factor in determining benthic community structure and carbon processing. Pomeroy et al. (2001), Rivkin et al. (1996) and White et al. (1991) reported limited heterotrophic bacterial activity and growth rate at low temperature at different time scales, depths and habitats. Moodley et al. (2005b) reported lower respiration rates at a slope station in the NE Atlantic (2170 m, 4 °C) than in the deep-sea sites in the Eastern Mediterranean (3859 m, 14 °C).

Our findings disagree with these previous studies and our experiments, carried out in the NE Atlantic and Western Mediterranean, showed that a similar quantity of carbon was processed in our two different sites (1900 m 4 °C versus 13 °C) in 7 days and that the different community structures did not drastically alter the fate of the tracer POM, which was dominated by respiration. Pfannkuche et al. (1993b) and Gooday et al. (2002) reported a strong relation between seasonal OM input and benthic standing stock, explaining higher activity of the benthos (in particular

the smaller size groups) to higher OM availability. Based on the results of these previous studies, we expected that NEA and WM would have shown differences in OM processing, having different benthic communities.

Despite this, in both stations the majority of the OM assimilation was carried out by bacteria. This suggests that both in the NEA and in the WM, metazoans are less efficient than bacteria in utilizing the OM and their contribution to the total benthic remineralisation is low when compared to bacteria. Our results corroborate what already found by Moodley et al. (2002), Heip et al. (2001) who reported >90% in biomass and about 80% in respiration dominance of microbiota in the Goban Spur and van Oevelen et al. (2011) who showed a 93% respiration by prokaryotes in the Hausgarten site (Fram Strait Greenland Sea, west of Svalbard).

Effect of OM type on sedimentary metabolism

In the settings we investigated we could show that in 7 days neither oxygen concentration, nor temperature, nor benthic community structure solely governed organic matter processing, with respiration being everywhere the major fate and oxygen-depleted setting fauna being more active than in normal oxygenated settings. Still, in some areas OM accumulates. The Arabian OMZ is a perfect example of OM accumulation and for years researches debated on the reasons why this accumulation happens. Demaison and Moore (1980) were among the first to suggest that oxygen depletion was responsible for organic matter (OM) accumulation in sediments, due to inefficient carbon processing and the lower oxidative power of anaerobic degradation pathways. This assumption has been debated: Calvert et al. (1995) and Pedersen and Calvert (1990), argued that other phenomena such as high organic matter delivery and sediment texture and dilution by other sedimentary components control accumulation of organic matter in sediments, rather than water-column anoxia. Our results also contrast with the oxygen depletion theory. Hedges and Keil (1995a) suggested that other, non-oxygen related factors such as OM quality and sorption to the inorganic mineral matrix were the primary causes for the OM enrichment in OMZ

sediment. Smallwood et al. (2000; 1999) and Jeffreys et al. (2009a) agree that mega, macro and meiofauna in the OMZ core affect OM quality by metabolic modification of the food they ingest, stripping away the more labile compounds and leaving behind poorer OM. This might be the case and our results indicate that inside the OMZ more OM processing is carried out than outside, but still OM accumulates. Wakeham et al. (2002) report that mid-water column fluxes do not reflect a steady degradation of OM along the Arabian Sea water column, implying that the material that sinks is already too refractory to be further remineralized.

If oxygen, temperature and biota are not responsible for a reduced organic matter remineralisation, then it is likely the quality or the form in which OM deposits on the sediment that hinders its processing.

Our experiments, conducted using DOM and POM in three different settings could provide some more information on this issue. The POM processing pathway involves metazoans ingesting and processing the organic matter in particulate form and consortia of bacteria degrading POM to DOM. The DOM processing pathway involves the microbial community that incorporates the DOM directly or after hydrolysis. We could show that in the Arabian Sea OMZ the POM was incorporated by all benthic groups as already shown by other studies carried out in the Indian and Pakistan margin of the OMZ using labeled phytodetritus (Andersson et al. 2008; Woulds et al. 2009). The DOM however, could be used only by bacteria and the other groups could not take any direct advantage from it. When comparing the values among treatments, our data suggest that the different biotic compartments relied on different food resources and that the DOM pathway is somewhat cut-off. The POM incorporation that we saw in the different benthic groups reflected direct uptake of the tracer rather than food web transfers. Other studies (Hoste et al. 2007; Iken et al. 2001; Vanreusel et al. 1995) pointed to the possibility of a transfer of OM from bacteria to nematodes via direct predation but our results did not confirm such hypothesis. The DOM incorporation data presented here show that OM transfer along the food chain, as fresh supplied DOM ingested by bacteria, in turn grazed upon by fauna, might not have been as important as the direct feeding on OM, be it POM or DOM, and that bacteria were not a principal food source at

least in the short term, in line with what was found by Guilini et al. (2010). Our results from the NEA and Western Mediterranean also show that significantly more OM was however processed or respired when OM was amended as DOM. These results show that the quality of OM added to core incubations determines its processing. POM that resembles *in situ* incoming OM of low quality, may not be so rapidly recycled in deep-sea sediments and the observed limited OM processing may be due to its low accessibility, both physical and biological. DOM is simply not used by the whole community, which causes an even longer processing time.

Effect of fauna on OM quality

Besides environmental conditions and constraints we saw that ultimately food is a very important factor in biotic metabolism. The famous quote "You are what you eat" has been the common conclusion of different studies on faunal biochemical composition. But is it always the case? Chapter 5 provides interesting insights on the fact that some animals like the common cockle, are rather flexible and do modify their food in order to remain what they are. Cockle metabolism masters FA and amino acids compound-selective accumulation, losses and synthesis and *C. edule* are in particular capable of specific FA and D-alanine *de novo* synthesis, as shown for other marine animal species like polychaetes. Our results are in line with what was shown by Woulds et al. (2012) and Braeckman et al. (2012) regarding macrofauna OM processing and degradation and also agree with previous work done by Jeffreys et al. (2009b) and Smallwood et al. (1999) that report on the importance of mega, macro and meiofauna in OM quality determination. Moreover, our results show some limitations to the use of FA as trophic markers to assess the diet of higher trophic levels, as it is constrained by the *de novo* biosynthesis and breakdown that marine animals are capable of, but the stable isotope labelling can offer further information on how much of the OM enters the fauna specific compounds (such as PLFAs and amino acids) and how much carbon comes instead from pre-existing storages.

The four studies presented in this thesis offer a view on biotic carbon processing, respiration and storage. Spanning from an oxygen minimum zone to well oxygenated and diverse deep-sea settings we could provide information on some mechanisms as well as quantitatively represent carbon cycling. Much more research is needed in order to fully

understand the dynamics of deep-sea benthic communities, their relation to the incoming OM and their potential for remineralization. I also think that many assumptions that endured till now regarding fauna metabolic requirements might be imprecise. In particular for nematodes, our data suggest that either all the previous and present work provided tracers that were not suitable to specifically label them, or their metabolism is much slower that what is currently believed. We demonstrated that in 7 days in the OMZ nematodes did not prey upon bacteria, implying that carbon consumed by bacteria is respired and not transferred up the food chain. We also showed that even when feeding directly on POM, nematodes never get highly labelled, suggesting either limited assimilation efficiency or very low feeding rates. Despite our attempts, nematodes are still elusive species and more dedicated work should be carried out in order to unravel their ecological importance. Our approach and understanding of megafauna also needs some improving. As our last study demonstrates, some assumptions commonly used regarding dietary needs and compound synthesis are not always valid and animal metabolism still escapes our full understanding.

I also believe that tracer experiments should evolve further, developing new methods for *in situ* studies. The limitations due to costs, water depth and pressure make it difficult to study the deep sea *in loco*, controlling experimental conditions manipulating oxygen and nutrients concentrations is almost impossible. But I strongly believe that these limitations are only technicalities that can be overcome developing new tools, prototypes and investing more funds in research and ship time.

SUMMARY

This thesis aimed at investigating deep-sea benthic ecosystems and biotic organic matter processing, using the powerful stable isotope tracer approach. Incubation and feeding experiments, together with compound-specific analysis and respiration measurements could offer an overview of benthic ecosystem functioning and resource allocation in bacteria, protozoans and metazoans.

In **Chapter 2** we investigated the effects of oxygen depletion on algal-derived particulate organic matter processing by sediment biota in the Arabian Sea OMZ. Our data clearly showed that fresh organic matter (OM) was processed and respired by the community in a very short time period (7 days). This study confirmed faunal abundance and activity, inside the OMZ, despite the scarcity of oxygen. Within the OMZ, the large polychaete *Linopherus sp.* dominated the community's tracer uptake (up to 17%), followed by bacteria and meiofauna. Outside the OMZ, the macrofaunal foraminifera's processing of fresh OM almost matched the bacterial one (in natural oxic conditions), even if values remain very low. Our experiments showed that subjecting samples from within the OMZ to higher oxygen seemed to decrease the uptake and therefore the activity in particular for meiofaunal nematodes and all the macrofauna. This indicated that OMZ communities are adapted to low oxygen conditions. Respiration in the OMZ station was higher than in the station outside the OMZ, In particular, in the latter respiration was higher in the manipulated suboxic treatment than in the natural oxic one, suggesting that the different groups had to process more OM to be able to incorporate less organic C. The evidence provided shows that the limited food supply and OM content of the sediment of the outside OMZ station are to be considered the major cause for the faunal low activity in terms of total uptake, biomass turnover and relative uptake when compared to OMZ sediment, not the oxygen supply. Moreover, relatively to the total amount of OM offered, the uptake and processing by the benthic community inside the OMZ was higher than outside the OMZ, once more demonstrating that inside the OMZ low oxygen does not impair the community functioning and demonstrating that the community inside the OMZ is more active that the community outside.

In **Chapter 3** we focussed on OM form and its role in benthic OM degradation and respiration. OM processing occurs along two pathways: the particulate organic matter (POM) pathway involves metazoans ingesting and processing the organic matter in particulate form and the bacteria degrading OM particles to dissolved organic matter (DOM); the DOM pathway involves the bacterial community that incorporates the OM directly. OM accumulates in oxygen minimum zones and we hypothesized that the main cause is that these two lanes are somewhat impaired. We specifically traced both pathways to assess if and where the malfunction happens, to ascertain which group(s) might be responsible for such lack of OM degradation and to identify what are the causes. Our results showed that: 1) fauna, protists and bacteria were present in both stations 2) short term OM processing in terms of uptake and respiration was recorded, with processing rates of the OMZ biota being comparable to oxygenated open slope and abyssal sediment, 3) there was an evident separation in DOM and POM processing pathways, 4) in 7 days no direct DOM derived-C transfer up the food chain due to predation occurred and bacteria seemed not to be a main food source for metazoans, 5) faunal activity in oxic sediment subjected to suboxic conditions was very low. Biota were active inside the OMZ as demonstrated by our data; short term processing of both POM and DOM was carried out in similar quantities to those of other settings. Therefore, oxygen-induced inactivity of biota cannot be claimed as the main cause for the OMZ organic matter accumulation. This led us to conclude that the probable cause for the OMZ OM accumulation must be the highly refractory nature of the OM delivered to the sediments.

In **Chapter 4** we investigated the OM processing of the deep benthic community of North Eastern Atlantic and Western Mediterranean, to assess similarities and differences. The environmental conditions of the two sites differed greatly: the Atlantic was colder (4 °C) and fauna dominated, the Mediterranean was warmer (14 °C) and bacteria dominated. Our results showed that there was no significant difference between the two sites with respect to the total amount of POM processed. This indicated that, on a 7-day time scale, OM recycling was not limited

by low temperature, contrasting with earlier findings. The community structure, different at the two sites, did not drastically alter the fate of the tracer POM, with respiration being the major component. In both stations in fact, the majority of the OM assimilation was carried out by bacteria, with a preference for DOM over POM. This suggested that, even when present in high biomass, metazoans were less efficient than bacteria in utilizing the OM and their contribution to the total benthic remineralisation was minimal. Significantly more OM was processed or respired when OM was amended as DOM than as POM. These results showed that the form of OM added to core incubations changed its processing. Bottom-water oxygen depletion did not significantly affect the total amount of POM processed. However, total sediment community assimilation efficiency was significantly lower under oxygen-depleted conditions. Lastly, unless metazoan fauna surfaced to take advantage of the deposited OM pulse and burrowed it immediately in the sediment, part of it may have been used by benthic boundary-layer (BBL) bacteria. Our water-only incubations demonstrated that water-column bacteria were similarly or even more active than sediment bacteria. This implied that part of the response recorded, at least in terms of respiration, may have been due to the biota living in the BBL.

In **Chapter 5** we examined macrobenthic processing of OM and in particular food-derived carbon allocation in tissues and respiration. Several studies dealt with marine animal energy budget and growth but we focused on a qualitative approach to investigate what a bivalve can really do with the molecules that compose its diet. We hypothesized that the bivalve uses different algal species in different metabolic pathways and transforms them into different biochemical compounds, depending on its needs. We also hypothesized that the necessity of a mixed diet arises from the fact that *C. edule* synthesizes *de novo* macromolecules (in particular phospholipid fatty acids -PLFAs) using diet- derived carbon. To investigate these hypotheses we run experiments with wild-caught cockles in thermo-controlled basins feeding the animals a mixture of diatoms and flagellates, cultured in ^{13}C labelled medium. The labelling allowed us to follow carbon as it entered the animal tissues and was incorporated in other compounds or respired. Total hydrolysable amino acid profiles were fairly similar among tissues but very different from the

diet and D/L- alanine ratios labelling changed according to treatment and tissue. Phospholipid-derived fatty acids (PLFA) were also examined in tissue samples and diets. Tissue profiles show 5 markers that have never been described earlier in cockles: C13-3methyl (found only in muscle tissue), C22:2a, C22:2b, C22:3a and C22:3b: these PLFAs are not found in the diet. Only few PLFAs were present in the diet and also in muscle and guts tissue of the cockles: C20:5ω3, 22:6ω3, C18:0, C16's. Some others like the C18:1ω7c, C18:3ω3 and C18:3ω4 were not in the consumer, and other compounds not present in the diet (such as the C20:s and C22:s) were instead found in the animals. Based on these findings, we could confirm that some PLFAs were produced *de novo* by *C. edule* and that cockles are very flexible in their PLFA metabolism. The differences in PLFA labelling profiles also showed preferential incorporation of diatom-derived than flagellate-derived carbon. These results contribute to changing our opinions regarding the famous quote "you are what you eat": I'd rather say "you are what you make of your food". This study also confirmed previous investigations reporting that macrofauna, and not only bacteria, can produce high levels of D-amino acids, probably used for osmotic regulation, especially in species subjected to high osmotic stress (i.e. in a tidal environment). We could also show the key role of fauna in OM quality determination via ingestion of food and excretion of biochemically modified, lower quality OM.

SAMENVATTING

Dit proefschrift richt zich op het verwerken van organisch materiaal in benthische ecosystemen in de diepzee gebruik makende van stabiele isotopen als biomerkers. Voedsel manipulatie experimenten zijn in combinatie met component specifieke isotoop analyses en respiratie metingen gebruikt om het integrale functioneren van het benthische ecosysteem te onderzoeken en de competitie voor voedsel tussen bacteriën, protozoa en meercelligen te bestuderen.

In **hoofdstuk 2** zijn de effecten van zuurstof beperking op de verwerking van phytodetritus door benthische organismen in de zuurstof minimum zone (Oxygen Minimum Zone, OMZ) van de Arabisch Zee onderzocht. Het blijkt dat vers organisch materiaal (OM) verwerkt en gerespireerd wordt binnen 1 week. Deze studie laat ook duidelijk zien dat lage zuurstofgehaltes in het bovenliggend bodemwater niet noodzakelijk de abundantie en activiteit van bentische fauna beperkt. De polychaet *Linopherus sp.* domineerde de merker opname in OMZ sedimenten, met een assimilatie bijdrage van 17%, gevolgd door bacteriën en meiofauna. Onder normale zuurstof condities droegen foraminiferen en bacteriën gelijkwaardig bij aan OM verwerking. Een toename van zuurstof in het bodemwater onderdrukte de activiteit van dieren (meio- en macrofauna) uit de OMZ. Benthische gemeenschappen in de OMZ zijn dus aangepast aan lage zuurstofgehaltes.

OM komt in particulaire (POM) en opgeloste (DOM) vorm voor. **Hoofdstuk 3** richt zich op het effect van de specifiek vorm van OM op afbraak en consumptie. De OM verwerking gebeurt langs twee wegen: particulair organisch materiaal wordt door meercelligen en eencelligen geconsumeerd, terwijl alleen de bacteriële gemeenschap direct opgelost organisch materiaal kan consumeren. OM hoopt zich op in zuurstof arme zones en we veronderstelden dat de belangrijkste oorzaak is dat deze twee wegen enigszins worden aangetast. We traceerden specifiek beide routes om te beoordelen of en waar de storing gebeurt, en om na te gaan welke groep(en) verantwoordelijk kunnen zijn voor een dergelijk gebrek aan OM verwerking. Onze resultaten laten zien dat fauna, protisten en bacteriën aanwezig zijn en functioneren in zowel zuurstofrijke als zuurstofarme stations en dat er een duidelijke scheiding is tussen DOM en POM verwerking. Er was geen substantiële transfer van opgeloste organisch materiaal via bacteriën naar meercelligen door middel van de “microbial loop” en predatie. Het bleek dat zuurstof-geïnduceerde inactiviteit van biota niet kan worden geclaimd als de belangrijkste oorzaak voor de OMZ organische stof accumulatie

De verwerking van OM door de benthische gemeenschappen van de Noord-Oost-Atlantische Oceaan en het westelijke Middellandse Zeegebied is onderzocht in **hoofdstuk 4** om overeenkomsten en

verschillen te beoordelen. De milieuomstandigheden van de twee locaties verschilden sterk: het sediment ecosysteem in de Atlantische Oceaan was koud (4 °C) en fauna gedomineerd, dat in de Middellandse Zee was warm (14 °C) en bacteriën gedomineerd. Onze resultaten laten zien dat er geen significant verschil is tussen de twee locaties wat betreft de totale hoeveelheid POM die wordt verwerkt. Dit geeft aan dat, op een 7 - daagse tijdschaal, de OM recycling niet werd beperkt door de lage temperatuur, in contrast met eerdere bevindingen met kortere incubatieduur. Ondanks verschillen in de structuur van de benthische gemeenschappen was de verwerking van het POM vergelijkbaar, met respiratie als belangrijkste term in het budget. Bovendien werd op in beide stations het merendeel van de OM assimilatie uitgevoerd door bacteriën, met een voorkeur voor DOM. Dit suggereert dat, zelfs indien aanwezig met hoge biomassa, meercelligen minder efficiënt waren dan bacteriën in het benutten van het OM. De bijdrage van fauna aan de totale benthische mineralisatie was minimaal. Bodemwater zuurstofdepletie had geen significante invloed op de totale hoeveelheid POM die verwerkt werd.

De focus van **Hoofdstuk 5** lag op de verwerking van organisch materiaal door de kokkel. De werk hypothese was dat deze tweekleppige verschillende algensoorten gebruikt in verschillende metabole routes en ze transformeert tot verschillende biochemische verbindingen, afhankelijk van de nutritionele behoeften. Om deze hypothese te onderzoeken werden kokkels in thermo-gecontroleerde bassins gevoed met een mengsel van diatomeeën en flagellaten, gekweekt in 13C-rijk medium. Het gebruik van 13C als merker liet ons toe om koolstof te volgen wanneer dit opgenomen werd in het dierlijke weefsel en vervolgens werd verwerkt in andere verbindingen of verbruikt voor respiratie. Totaal hydrolyseerbare aminozurenprofielen waren vergelijkbaar tussen weefsels maar zeer verschillend ten opzichte van algen. Deze studie bevestigt eerdere bevindingen dat macrofauna, en niet alleen bacteriën, D-aminozuren kunnen produceren. Waarschijnlijk worden deze D-aminozuren gebruikt voor osmotische regulatie en zijn ze nodig om in een getijden omgeving te overleven. Fosfolipide-afgeleide vetzuren (PLFA) werden onderzocht in weefselmonsters en diëten. We hebben 5 PLFAs gevonden die nog nooit eerder voor kokkels gerapporteerd zijn: C13-3methyl (alleen te vinden in spierweefsel),

C22:2a, C22:2b, C22:3a en C22:3b. Deze PLFAs waren niet aanwezig in de algen. Slechts enkele PLFAs waren aanwezig in de voeding en ook in de spieren en de ingewanden van de kokkels: C20:5ω3, 22:6ω3, C18:0, C16's. De verschillen in PLFA ^{13}C profielen toonden preferentiële incorporatie van diatomeeën-afgeleide koolstof ten opzichte van flagellaten afgeleide koolstof. Deze resultaten suggereren dat het beroemde citaat "je bent wat je eet" beter aangepast kan worden tot "je bent wat je zelf van je voedsel maakt".

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I fell in love with marine biology when I was a child of four, spending hours in front of the TV watching J. Cousteau's documentaries and being deeply moved by a movie, *Orca: The Killer Whale* by Michael Anderson. My big dream was to become a marine biologist.

Now that this dream becomes real, that I made it to the top of this steep hill and I get my prize, I feel the urge to look back and sideways and thank all the people that supported me and pushed me forward. Because "Winter is coming", as George R.R. Martin says, and "sic transit Gloria mundi", which was whispered into the ear of victorious Roman generals during their parade of triumph, to remind them that all earthly success is fleeting.

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