GEOLOGICA ULTRAIECTINA

Mededelingen van de

Faculteit Geowetenschappen

Universiteit Utrecht

No. 277

Assessing the fate of organic matter in subtidal sandy sediments using carbon and nitrogen stable isotopes as deliberate tracers

Victor Evrard

ISBN: 978-90-5744-142-4

Assessing the fate of organic matter in subtidal sandy sediments using carbon and nitrogen stable isotopes as deliberate tracers

Stabiele isotopen van koolstof en stikstof als merkers voor de transformaties van organisch materiaal in subtidale zandbodems

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht, op gezag van de rector magnificus prof.dr. W. H. Gispen, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op maandag 27 augustus 2007 des ochtends te 10.30 uur

door

Victor Pierre Edouard Evrard

geboren op 9 april 1976 te Parijs, Frankrijk

Promotoren: Prof.dr. J.J. Middelburg

Prof.dr. C.H.R. Heip

Co-promotor: Dr. K. Soetaert

This thesis was supported by the European Union (COSA project, EVK#3-CT-2002-00076) and the Netherlands Organisation for Scientific Research (PIONIER 833.02.002)

A la mémoire d’Agalion,

Contents

Chapter 1
General Introduction ... 1

Chapter 2
Nutrient dynamics of seagrass ecosystems: ^{15}N evidence for the importance of particulate organic matter and root systems ... 13

Chapter 3
Tracing incorporation and pathways of carbon and nitrogen in microbial communities of photic subtidal sands ... 23

Chapter 4
The pivotal role of benthic primary producers in moderating carbon and nitrogen flows through food webs in sandy sediments ... 43

Chapter 5
Relative significance of phytodetritus deposition and microphytobenthos for the heterotrophic community of a shallow subtidal sandy sediment. ... 63

References ... 89

Summary ... 107

Samenvatting ... 111

Acknowledgments ... 115

Curriculum vitae ... 119

Chapter 1
General Introduction

1.1 Marine sediments

Shelf sediments can be described according to many criteria. However, most studies use definitions based on geological or biogeochemical characteristics, depending on the issue at hand. For example, according to Hal (2002), sediment typology can be easily categorised with regard to grain size and origin into mud, sand, rock/gravel, coral and, shell deposits. Alternatively, Middelburg et al. (2005) propose a classification from a biogeochemical point of view and make a distinction between (i) steadily accreting silty and muddy sediment, (ii) highly mobile, silty, and muddy sediments (fluidized bed reactors), (iii) non-accumulating or eroding sediments (bypass zones), and (iv) sandy, permeable sediments. These shelf sediments can also be divided into two groups: those that receive enough light to sustain photosynthesis, the photic sediments, and those that do not, the aphotic sediments. This list of definitions is far from being exhaustive and mainly serves to illustrate the various topic-based approaches used to study the shelf environment.

It is known that a large fraction of global ocean photosynthesis occurs in the coastal area and that coastal primary producers thus contribute substantially to the primary production of the biosphere (Field et al. 1998). While the relative contribution of benthic and pelagic primary production in coastal systems may be difficult to assess, Gattuso et al. (2006) demonstrate that benthic primary production may take place on more than 33% of the global shelf area, suggesting that the contribution of benthic primary production may have been previously underestimated. Bound together by perpetual feedback systems, benthic primary producers, bacteria and benthic fauna are important, not only biogeochemically, but also ecologically, because the benthos represents a substantial source of energy for demersal species of shrimps and fish, and also for birds (Reise 1977,

Edgar & Shaw 1995a, b, Bertness & Leonard 1997). These trophic interactions confer on benthic environments various value added benefits (Hall 2002).

1.2 The benthic community structure

The benthic community is a complex system that can be simplified by considering a trophic structure made of different resources and consumers (Fig. 1.1)

1.2.1 The resources: primary production and external subsidies

The microphytobenthos (MPB), which consists of the microscopic photosynthetic organisms present at the sediment surface, contributes significantly to the shelf primary production in various areas of the globe, such as intertidal areas (MacIntyre et al. 1996, Barranguet et al. 1998, Underwood & Kromkamp 1999) or subtidal areas (Jahnke et al. 2000, Glud et al. 2002). The term MPB usually refers to benthic microalgae (BMA), which primarily include Bacillariophyceae (diatoms) and Chlorophyceae (i.e. green algae), and also phototrophic prokaryotes, the cyanobacteria. These photosynthetic micro-organisms constitute a significant fraction of the sediment vegetation in terms of biomass, production and with respect to area covered (Duarte & Cebrian 1996, Middelburg et al. 2005, Middelburg & Soetaert 2005). Cahoon (1999) estimated a global benthic microalgal production of 500 Tg C yr^{-1}. The importance of MPB to the benthic community functioning is threefold: it influences nutrient, oxygen and carbon dioxide exchange at the sediment surface; by forming a biofilm it can play a role in the stability of the sediment by enhancing the cohesion of sand grains (Miller et al. 1996, Middelburg et al. 2000); and, it constitutes a significant resource for the heterotrophic organisms (Miller et al. 1996, Herman et al. 2000).

In addition to the MPB, other benthic photosynthetic organisms contribute to the global primary production of the shelf. Macrophytes, which include macroalgae and phanerogams (seagrasses), are responsible for up to ~5800 Tg C yr^{-1} (Duarte & Cebrian 1996, Duarte et al. 2005). However, macroalgae mainly colonize rocky shores and thus contribute only modestly to the global primary production of the shelf. The fate of macrophytes is mainly export and burial (Duarte & Cebrian 1996). Similarly, salt marshes, mangroves, and terrestrial organic matter through river discharge, can have an important influence on the organic matter content of shelf sediments (Mann 1988). Altogether, these different compartments of coastal primary producers constitute a resource for benthic fauna and bacteria.

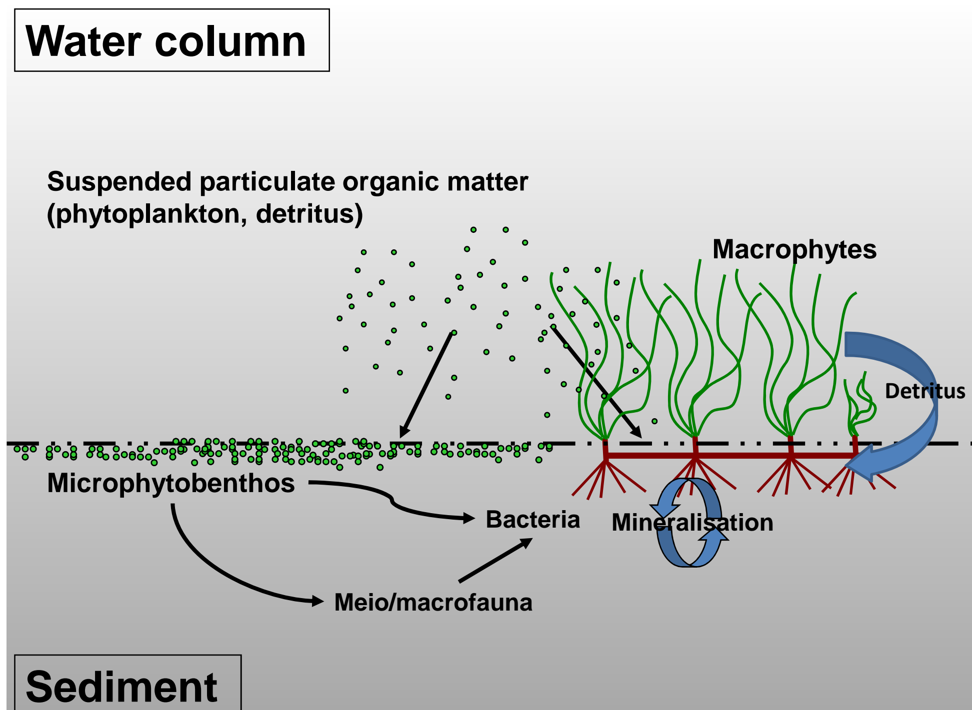

Figure 1.1. Origin and fate of organic matter in the photic sediment. The production of organic matter by the microphytobenthos and macrophytes occurs in or on the sediment and supports the benthic fauna (meiofauna and macrofauna) and bacterial secondary production. In addition, settling particulate organic matter can contribute to the benthic organic matter.

In addition to autochthonous production, depending on locations and hydrodynamic conditions, marine shelf sediments can receive substantial loads of suspended particulate organic matter (SPM). Settling SPM consists mostly of pelagic detritus and phytoplankton but also MPB following resuspension by currents (de Jonge & Vanbeusekom 1995). While settling SPM constitutes the only significant resource for deep sea sediment communities, it also represents a significant input in coastal areas. SPM, which represents the major fraction in the diet of benthic suspension feeders, also largely contributes to the diet of deposit feeders in tidal estuaries (Heip et al. 1995, Herman et al. 1999). However, this can also be the case for subtidal permeable sediments where the action of currents and waves create advection of porewater loaded with organic particles into the sediment (Precht & Huettel 2003, Wild et al. 2004, Huettel et al. 2007).

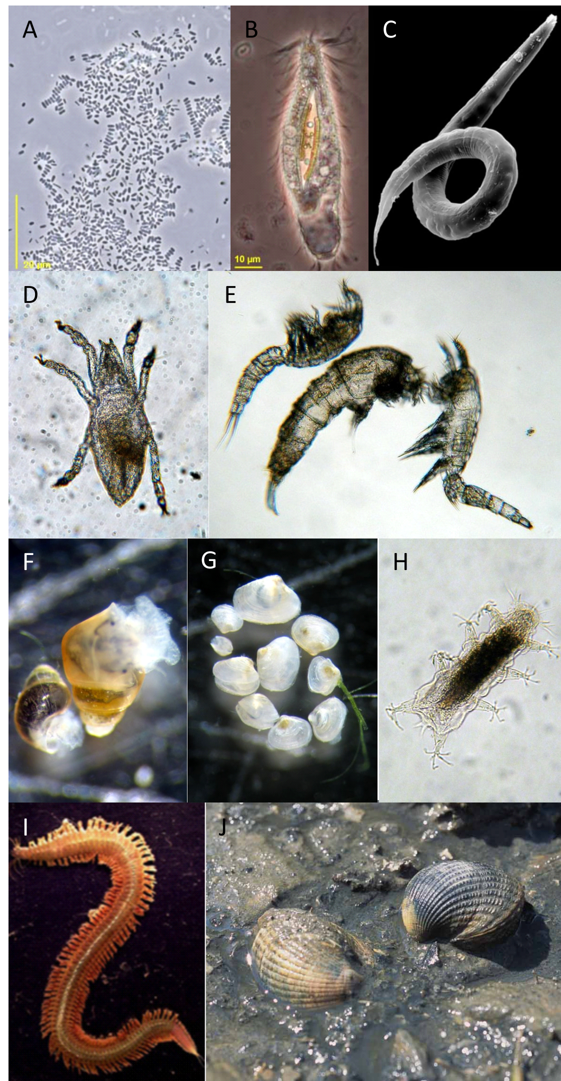

Figure 1.2. The benthic heterotrophic compartment is composed of (A) bacteria, (B) ciliates, (C) nematodes, (D) halacarids, (E) harpacticoid copepods, juveniles stages of (F) gastropods and (G) bivalves, (H) tardigrades, (I) polychaetes and cockles (J).

1.2.2 Heterotrophic community

The benthic heterotrophic community living in the sediment is composed of complex assemblages of organisms (Fig. 1.2), commonly and conveniently classified into size classes (Schwinghamer 1981).

Archaea and bacteria are the smallest microorganisms, their size ranges from 0.5µm to 4µm. The distribution of bacteria is mainly correlated to sediment grain size (Dale 1974) in the order of 10^9 cm^{-3} of sediment. Their metabolism can be either anaerobic or aerobic. Bacteria substantially depend on primary production, as phytodetritus or photosynthesis-produced carbohydrates represent their main substrate (Goto et al. 2001, Rusch et al. 2003).

The microfauna, which usually refers to protozoan (e.g. flagellates and ciliates), includes microorganisms usually larger than bacteria. Their size is variable but they are normally smaller than ~60 µm and they show densities in the order of $\sim 10^3$ cm^{-3}. They rely significantly on bacteria as their food source (Fenchel 1978, Sherr & Sherr 1987).

The meiofauna constitutes an important fraction of the interstitial fauna biomass of the sediment, with very variable densities (in the order of $\sim 10^6$ m^{-2} of sediment). The meiofauna is composed of microscopic organisms that cannot be handpicked as they are too small to be seen with the naked eye. They are commonly separated from other fractions of the sediment on a 38 µm mesh size sieve. The most abundant and omnipresent taxonomic groups include nematodes, copepods, foraminifera and ostracods. They show different trophic behaviours with numerous deposit feeders, suspension feeders or bacterivorous species. The role of meiofauna in benthic environments is manifold: it enhances mineralisation, directly through grazing (Coull 1999) but also by stimulating bacterial activity through bioturbation and pre-processing fresh organic matter (Gerlach 1978, Aller & Aller 1998); it provides a significant source of food for various species of crustaceans and fish; and, it serves as an indicator of environmental changes and levels of pollution, and thus can serve as a convenient tool for assessing impacted environments (Coull & Chandler 1992, Warwick & Clarke 1998).

Finally the macrofauna, which consists of most the largest organisms living on or in the sediment, includes all metazoans retained on a 1 mm mesh size sieve. Macrofauna density and biomass are found to be negatively correlated with sediment grain size in the North Sea (Heip et al. 1992). However, this cannot be generalised as most macrofauna studies have been carried out in temperate regions of the globe or in very specific ecosystems, leaving for instance the situation in tropical regions poorly documented. Macrofauna species, which represent a major source of food for higher trophic levels (e.g. crustaceans, fish and birds), show the same variety of trophic interactions as those of

meiofauna: polychaete worms, which usually live in burrow or in the sediment, show both deposit feeding and suspension feeding; bivalve species (e.g. cockles) are primarily suspension feeders, feeding on SPM while gastropods are mainly deposit feeders, feeding on microalgae. Certain species also selectively feed on one type of food. Macrofauna diversity and biomass are highly structured by food availability and quality (Herman et al. 1999). In addition, macrofauna also enhances bacterial activity through bioturbation. In summary, macrofauna shows similar characteristics to those of meiofauna but on a larger scale.

It is important to remember that the segregation of benthic organisms by size, function or morphology is purely convenient and that classifications are not exhaustive. Many organisms present characteristics of different compartments. For example foraminifera, which are usually included in the meiofauna compartment, are protist organisms with characteristics of the microfauna with some individuals large enough to be classified in the macrofauna; similarly, nematodes which are integrated in the meiofauna compartment show sizes ranging from µm to mm, characteristic of macrofauna; finally, juveniles of many macrofauna species (e.g. bivalves and gastropods) can be classified into the meiofauna compartment (temporary meiofauna) as they are extremely small and not retained on a 1 mm sieve.

1.3 Stable isotopes and biomarkers

1.3.1 Stable isotopes natural abundance

Isotopes are the various forms a chemical element can take with regard to the number of neutrons the nucleus contains. These forms have different atomic masses (i.e. the greater the number of neutrons, the heavier the isotope). Contrary to radioactive isotopes, which are unstable as their decaying nuclei lose energy through radiations of particles or electromagnetic waves, stable isotopes don't emit radiations. Heavy isotopes are rare: they represent a small percentage of the naturally abundant light ones but their chemical properties are virtually identical. Because of their identical chemical properties and their paucity, stable isotopes have many applications. For example, radioactive and stable isotopes can be used as a tracer in chemical reactions and, radiocarbon dating is a widely used technique to estimate the age of different carbonaceous materials. As stable isotopes are safe to use and relatively easy to measure (through the use of isotope ratio mass spectrometry, IRMS), they represent an important tool for ecologists and biogeochemists. Hydrogen (^{1}H, ^{2}H), carbon (^{12}C, ^{13}C), oxygen (^{16}O, ^{17}O, ^{18}O), nitrogen (^{14}N, ^{15}N) and sulphur (^{32}S, ^{33}S, ^{34}S, ^{36}S) are the most commonly analysed stable isotopes. Isotope

abundances (commonly referred to as "isotopic signatures") can be presented in terms of atom percent. For example, atom percent $^{13}C = [^{13}C/(^{12}C+^{13}C)]\times100$. In addition, the absolute abundance can be quantified by the isotope fraction $F = ^{13}C/(^{12}C+^{13}C)$. Normally, isotopes are presented in terms of their isotope ratio ($R = ^{13}C/^{12}C$) because IRMS measurements provide isotopic ratios rather than abundances. As most isotopic measurements are performed to describe isotopic signature differences and those differences are significantly small (typically beyond the third decimal figure), the conventional delta notation (δ) is generally used. The δ notation is defined in per mille (‰), relative to a standard ratio as follow: $\delta_1 = [(R_1 - R_S)/R_S]\times1000$, where R_1 is the ratio of the sample being measured for a stable isotope (e.g. ^{13}C or ^{15}N) and R_S is that of the standard. For example, $\delta^{13}C$ is expressed relative to the Vienna-Pee Dee Belemnite (VPDB) ratio of NBS-19 calcite ($R_S = R_{VPDB} = 0.0111797$) and $\delta^{15}N$ relative to the ratio ($^{15}N/^{14}N$) of the air ($R_S = R_{Air} = 0.0036765$).

The use of stable isotopes in ecology is based on the precept "you are what you eat", i.e. the isotope value of the consumer reflects that of the resource. Bearing in mind that chemical reactions involved in the transfer of chemical elements from substrate to product may shift the isotopic ratio by a few per mille, it is possible to infer sources and consumers in trophic ecology by comparing their isotopic values. This shift, commonly known as fractionation, is mass-dependent and hence varies more or less depending on the chemical element. For primary producers during carbon fixation for example, the fractionation from their inorganic C source can be significant (~20 ‰ for C3 plants) (Peterson & Fry 1987, Dawson et al. 2002). However, fractionation between primary producers and their consumers is usually negligible (< 1 ‰). Nitrogen stable isotope signatures ($\delta^{15}N$) can be used to derive trophic levels and elemental flows within the food web (Phillips 2001, Post 2002) as a steady 3-4 ‰ fractionation is found between resource and consumers (Deniro & Epstein 1981, Minagawa & Wada 1984). In general, stable isotopes have proven to be very helpful in the investigation of elemental flows in various ecosystems (Peterson & Fry 1987, Ben-David et al. 1997, Ponsard & Arditi 2000, Carman & Fry 2002). However, when the number of potential sources for a consumer increases, mixing models do not allow for an unequivocal differentiation between these sources and it is then necessary to take into account more stable isotopes (Fry & Sherr 1984, Peterson & Fry 1987, Peterson & Howarth 1987). In many studies though, it appears that the number of sources is greater than the number of stable isotopes that can be measured. It is possible to constrain the number of unknowns by looking at additional variables (e.g. stomach content investigation) or determine a range of solutions (Phillips & Gregg 2003). However, an alternative, complementary and more powerful way of investigating elemental flows within and through food webs is through the addition of stable isotopes as tracers.

1.3.2 Tracer experiments

Deliberate tracer experiments consist of adding enriched inorganic (e.g. ^{13}C-bicarbonate or ^{15}N-nitrate) or organic (e.g. ^{13}C-labelled diatoms) material directly to the environment of interest. This method allows one to follow the fate of the enriched material through the entire range of biologic compartments present in that environment. As the level of labelling of these compartments will be relative to the affinity for their substrate, it increases the resolution of the different elemental pathways from the source to the consumers and successive trophic levels. The level of labelling is usually expressed through the specific uptake (noted $\Delta\delta$, in ‰) which is the δ value difference between the enriched sample and that of the background (unlabelled). $\Delta\delta_1 = \delta_1 - \delta_0 = 1000\times(R_1 - R_0)/R_S$, where δ_1 correspond to the δ value of the sample 1, δ_0 is that of the background sample, R_1 and R_0 are the ratio of sample 1 and the background respectively and, R_S is the ratio of the standard.

In this thesis, a more precise notation, originally proposed by Maddi et al.(2006) is adopted. The enrichment, noted δ^E (in ‰) describes the level of labelling as the difference of the ratio between one sample and the background, relative to the background, as follows: $\delta^E = 1000\times(R_1 - R_0)/R_0 = [(\delta_1+1000)/(\delta_0+1000)-1]\times1000$. In trophic ecology, these notations provide quantitative data with regard to the affinity of certain organisms or chemical reactions for certain resources or substrates.

However, when biomasses of the different compartments are available, it is possible to derive accurately the flows of chemical elements through the calculation of the isotope incorporation (I) as I = E×biomass, where E is the excess label (e.g. ^{13}C or ^{15}N). $E = F_1 - F_0$, where F_1 and F_0 are the fractions in the sample and the background respectively. The biomasses typically investigated in trophic ecology are those of the different consumers. Some compartments are microscopic (MPB, bacteria) and it is not possible to physically separate them. However, some microbial biomarkers allow assessing microbial composition, biomass and, through compound specific isotope measurements, the elemental incorporation (Boschker & Middelburg 2002, Veuger et al. 2005). Ultimately, if the initial isotope dilution of the source is known, it is possible to estimate the total transfer of organic matter to and through the different biologic compartments.

As stated earlier, there are different ways to investigate organic matter flows. It is possible to label an autochthonous resource such as MPB *in situ* via ^{13C}-bicarbonate labelling (Middelburg et al. 2000) or label some organic material (e.g. microalgae) in cultures beforehand and then add it to the environment (Blair et al. 1996, Moodley et al. 2000, Witte et al. 2003). These isotope tracer techniques can also be modified and combined. For example, Herman et al. (2000) used differential labelling (^{13}C-labelled

MPB and ^{15}N-labelled phytoplankton) to assess the relative importance of MPB and phytoplankton.

Contrary to radioactive isotopes, stable isotopes are convenient as they are safe for the researcher and for the environment and can thus be used in the laboratory as well as *in situ*. Altogether, natural abundance and tracer studies have provided unique and crucial ecological information, particularly in the field of food web studies. Research combining the investigation of the natural abundance of stable isotopes and stable isotope tracer experiment is regarded as deserving the "golden spike award" (Fry 2006).

1.4 Rationale and thesis layout

Stable isotope approaches have been widely used in the marine environment to investigate food web ecology. Shelf sediments are important as they are biologically active and potentially contributing significantly to global biogeochemical cycles. A review of the literature shows that research interests have been mainly concentrated on the most active silty/muddy environments. Also, emphasis has been on intertidal mud flats, estuaries and coastal lagoons, probably for practical (logistic) reasons (Hentschel 1998, Hughes et al. 2000, Middelburg et al. 2000, van Oevelen et al. 2006b, van Oevelen et al. 2006c). These tidal environments, which are dominated by fine sediments, undergo long periods of emersion providing enough light to sustain a significant primary production (Underwood & Kromkamp 1999). In contrast, sandy sediments, many of which are subtidal, constitute ~70% of the shelf sediment (Emery 1968). They have often been mistakenly regarded as poorly active and biogeochemically limited due to their low organic content (Boudreau et al. 2001) and, have received limited attention (Steele & Baird 1968, Koop & Griffiths 1982, Dexter 1992, Webb 1996). However, recent studies have highlighted the significance of sandy sediments for their potentially fast organic matter turnover (Sundback et al. 1996, Dauwe & Middelburg 1998, Huettel & Rusch 2000, Jahnke et al. 2000, Ehrenhauss et al. 2004a, de Beer et al. 2005, Buhring et al. 2006). Typically, there seems to be no relation between sediment grain size and reaction rates (e.g. respiration, Fig. 1.3). The hypothesis is that remineralisation rates are dependent on the quality of organic matter which should increase with sediment grain size and negatively correlate with its age (Dauwe & Middelburg 1998). Another hypothesis is that remineralisation occurs deeper in coarser sediments.

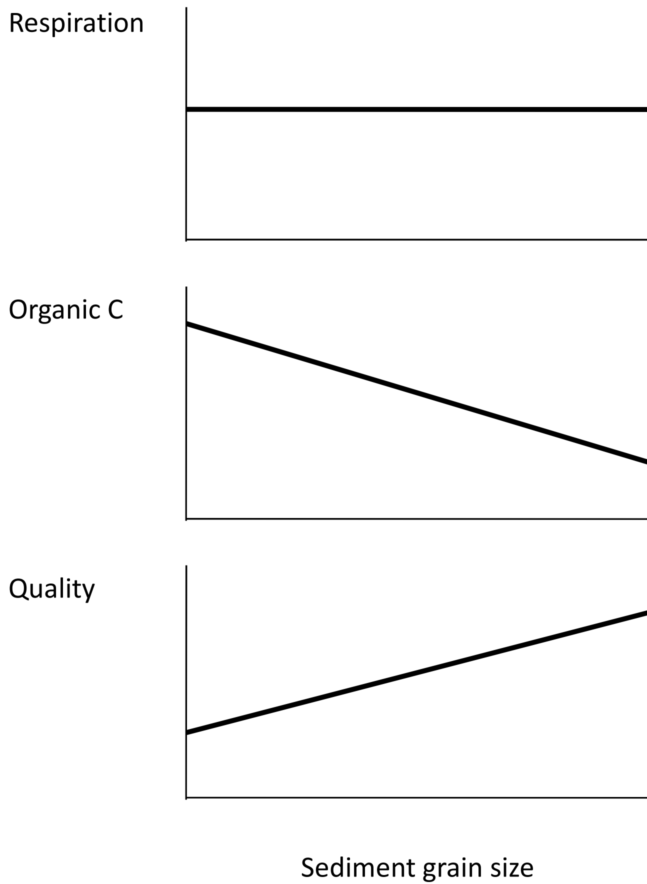

Figure 1.3. Remineralisation rates (i.e. respiration) depend on the quality of the organic matter and the quantity of organic matter. The quality of organic matter (degradability), negatively correlated with its age, is expected to increased with sediment grain size and overcome the lower quantities.

The research presented in this thesis aims to provide an updated picture of the different processes involved in carbon and nitrogen flows in subtidal sandy sediments. With a clear focus first on the significance of the microbial compartment (chapters 2 and 3) and second on the processes occurring in the food web (chapters 4 and 5), I aim to resolve several research questions:

- What contributes to the sustainability of seagrass plants in oligotrophic environments?
- How much C and N can be fixed and assimilated by the microbial compartment of subtidal sandy sediments in the photic zone?
- What is the significance of MPB production for the benthic food web?
- What is the relative significance of MPB and phytodetritus for the benthic food web of carbon-poor subtidal sandy sediment?

Chapter 2 provides evidence for the importance of the bacterial compartment of a tropical seagrass meadow. In an *in situ* setting, ^{15}N-labelled phytodetritus was injected in the rhizosphere of a subtidal heterogeneous seagrass meadow. The subsequent gradual ^{15}N-enrichment of seagrasses provided strong evidence of active recycling of organic matter and nutrient release rapidly made available to the plants. This illustrates the tight coupling between the microbial compartment and the seagrass meadow.

Chapter 3 shows, through a dual labelling experiment (^{13}C and ^{15}N), the pathways and fate of C and N through the microbial compartment of a sandy sediment. This study emphasises the various autotrophic and heterotrophic processes involved, through a thorough use of state-of-the-art microbial biomarker tools. The study highlights the fast transfers of inorganic C and N to the MPB, the significant release of extracellular polymeric substance and the bacterial heterotrophic and chemoautotrophic activities.

Chapter 4 is based on the same experiment as chapter 3 and focuses on the benthic food web structure and its reliance on C and N freshly produced by the MPB. The faunal compartments and the bacteria are investigated, and their relative biomass and contribution to the processing of organic matter are estimated. Coupled to a detailed food web survey based on the natural abundance of stable isotopes, this comprehensive study provides new evidence of the preeminence of MPB (vs. SPM) as a food source.

Chapter 5 supports the main conclusion of chapter 4. In a sandy sediment with low organic C content and thus very little detritus, the hypothesis of a greater contribution of settled phytodetritus to the benthic food web is tested. The relative contributions of MPB and phytodetritus are assessed through a dual ^{13}C-tracer approach and the results are discussed with regards to the detailed study of the ^{13}C natural abundance of the food web.

Chapter 2

Nutrient dynamics of seagrass ecosystems: ^{15}N evidence for the importance of particulate organic matter and root systems

Published in Marine Ecology Progress Series*

2.1 Introduction

Seagrass meadows are among the most efficient ecosystems in terms of the value-added benefits of the services they provide. Their ecosystem's services are estimated at about $19,000.ha^{-1} yr^{-1} (Costanza et al. 1997) and they contribute about 12% of the net ecosystem production (Duarte & Cebrian 1996), about 5% of respiration (Middelburg et al. 2005) and about 25% of carbon burial (Duarte et al. 2005) in the coastal areas. Despite the obvious importance of the high productivity of seagrass meadows, current knowledge of their nutrient dynamics remains poor. Compared to most angiosperms, seagrasses are rather inefficient in re-using their internal nutrient pool (Hemminga et al. 1999); that is, seagrasses living in a physical dynamic environment lose a lot of nutrients when senescent leaves are ripped off from their meadow. Inefficient internal recycling is a typical trait for plants from nutrient-rich environments. This raises the question as to how seagrass

---

* Evrard V, Kiswara W, Bouma TJ, Middelburg JJ (2005) Nutrient dynamics of seagrass ecosystems: ^{15}N evidence for the importance of particulate organic matter and root systems. Mar Ecol Prog Ser 295:49-55

meadows are able to maintain such a high level of productivity in oligotrophic environments.

Seagrasses can take up nutrients through both their leaves and roots. The relative importance of roots and leaves for nutrient uptake depends on a number of factors including the concentrations and availability in the water column and sediment reservoirs and can vary from leave-dominated to root uptake-dominated (Carignan & Kalff 1980). Foliar uptake supplies around 50% of the overall N requirement of *Thalassia testudinum* (Lee & Dunton 1999) and *Thalassia hemprichii* (Stapel et al. 1996); between 30 to 90% of the overall N requirement of *Zostera marina* (Iizumi & Hattori 1982, Short & McRoy 1984, Pedersen & Borum 1992, 1993); and in extreme cases, the complete N requirement of *Phyllospadix torreyi* (Terrados & Williams 1997). The assumed minor importance of roots for nutrient acquisition is remarkable considering that below-ground biomass is a major carbon investment with respect to tissue construction and maintenance. Below-ground carbon investments have even been identified as an important factor in the vulnerability of seagrasses to low-light conditions, which is one of the main reasons for the world-wide decline of seagrass meadows (Hemminga 1998). The relatively high carbon costs associated with seagrass roots combined with carbon and nutrient mass balance constraints (Erftemeijer & Middelburg 1995) raises the question as to whether current understanding of the importance of seagrass roots for nutrient acquisition is correct.

There are two reasons to re-assess the current view on nutrient cycling in seagrass meadows. Firstly, most studies on nutrient uptake by seagrass plants focus solely on inorganic nutrients and very little is known about uptake of organic nutrients (McRoy & Goering 1974, Bird et al. 1998). Consequently, we lack insight into the role of (1) dissolved organic nutrients that are usually more abundant than dissolved inorganic nutrients in oligotrophic environments and (2) nutrient recycling within seagrass meadows via microbial decomposition of POM in the water column and/or in the sediment. The organic matter that is mineralised in seagrass meadows may either be produced in the meadow (recycled production) or be imported from adjacent ecosystems (new production). A number of studies have focused on the complex biogeochemical interactions in sediments inhabited by seagrasses (e.g. Holmer et al. 1999, Holmer et al. 2001). Secondly, seagrasses often grow on sandy sediments and Huettel and co-workers (Huettel & Gust 1992b, a, Huettel & Rusch 2000, Rusch & Huettel 2000) have reported high rates of water exchange between permeable, sandy sediments and overlying water. These enhanced rates of water exchange relieve diffusive limitations of nutrient transport into the sediment towards seagrass roots and result in the transfer of small particles (including algae and bacteria) from the water column to the sediment. In the sediment, these particles may be trapped and retained depending on the relative sizes of pore spaces

and particles but also the topography of the sediment. Consequently, POM transported and trapped into sandy sediments and the subsequent regeneration and release of dissolved nutrients may be a pathway of nutrient supply to seagrasses.

The objective of our study was to asses if seagrasses may derive nutrients from organic nutrient sources such as POM that is transported into sandy sediments. Using an *in situ* isotopic enrichment experiment, we demonstrate for 5 tropical species that seagrass roots enable a rapid transfer of nutrients from degrading phytodetritus trapped in the sediment to the seagrass shoots.

2.2 Materials and methods

2.2.1 Study site

The experiment was carried out at Derawan Island (2°16’40”N, 118°16’40”E), situated in the Berau delta in East Kalimantan, Indonesia. The area closer to the Berau is strongly influenced by the river discharge, which can transport substantial amounts of terrigenous organic materials, sediment and nutrient loads. More outward in the estuary, the river influence is limited, and oligotrophic tropical coral islands are present. The vegetative cover at the experimental site at Derawan Island is a heterogeneous and sparse mix of *Thalassia hemprichii*, *Cymodocea rotundifolia*, *Halodule uninervis*, *H. pinifolia*, *Halophila ovalis/ovata* and *Syringodium isoetifolium*. The meadow was short due to grazing by the green sea turtle, *Chelonia mydas*.

2.2.2 *In situ* labelling

The experiment was carried out in a shallow, but continuously submerged, seagrass meadow. Within this meadow, a 3 × 3 m square research area was protected against turtle grazing by a 3 m high cage made out of white (i.e. visible to the turtles) fishing net (50 mm mesh size). The subtidal location allowed us to work in the cage without causing any damage to the vegetation or disrupting the sediment. Within the turtle exclosure, we equally subdivided 3 plots: 2 replicate plots for labelling and 1 plot as a control. In each plot, 6 flagged bamboo sticks were sparsely distributed.

An axenic clone of the diatom *Amphora coffeaeformis* (UTCC 58) was cultured at 16°C under 32 watt incandescent lights. The artificial seawater (F2 medium) contained 50% ^{15}N-enriched nitrate (98% ^{15}N, Isotech) to label the diatoms. After 3 wk, the labelled diatoms were concentrated by centrifugation, washed several times to remove adhering

$^{15}N\text{-}NO_3$ and subsequently freeze-dried. The axenic state of the diatom culture was verified microscopically. The freeze-dried phytodetritus was subsequently distributed over 12 Eppendorf tubes (2 mL), containing 35 mg dried material each. At the field site, the diatoms were re-suspended in 2 mL seawater and gently homogenized by sucking the solution in and out 3 times with a syringe. At low tide, the re-suspended solution was injected in the upper 50 mm of sediment, at 4 equidistant points 50 mm around each bamboo stick of the 2 replicate labelling plots (day 0).

2.2.3 Sample collection and analysis

Seagrass samples (2 replicates and 1 control) were retrieved during low tide at 1, 2, 4 and 8 days after label addition. Each flagged subplot was sampled digging a deep groove (150 mm deep) with a long blade knife. A 150 × 150 mm square clump of seagrass around the bamboo stick was extracted from the sediment and poured into a plastic bag. Seagrass samples were first sorted by species present at each subplot. Due to the sparse and heterogeneous seagrass cover, it was not always possible to recover all different species (e.g. *Halodule pinifolia* and *Halophila ovalis*). Subsequently and respectively to their subplot (the 2 replicates and the control), plants were dissected to isolate their roots, rhizomes and leaves. In the case of *Thalassia hemprichii*, we also isolated the sheath. Epiphytes were carefully removed from the leaves scraping them clean with a razor blade and all the samples were rinsed twice in seawater. All separated parts were placed into 20 mL glass vials and dried in a stove at 60°C for 72 h. Dried samples were ground using an agate mortar.

The stable isotope measurements were made using a Finnigan Delta S isotope ratio mass spectrometer coupled on-line via a conflo interface with a Carlo Erba/Fisons/Interscience elemental analyser. The nitrogen isotopic composition was expressed in the delta notation using the equation:

$$\delta^{15}\mathrm{N}(‰) = \left(\frac{R_{sam}}{R_{air}} - 1\right) \times 1000$$

With $R_{sam} = {}^{15}N/{}^{14}N$ (ratio of the sample) and $\delta^{15}N$ expressed relative to atmospheric nitrogen ratio ($R_{air} = 0.0036765$).

$\delta^{15}N$ values were averaged between the 2 replicate subplots for the different seagrass fractions isolated for each species. Background values were obtained by averaging the controls $\delta^{15}N$ values over the 4 sampling days.

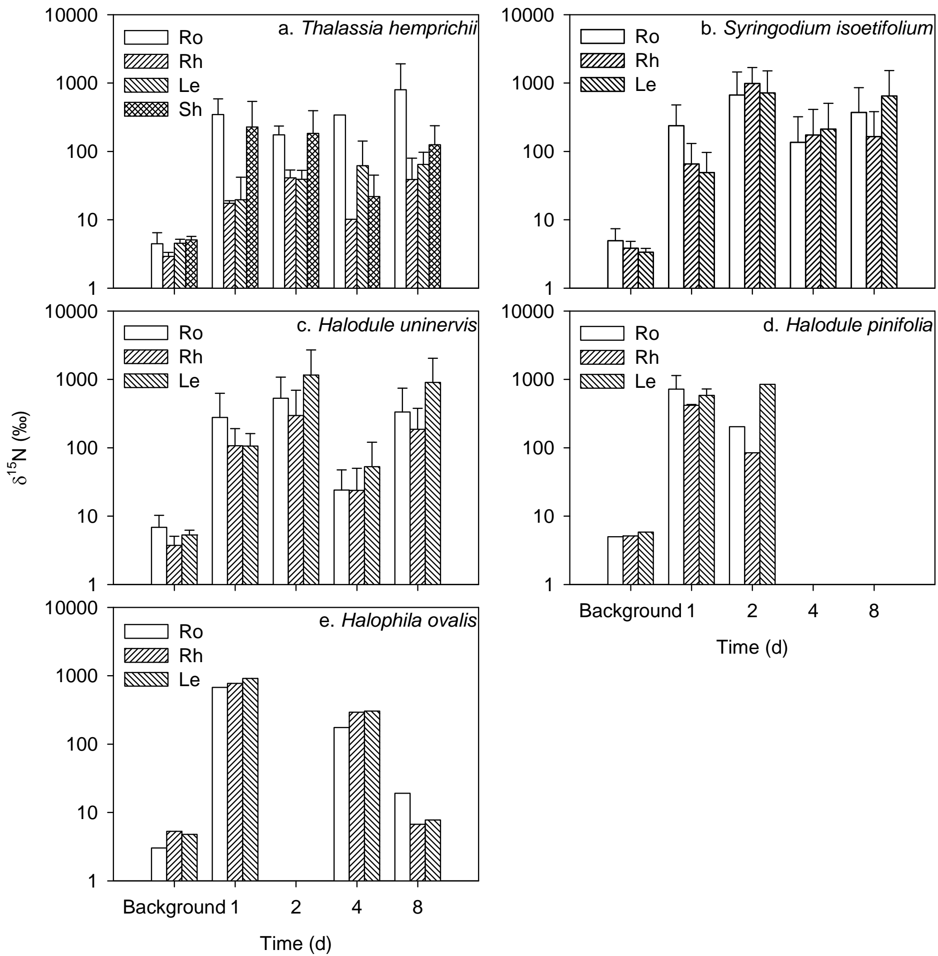

Figure 2.1. Time series over 8 d of the δ^{15}N values (mean + SD, log-scaled) in the roots (Ro), the rhizomes (Rh), the leaves (Le) and the sheaths (Sh) of (a) *Thalassia hemprichii*, (b) *Syringodium isoetifolium*, (c) *Halodule uninervis*, (d) *Halodule pinifolia*, and (e) *Halophila ovalis*. The uptake of ^{15}N can be followed up 1, 2, 4 and 8 d after its injection in the sediment. Background values show the δ^{15}N natural abundances.

Table 2.1. Natural $\delta^{15}N$ abundance (i.e. background value) and the $\delta^{15}N$ values as measured 1 day after label addition; mean ± SD (n). Natural ^{15}N abundance in seagrass leaves agreed well with literature values for a wide range of species (Udy et al. 1999, Marba et al. 2002, Anderson & Fourqurean 2003, Yamamuro et al. 2003).

	Roots		Rhizomes		Leaves	
	Background	After 1 d	Background	After 1 d	Background	After 1 d
Thalassia hemprichii	4.47 ± 2.02 (2)	346.8 ± 242.7 (2)	2.9 ± 0.4 (2)	17.5 ± 1.5 (2)	4.6 ± 0.6 (2)	19.6 ± 22.5 (2)
Syringodium isoetifolium	5 ± 2.4 (4)	238 ± 239.8 (2)	3.9 ± 1 (4)	65.7 ± 65 (2)	3.4 ± 0.5 (4)	49.1 ± 47.5 (2)
Halodule uninervis	6.9 ± 3.4 (4)	278.6 ± 347.9 (2)	3.8 ± 1.3 (4)	107.2 ± 83.2 (2)	3.8 ± 1.3 (4)	106 ± 55.5 (2)
Halodule pinifolia	5	720 ± 417.2 (2)	5.1	417.6 ± 13.3 (2)	5.9	583.1 ± 143.2 (2)
Halophila ovalis	3	676	5.3	774.3	4.8	913.2

2.3 Results

Transfer of ^{15}N from the labelled phytodetritus to seagrass plants was very rapid (Table 2.1, Fig. 2.1). Within 24 h after injecting the ^{15}N-labelled phytodetritus in the sediment, all exposed seagrass plants showed an important increase in $\delta^{15}N$ values, despite differences in turnover, size and standing biomass between species. The response of the relatively large *Thalassia hemprichii* plants seems somewhat slower than that of the relatively small *Halophila ovalis* plants, which we ascribe to differences in biomass and biomass turnover rates. The relatively low $\delta^{15}N$ values of *T. hemprichii* leaves is partly due to the separation of leaves and sheaths, which were not separated for the other species. As the sheath contains the growing part of the leaves, it may be expected that labelling would be greater there. Although the sheaths were indeed labelled to a greater extent than the leaves (Fig. 2.1a), *T. hemprichii* was labelled to a smaller extent than the other species.

For all species except *Halophila ovalis*, the roots were initially labelled to a much greater extent than the rest of the plant (Table 2.1). This suggests that the rapid nutrient transfer from phytodetritus in the sediment to seagrass rhizomes and shoots occurred via

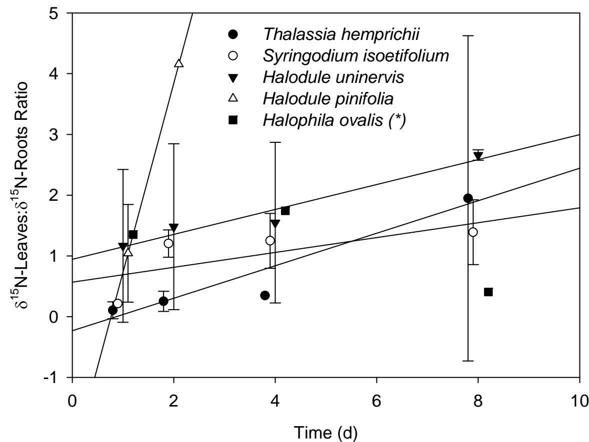

Figure 2.2. Ratio (mean ± SD and linear regression) of leave to root $\delta^{15}N$ signatures over time. An increasing ratio indicates that there is a transfer of ^{15}N from the roots to the leaves. Points at each time step were slightly shifted to enhance readability. Coefficients of determination (r^2) for the regression lines are 0.91, 0.50 and 0.95 for *Thalassia hemprichii*, *Syringodium isoetifolium* and *Halodule uninervis* respectively. **Halophila ovalis* linear regression is not represented here as the very low value of the last data point might be erroneous.

the roots. This nutrient transfer pathway is further confirmed by calculating from the time series the ratio of $\delta^{15}N$ in the leaves to $\delta^{15}N$ in the roots (Fig. 2.2). For all the seagrass except for *H. ovalis*, this ratio increased with time, indicating that, over time, leaves became enriched relative to the roots.

2.4 Discussion

The *in situ* deliberate tracer experiment convincingly demonstrated the potential role of sediment POM as a nutrient source for seagrass productivity in oligotrophic systems. In addition, we demonstrated that seagrass roots can play a major role in nutrient uptake once POM is trapped in the sediment. Before discussing in detail the results and their implications, it is instructive to evaluate the experimental approach. The pulse-chase experiment was performed *in situ* rather than in the laboratory or mesocosm in order to minimize disturbances and deviations from field conditions. Particle transport to seagrass sediment was simulated by injecting algae in the upper 50 mm sediment. Although the resulting depth distributions within the sediment may differ from natural particle trapping depth distributions, the retention and subsequent fate of this material was similar to particles delivered by natural processes. As with most tracer studies, the labelled phytodetritus material added may not be the same as that in the field in terms of size and degradability. *In situ* deliberate tracer experiments provide essential and unequivocal information on nitrogen flows, even though interpretation may be somewhat complicated by heterogeneity as reflected in the relative large standard errors (Fig. 2.1 & 2.2, Table 2.1). Heterogeneity is related to a number of causes, including differences in the quantity and depth distribution of tracer injection, variability in sediment grain size (causing differences in tracer retention after injection) and heterogeneity of the seagrass meadow in terms of plant, zoobenthos and micro-organism biomass. While *in situ* tracer experiments can unequivocally identify the flows of ^{15}N from sediment POM to seagrass tissues, they don't provide any resolution whether dissolved inorganic or organic nitrogen is assimilated.

Solute exchange between the water column and sediments has been studied extensively. Hydrodynamics play a critical role in the advective transport and the degradation of organic matter in sediment, as was shown for permeable bare sediments in the field, in flumes and in chamber experiments (Huettel & Gust 1992a, Huettel & Rusch 2000, Rusch & Huettel 2000, Ehrenhauss et al. 2004a, Ehrenhauss et al. 2004b). Permeable sediments act as biocatalytic filters where algal material, flocs and detritus are trapped. In the ecosystem we studied (seagrass meadow behind coral reef), particle trapping can be considerably enhanced, as exuded coral mucus traps POM in the water column and concentrate it in the lagoon with tidal currents (Wild et al. 2004). Moreover,

seagrass canopies strongly enhance sedimentation by reducing current velocity and wave energy. Canopy trapping has been demonstrated in temperate areas (Koch 1999, Terrados & Duarte 2000, Gacia & Duarte 2001, Granata et al. 2001, Koch 2001, Gacia et al. 2002) as well as in tropical ones (Agawin & Duarte 2002, Gacia et al. 2003). In a seagrass meadow dominated by *Thalassia hemprichii*, Agawin and Duarte (2002) showed that particle loss is 4 times higher than in unvegetated areas, suggesting an important transfer of planktonic production to the seagrass meadow. Even though one may show that, in extreme conditions seagrass meadows become a source of suspended particulate matter (Koch 1999), particle resuspension is generally limited (Terrados & Duarte 2000, Gacia & Duarte 2001, Granata et al. 2001); thus, the literature suggests that POM will be transported to and trapped in sediments, as was mimicked by the label addition applied in this experiment.

The POM trapped in seagrass sediments enters the benthic food web as a resource for macrofauna, meiofauna and bacteria. The rapid appearance of ^{15}N in the seagrass plants following the addition of the labelled material suggests that bacterial mineralization dominates over bacterial immobilisation in our system. While in temperate zones or sparse vegetations bacterial growth may not be linked directly to seagrass production (Boschker et al. 2000), it seems to be the opposite in tropical zones or dense vegetations where bacterial growth can be enhanced in seagrass sediments (Holmer et al. 1999, Jones et al. 2003). For instance, Blaabjerg et al. (1998) reported diurnal cycles in sulphate reduction in sediments with dense cover of *Zostera marina*. In our experiment, the bacterial community immediately responded to the addition of phytodetritus and thus regenerated nutrients which were then taken up by the plants. The steady increase in $\delta^{15}N$ over the 8 day period reveals persistent retention and mineralization of the labelled material and excludes the possibility that the entire ^{15}N uptake is due to the assimilation of an early release of residual dissolved nitrogen that might have been inadvertently added with the introduced phytodetritus. Moreover, the steady increase in $\delta^{15}N$ of leaves relative to roots indicates that the majority of uptake occurs via the roots (Fig. 2.2).

Recycling internal nutrient pools (nutrient resorption) through the remobilization of older senescent parts was shown to have a rather low efficiency of about 15% (Stapel & Hemminga 1997, Hemminga et al. 1999). Perhaps nutrient resorption can be more efficient under specific conditions, but is unlikely to be sufficient enough to maintain the high productivities observed in oligotrophic tropical offshore coral islands. It appears that POM trapped in the sediment offers an important nutrient source that seagrasses take up via their roots. These findings corroborate the results of a nutrient mass balance model that underlined the importance of organic matter mineralization for nutrient supply and seagrass roots for nutrient uptake (Erftemeijer & Middelburg 1995).

In this paper we identified that phytodetritus and POM trapping in the sediment, either by advective transport or due to canopy friction, in combination with root nutrient uptake are among the key processes involved in sustaining nutrient supply to seagrass meadows in oligotrophic environments. Although our *in situ* experiment does not allow us to derive a precise nutrient budget, it clearly demonstrates that the roots provide seagrasses with a competitive advantage over other primary producers because additional nutrients, i.e. those in particulate organic form in the sediments, can be utilised. This issue needs further assessment for a better understanding of systems where seagrass meadows dominate over other primary producers.

Chapter 3
Tracing incorporation and pathways of carbon and nitrogen in microbial communities of photic subtidal sands

In revision for Aquatic Microbial Ecology*

3.1 Introduction

Coastal sediments have traditionally been considered donor-controlled ecosystems in which supplied detritus of variable quality is mineralised. During the last two decades, there is growing awareness that primary production by microphytobenthos (MPB, or the secret garden) can contribute significantly to organic carbon production (MacIntyre et al. 1996) and moderate sediment carbon flows (Middelburg et al. 2000) in the coastal zone. This key-role of MPB in coastal primary production and benthic C flows has been documented particularly for intertidal flats that receive abundant light during exposure (MacIntyre et al. 1996, Underwood & Kromkamp 1999). Recently, Gattuso et al. (2006) suggested that net benthic primary production may take place on more than 33% of the global shelf area. This implies that the contribution of MPB to the global cycles of carbon may have been underestimated.

Subtidal sediments in the photic zone typically show three major metabolic pathways. The first one is phototrophy and concerns primarily carbon dioxide fixation by benthic microalgae (BMA) and cyanobacteria, the two major components of the MPB

---

* Evrard V, Cook PLM, Veuger B, Huettel M, Middelburg JJ (in revision) Tracing incorporation and pathways of carbon and nitrogen in microbial communities of photic subtidal sands. Aquat Microb Ecol

(terminology listed in Table 3.1). In the surface layer, the MPB often represents the most important compartment in terms of biomass and turnover and, modulates C and N transfer across the sediment-water interface (MacIntyre et al. 1996, Sundback et al. 1996). Although C fixation and N assimilation are often closely related to the build up of biomass, a large part of the carbon fixed may be diverted to synthesis of extracellular polymeric substances (EPS) that can account for a great fraction of organic carbon present in the sediment (Smith & Underwood 1998, Goto et al. 1999). The second major pathway is heterotrophy: the utilisation of detritus, MPB, EPS and other organic substrates by heterotrophic bacteria (Hbac), meiofauna and macrofauna. Heterotrophic benthic organisms produce new biomass (secondary production) and govern mineralization of organic matter. The third major metabolic pathway is chemoautotrophy: the fixation of carbon dioxide using the energy released upon oxidation of reduced inorganic compounds. This topic has received limited attention in the literature; yet it may constitute a significant pathway close to redox boundaries (Kristensen & Hansen 1995, Thomsen & Kristensen 1997).

Although the biological importance of subtidal permeable sediments has been recognized decades ago (Riedl et al. 1972), these systems, in particular subtidal ones, remain poorly documented. In contrast to fine grained sediments, which are characterised by large stocks of organic matter, permeable sediments have long been wrongly considered as biogeochemically limited (Boudreau et al. 2001), because of their low concentrations of nutrients in pore water and low organic C contents. Nevertheless, recent studies have highlighted the biogeochemical importance of permeable sediments, where interfacial water flows strongly facilitate pore water and organic matter exchange between the sediment and the water column, thus enhancing organic matter supply to the sands and remineralization (Huettel & Gust 1992b, a, Huettel et al. 1996, Huettel & Rusch 2000, Rusch & Huettel 2000, Precht & Huettel 2003). Advective pore water flows allow reactive compounds to be transported more efficiently into the sediment and made available to bacterial community attached to the sand grains (Rusch et al. 2001, Franke et al. 2006), conferring these sediments with important biocatalytic filter properties (Huettel et al. 2003, Ehrenhauss et al. 2004a). Evidence is accumulating that hydrodynamics might be the principal factor governing the high remineralization rates found in permeable sediments (de Beer et al. 2005). The low C content of sandy, permeable sediments is not the consequence of sediment inactivity but rather the consequence of high organic carbon turnover (Boudreau et al. 2001, Dauwe et al. 2001, de Beer et al. 2005) which in the end has an important influence on the overall oceanic C cycle (Shum & Sundby 1996).

Sandy sediments cover about 70% of the continental shelf (Hall, 2002) and a major part of these sediments receives enough light to sustain primary production (Jahnke et al., 2000; Gattuso et al., 2006). These sands thus may contribute significantly to the shelf and

global carbon cycle. Therefore quantitative data on the flow of carbon within subtidal permeable sediments inhabited by MPB in which phototrophic, heterotrophic and chemoautotrophic pathways co-occur are of great interest, e.g. as input data for coastal and global models of carbon and nutrient cycles.

In this study, we follow the fate of fixed C and assimilated N through the benthic microbial compartment after a deliberate tracer addition of ^{13}C-bicarbonate and ^{15}N-nitrate into sediment cores. Through the use of stable isotopes coupled with microbial biomarker techniques involving phospholipid-derived fatty acids (Middelburg et al. 2000, Boschker & Middelburg 2002, Boschker et al. 2005) and hydrolysable amino acids including bacterial biomarker D-alanine (Veuger et al. 2005), we can follow the fate of C and N within the different microbial components, assess the relative contribution of the different metabolic pathways and compare our observations with those of other benthic environments. This study and its companion paper on the benthic food web Evrard et al. (Chapter 4) offer an unprecedented view on the trophic interactions in a subtidal permeable sediment receiving sufficient light to support MPB growth.

Table 3.1. Terminology and correspondences for biomass estimations

<table>
<tr><th colspan="3">Terminology</th><th>Biologic compartment</th><th colspan="5">Biomass</th></tr>
<tr><th></th><th></th><th></th><th></th><th colspan="3">C and N</th><th colspan="2">C only</th></tr>
<tr><td rowspan="5">Benthic Microbial Community</td><td></td><td rowspan="3">Bacteria</td><td>Heterotrophic Bacteria (HBac)</td><td rowspan="8">Bulk Sediment</td><td rowspan="7">THAA</td><td rowspan="3">D-Ala</td><td></td><td rowspan="2">$PLFA_{Bac}$</td></tr>
<tr><td></td><td>Chemo-autotrophic Bacteria (CBac)</td><td></td></tr>
<tr><td rowspan="3">MPB</td><td>Cyanobacteria (Cy)</td><td rowspan="3">Chl*a*</td><td rowspan="3">$PLFA_{All} - PLFA_{Bac}$</td></tr>
<tr><td rowspan="2">BMA</td><td>Diatoms</td><td></td></tr>
<tr><td>Haptophytes</td><td>(grey)</td></tr>
<tr><td colspan="4">Meiofauna and microfauna</td><td colspan="3">(grey)</td></tr>
<tr><td colspan="4">Detritus</td><td colspan="3">(grey)</td></tr>
<tr><td colspan="4">EPS</td><td colspan="4"></td></tr>
</table>

BMA: benthic microalgae
Chl*a*: chlorophyll *a*
D-Ala: D-alanine
EPS: extracellular polymeric substances
MPB: microphytobenthos
$PLFA_{All}$: all phospholipid-derived fatty acids
$PLFA_{Bac}$: bacterial-specific phospholipid-derived fatty acids (iC14:0, iC15:0, aC15:0 and iC16:0)
THAA: total hydrolysable amino-acids
(grey box): negligible relative contribution for estimation of stable isotope transfer

3.2 Material and methods

3.2.1 Study site.

The research was carried out in List, on the Island of Sylt (Germany) in July 2004. The sampling site, the Hausstrand, was situated south of List Harbour close to a site studied earlier by de Beer et al. (2005). The area is exposed to the east and strongly influenced by North-South tidal currents parallel to the shore and wind driven waves. The sediment is a silicate sand with a median grain size of 350 µm, a porosity of 0.42 and an organic carbon content of ~0.2% (250 g C m^{-2}).

3.2.2 Incubation experiment

Five transparent and one opaque acrylic cylinders (19 cm inner diameter x 33 cm high) were used to sample a set of 6 sediment cores with their overlying water. They were retrieved at low tide from the study site, at approximately 1.5 m water depth. The cores (approximately 15 cm of sediment and 18 cm of overlying water) were immediately transferred to the laboratory where the cylinders were closed by acrylic lids. Each lid carried an electric motor that propelled a 15 cm diameter horizontal rotating disc that stirred the water column at 15 cm above the sediment water interface. The motor maintained an electronically controlled angular velocity set to 40 rpm which generated a pressure gradient of 1.9 Pa (~0.2 Pa cm^{-1}) between the circumference and the centre of the sediment surface. This pressure gradient corresponds to the gradient produced by slow bottom currents (~10 cm s^{-1}) interacting with sediment ripple topography (15 mm ripple amplitude) as were typical for the study site during our investigations. The pressure gradient forces water through the upper layer of the permeable sand that enters in the sediment in the ripple troughs and emerges at the ripple crests, and in the chamber enters the sediment near the chamber wall and emerges from the centre of the core. The functioning and deployment of these chambers has been described in detail by Huettel & Gust (1992b), Huettel et al. (1996), Janssen et al. (2005a) and Janssen et al. (2005b)

The set of chambers was immersed in a trough of ~200L, filled with sea water from the field site, maintained under *in situ* temperature (20°C) and constantly aerated. Each chamber's upper lid with stirrer was elevated about 1 cm above the chamber's edge, allowing circulation of water within the whole system. The whole circulation of water was enhanced by a submersible water pump placed at the bottom of the trough. The system was left to settle 24 h before the experiment started. On the following day, one transparent core was immediately sampled to provide background values (T=0). The other cores were hermetically closed and received a pulse of 850 mmol $NaH^{13}CO_3$, equivalent to a 10%

^{13}C-labelling of the DIC pool, and 170 mmol $Na^{15}NO_3$. The four transparent cores were illuminated by an artificial light providing an irradiance of 185 µmol quanta m^{-2} s^{-1} at the sediment surface which corresponded to the in-situ light intensities. Primary production was estimated on the first day from the difference in oxygen increase in the light and oxygen decrease in the dark. Oxygen concentrations were measured using Winkler titrations (Grasshoff et al. 1999), in the light (n=3) and in the dark (n=1) at t =0, 2.1, 3.4 and 4.9 h from the beginning of the illumination period. After 9 hours, the light was switched off and the water column of the 5 cores was flushed twice to remove the added tracers and replaced with seawater from the study site. Care was taken to avoid disrupting the sediment surface. A 9:15 h light:dark cycle was kept during the 4 days of the experiment. The cores were always kept open when in the dark and were submerged in the trough to allow aeration and mixing of the water within the whole system.

At each time step after the labelling period (T = 1, 2, 3 and 4 d), just before the period of illumination, one core was removed from the trough and its sediment sampled as follows. Four subsamples were taken from the core using 4 small core liners (3.56 cm diameter ~10 cm^2). For organic C, N, phospholipid-derived fatty acids (PLFA) and total hydrolysable amino acids (THAA) content and analysis, 3 cores were sliced (0-1, 1-2, 2-3, 3-4, 4-5 and 5-10 cm) and layers pooled together to limit heterogeneity inherent to microbial communities in sandy sediments as much as possible. The remaining subsample core was sliced (0-2, 2-4, 4-6, 6-8, 8-10 and 10-20 mm) for pigments distribution analysis. All sediment samples were freeze-dried and stored in a freezer until analysed.

3.2.3 Analyses and data handling

Small fractions of the sediment samples were ground in agate mortars to obtain a fine and homogeneous sediment powder. Nitrogen and organic carbon contents (remaining after acidification) and their isotopic compositions (^{13}C and ^{15}N) were measured from those fractions using a Carlo Erba/Fisons/Interscience elemental analyser coupled via a conflo II interface to a Finnigan Delta S isotope ratio mass spectrometer (EA-IRMS).

PLFA for all layers down to 5 cm were extracted from approximately 6 g of dry sediment per layer, following the method of Boschker et al. (1999) and Middelburg et al. (2000), and their concentrations were determined by gas chromatography with flame ionization detection (GC-FID, Carlo Erba HRGC mega 2 GC). PLFA stable carbon isotopic composition was determined by gas-chromatography-combustion-isotope ratio mass spectrometry (GC-c-IRMS, Hewlett Packard 6890 GC coupled via a Thermo combustion interface III to a Thermo Delta Plus isotope ratio mass spectrometer). Bacterial and MPB carbon contents in the sediment were estimated from the PLFA concentrations. Bacterial biomass (BAC, expressed in mmol C m^{-2}) was calculated from

bacterial PLFA ($PLFA_{bac}$) as $BAC = PLFA_{bac}/a$ where a is the average PLFA concentration in bacteria (0.073 mmol of carbon PLFA per mmol of carbon bacteria for aerobic sediments; Brinch-Iversen & King 1990; Moodley et al. 2000). $PLFA_{bac}$ was estimated from the bacteria-specific PLFA pool ($PLFA_{bacsp}$: iC14:0, iC15:0, aiC15:0 and iC16:0; specific of both heterotrophic and chemoautotrophic bacteria) as $PLFA_{bac} = \Sigma PLFA_{bacsp}/b$, where b is the average fraction-specific bacterial PLFA (0.14 mmol of carbon bacterial-specific PLFA per mmol of carbon bacterial PLFA; Moodley et al. 2000). MPB biomass (expressed in mmol C m^{-2}) was calculated from the difference between total PLFA ($PLFA_{all}$) and the $PLFA_{bac}$ as $MPB = (\Sigma PLFA_{all} - PLFA_{bac})/c$, where c is the average PLFA concentration in MPB (0.053 mmol of carbon PLFA per mmol MPB). c was calculated as the weighted average PLFA concentration in a mix of different phytoplankton monocultures similar to the MPB composition (Dijkman & Kromkamp 2006; Dijkman, N.A. pers. comm.).

Hydrolysable amino acid (HAA) concentrations and their ^{13}C and ^{15}N enrichment were measured for the first cm layer by GC-c-IRMS (Veuger et al. 2005, Veuger et al. 2007b). ^{13}C and ^{15}N incorporation into the bacteria-specific amino acid D-alanine (D-Ala) is expressed relative to that into the common protein amino acid L-alanine (L-Ala, a stable constituent of the HAA pool of all organisms) as the ^{13}C or ^{15}N D/L-Ala incorporation ratio (^{13}C or ^{15}N incorporation in D-Ala / ^{13}C or ^{15}N incorporation in L-Ala) to obtain an estimate of the bacterial contribution to total ^{13}C and ^{15}N incorporation.

Stable isotope data were expressed in the delta notation ($\delta^{13}C$ and $\delta^{15}N$) relative to Vienna Pee Dee Belemnite (VPDB) and air, for carbon and nitrogen respectively. Following Maddi et al. (2006) we use the enrichment (δ^E notation), as a measure of label enrichment. $X\delta^E = ((\delta X_s+1000)/(\delta X_b+1000)-1)\times 1000$, where X is ^{13}C or ^{15}N and where b stands for background (natural abundance) and s for sample (enriched). The incorporation (I, the total uptake of label), as defined by Middelburg et al. (2000), was expressed in mmol X m^{-2}, as the product of the atomic excess (E) and a quantity (organic C, N, C-PLFA or C- and N-amino acid). $E = F_s - F_b$, is the difference between the stable isotope fraction of the sample and that of the background. $F = R/(R+1)$, where R is the stable isotope ratio calculated from the δ-value. $R = (\delta X/1000+1)\times R_{VPDB/Air}$ with $R_{VPDB} = 0.0111797$ and $R_{Air} = 0.0036765$.

Pigment samples were analysed by reverse-phase high-performance liquid chromatography (Barranguet et al. 1998), and data expressed in µg g sed^{-1}. Relative fractions of microphytobenthic taxa were estimated using the CHEMTAX program (Mackey et al. 1996).

3.3 Results

3.3.1 MPB composition, biomass and production

With ~4800 and 11600 mmol C m^{-2} for the top 2 and 5 cm of sediment, organic C content was relatively high for sandy sediments (~0.21% of dry weight for the top cm). The vertical concentration profiles of pigments over the first 20 mm of sediment suggest a homogenous distribution of MPB within the photic zone and below (Fig. 3.1). Average Chl *a* concentrations were 82 ± 10 and 793 ± 70 mg m^{-2} for the top 2 mm and the upper 20 mm respectively and did not significantly change over time. Pigment data relevant for MPB group identification are shown in Fig. 3.1A. Chl *b* and lutein were not detected indicating the absence of green algae. Chl *a* concentrations appeared to be correlated to fucoxanthin and, together with the presence of Chl *c* and diadinoxanthin, suggested that the MPB was dominated by diatoms. This was confirmed by the output of the CHEMTAX analysis (Fig. 3.1B) indicating that diatoms represented approximately 60% and 55% of the MPB for the depth layers 0-10 and 10-20 mm, respectively. Cyanobacteria accounted for approximately 30% of the MPB biomass and haptophytes contributed for the

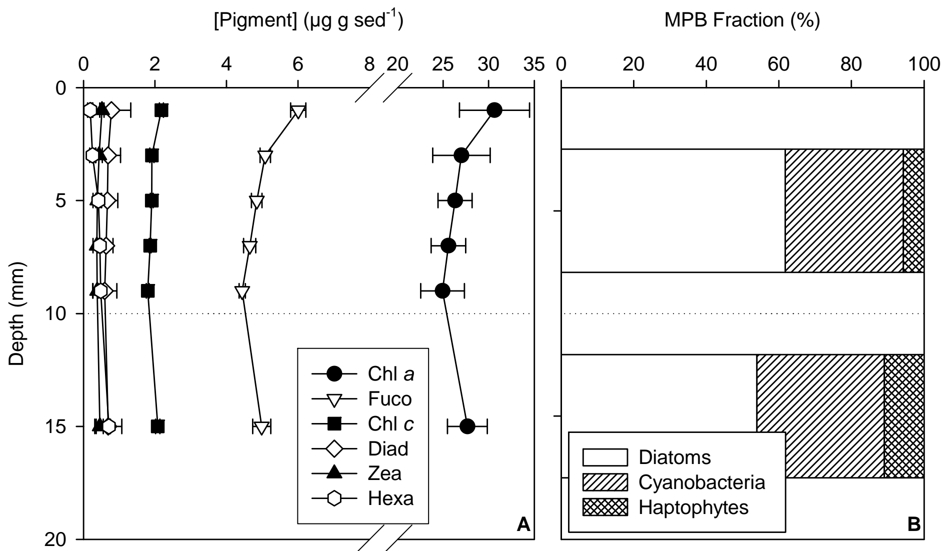

Figure 3.1. Pigment (A) and MPB (B) composition of the sediment. (A) Depth profiles of chlorophyll a (Chl *a*), Fucoxanthin (Fuco), chlorophyll c (Chl *c*), diadinoxanthin (Diad), zeaxanthin (Zea) and 19-hexanoyloxyfucoxanthin (Hexa). (B) MPB composition calculated by the CHEMTAX program for the 0-10 and 10-2- mm layers.

remaining ~10%. The latter group is supposedly part of the phytoplankton and has likely settled onto the sediment prior to sampling. Considering their small contribution and to simplify further discussion, the haptophytes will be neglected and only diatoms and cyanobacteria will be taken into account with proportions of 66% and 33%, respectively. Depth integrated algal biomass estimated from PLFA concentrations were 1411 and 3400 mmol C m^{-2} for the 0-2 and 0-5 cm layers, respectively. Finally, primary production based on oxygen evolution was 1.68 mmol m^{-2} h^{-1}.

3.3.2 Label transfer

Enrichment of ^{13}C and ^{15}N in bulk sediment was initially rapid and slightly decreased after day 2 (Fig. 3.2A,B). Enrichment mainly occurred at the sediment surface with maximal δ^E values of 73 and 1220‰ for ^{13}C and ^{15}N reached on day 2. The deeper layers were much less enriched and, for the layer 1-2 cm, ^{13}C and ^{15}N enrichment maxima were reached on the third day, which may be ascribed to the burial of labelled particulate organic matter due to porewater advection or bioturbation. The final comparison of C and N uptake in light and dark cores on day 4 showed a striking difference in label uptake between C and N because in contrast to C, N uptake occurred also in the dark. ^{15}N dark enrichment reached 75% of the value measured for the light incubation. Further processes that may explain the differences between the δ^E values of ^{13}C and ^{15}N enrichments are differences in label addition and uptake pathways between bicarbonate and nitrate.

PLFA ^{13}C enrichment was significant and rapid from day 1 (Fig. 3.2C,D). Within the first cm layer (Fig. 3.2C), the diatom-specific biomarker C20:5ω3 was the most abundant (data not shown) and the relatively strong enrichment reached a maximum of 67‰ on day 2. The presence of C18:3ω3 and C18:3ω4, in the absence of green algae, can be attributed to cyanobacteria. C18:3ω3 and C18:3ω4 enrichments were highest on day 2 with δ^E values of 225 and 159‰, respectively. Cyanobacteria-related PLFAs of the underlying layer were far less enriched but increased slightly over time, suggesting a gradual burial (Fig. 3.2D). The diatoms-specific PLFA showed very low enrichment in the subsurface. MPB PLFA enrichment in the dark was negligible both at the sediment surface and deeper in the sediment (Fig. 3.2C and D). Bacterial biomass, which was low at the sediment surface and increased with depth, represented 318 and 884 mmol C m^{-2} for the top 2 and 5 cm of sediment respectively. The presence of chemoautotrophic bacteria was evident as a white biofilm (indicative for sulphide oxidizers that accumulate whitish elemental sulphur in their cells) was visible from day 3 at the sediment surface in the centre of the cores. In this zone outflow of reduced pore water created an interface between anoxic sediment and the oxic water column. Underneath the white biofilm in the centre of each core was a ~15 mm diameter black spot at the sediment surface, characteristic of reduced compounds,

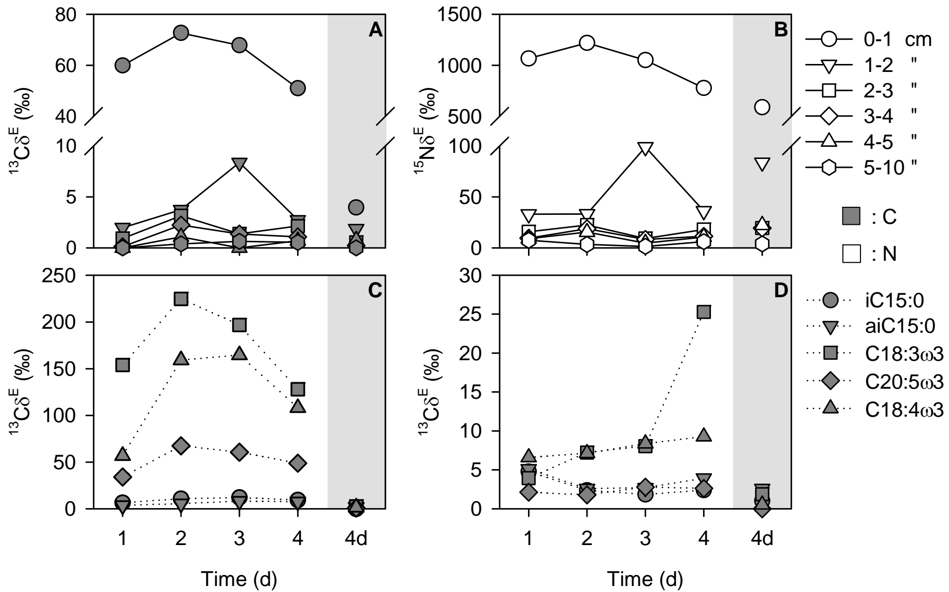

Figure 3.2. ^{13}C and ^{15}N enrichment over time in bulk sediment (A and B respectively) for the different sediment layers. ^{13}C enrichment over time in bacterial specific PLFAs (iC15:0, aC15:0), diatoms specific PLFAs (C20:5ω3) and cyanobacterial PLFA (C18:3ω3 and C184:4ω3) for the 0-1 and 1-2 cm layers (C and D respectively). The dark incubation on day 4 (4d) is greyed.

which contrasted with the rest of the brown sediment surface. Bacterial-specific PLFAs at the sediment surface showed less enrichment (only iC15:0 and aiC15:0 were represented with maximal δ^E of 12 and 8‰ respectively) than those of MPB and followed roughly the same enrichment pattern as the algal ones although they reached their maximum on day 3 (Fig. 3.2C). The enrichment of bacterial PLFA for the 1-2 cm layer showed the opposite trend, with a maximum enrichment on day one followed by a decrease over time (Fig. 3.2D). Enrichment in the dark was very low but noticeable, particularly in the 1-2 cm layer where the δ^E value for aiC15:0 was the highest of all specific PLFA.

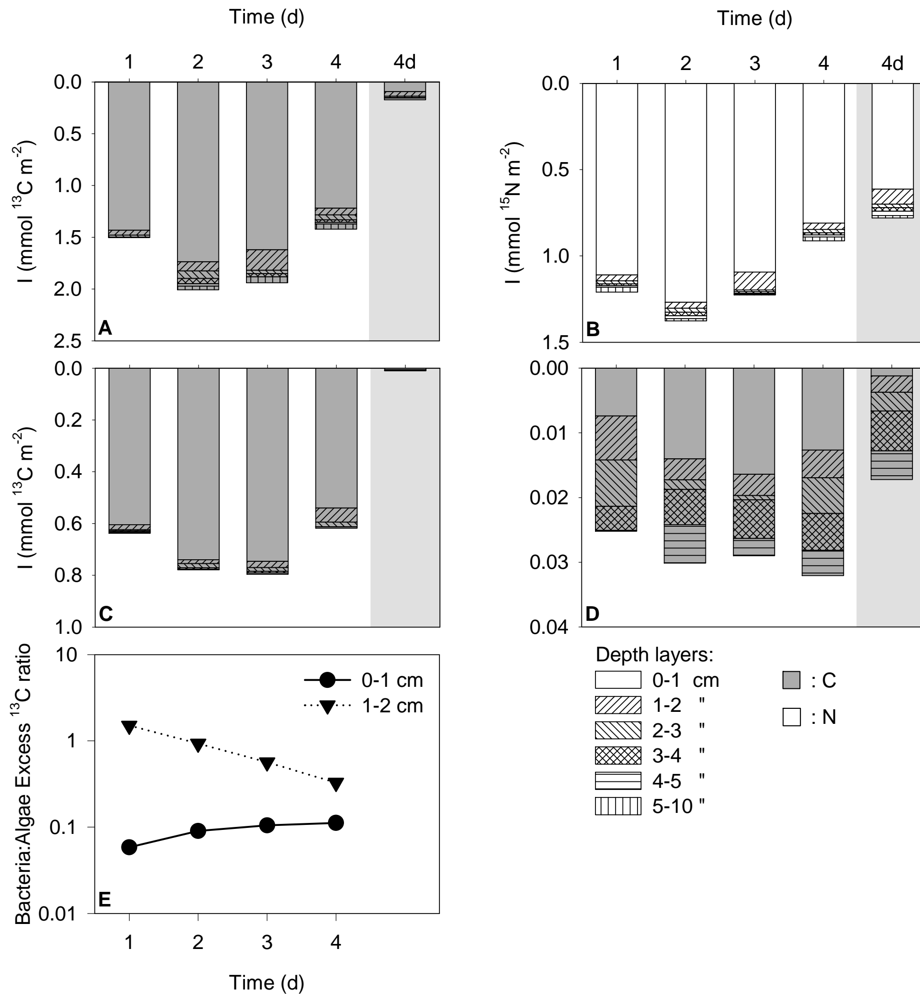

Figure 3.3. ^{13}C and ^{15}N incorporation over time in bulk sediment (A and B respectively) for the different layers. MPB (C) and bacteria (D) ^{13}C incorporation estimated from PLFA. (E) Bacteria:BMA excess ^{13}C ratio over time in the first and second cm layer of sediment.

3.3.3 C and N assimilation

Bulk sediment incorporation of ^{13}C and ^{15}N showed similar dynamics with maximal incorporation on day 2 (Fig. 3.3 A,B), which is in agreement with bulk sediment specific uptake (Fig. 3.2 A,B). While the enrichments depicted in Fig. 3.2 A,B reflect the efficiencies of label uptake, Fig. 3.3 A,B show the actual amounts of tracer that were

measured in the sediments on the 4 days of the experiment. Organic C and N concentrations did not significantly vary over time (data not shown). Maximum ^{13}C incorporation (2 mmol ^{13}C m^{-2}) integrated over the upper 10 cm of sediment was greater than that of ^{15}N (1.4 mmol ^{15}N m^{-2}). On day 1, after 9 hours of labelling, there was no incorporation below 4 cm and the total depth integrated incorporation was 1.5 mmol ^{13}C m^{-2}. Considering that 10% of the DIC pool had been labelled and that all carbon remained in the sediment (i.e. respired ^{13}C was negligible compared to the sediment incorporation, see Chapter 4, and there was no resuspension), the total carbon fixed was 1.5/(0.1 x 9) = 1.66 mmol m^{-2} h^{-1}. The close agreement with the oxygen flux measurements (1.68 mmol m^{-2} h^{-1}) confirms that the carbon fixation was mostly caused by phototrophs with only negligible contribution of the chemoautotrophic bacteria, thus, very little ^{13}C incorporation occurred in the dark (0.17 mmol m^{-2}) at day 4. In contrast, total depth integrated ^{15}N incorporation in the dark at day 4 was very similar to that in the light (0.77 and 0.89 mmol ^{15}N m^{-2} respectively).

Algal incorporation of ^{13}C (Fig. 3.3C) was significant and rapid within the first centimetre layer and, slightly increased between days 1 and 3. Although most of the incorporation was restricted to the sediment surface, the deeper layers (1-3 cm) showed a slight increase until day 4, confirming burial of MPB as suggested earlier. C assimilation into MPB based on PLFA amounted to 44 ± 2% that of total C fixation. MPB carbon incorporation in the dark was small (1.6% that of in the light). Bacterial ^{13}C incorporation was rather small (Fig. 3.3D) and showed dynamics distinct from that of the MPB. Some ^{13}C was incorporated by bacteria in the first centimetre but most of the bacterial ^{13}C incorporation occurred in the deeper layers beginning on the first day. The fact that most of the incorporation of label was found in the deeper layer from the first day onwards and in the same proportion as the incorporation in the dark incubation, suggests that there was a direct uptake of ^{13}C-bicarbonate by the bacterial community. The difference of ^{13}C incorporation pathways of bacteria in the surface sediment and those inhabiting deeper layers was further supported by the data shown in Fig. 3.3E. In the same way as δ^E, the atomic excess ^{13}C reveals the label incorporation efficiency (i.e. incorporation normalized by the biomass) and the bacteria/algae ^{13}C excess ratio reflects their relative efficiencies. The increasing ratio for the 0-1 cm layer after the first day illustrates the transfer of ^{13}C from algae to bacteria, while the ratio greater than 1 on day 1 for the subsurface layer confirms the direct uptake of ^{13}C-bicarbonate. The decreasing ratio for the subsurface layer on the following days reflects a slow burial of labelled algae.

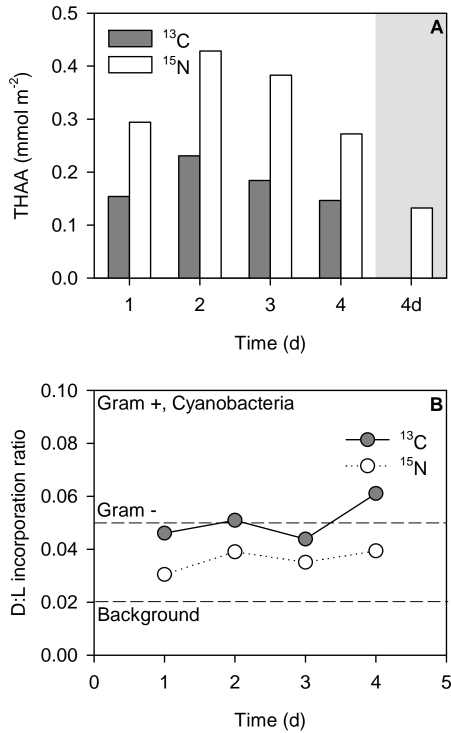

Figure 3.4. (A) ^{13}C and ^{15}N incorporation over time in THAA in the 0-1 cm layer. (B) D:L-Ala ratio of incorporation of ^{13}C and ^{15}N in the 0-1 cm layer.

Finally, ^{13}C and ^{15}N incorporation into THAA was assessed for the first centimetre layer (Fig. 3.4A). ^{13}C and ^{15}N incorporation into THAA followed the same dynamics, with a maximum on day 4, and concentrations were much lower than those of the bulk sediment. Conversely to bulk sediment, ^{15}N incorporation in THAA was higher than that of ^{13}C. D:L-Ala incorporation ratios for ^{13}C and ^{15}N (Fig. 3.4B) were well above the racemisation background value of ~0.017 (representing D-Ala produced by racemisation during sample hydrolysis) and increased slightly over time, implying an increasing contribution of prokaryotes in the label incorporation from day 1 onward.

3.4 Discussion

The experiment presented here reveals pathways of C and N through the microbial communities of permeable marine sands in the photic zone. The use of stable C and N isotopes permitted quantification of the different microbial activities that lead to the incorporation of inorganic C and N into sandy benthic biomass. The results show the dominance of photosynthetic carbon fixation and the independence of nitrogen assimilation from light conditions. The study also highlights the role of EPS for the transfer of energy from MPB to the heterotrophic communities of photic sublittoral sands.

Before discussing our findings, we examine the experimental approach. Although the chambers used were suitable for *in situ* work, the experiment was conducted in the laboratory to minimize potential losses of material, to prevent disturbance of the experimental settings by currents and waves, and to facilitate the periodical sampling. Extreme care was taken during sampling in the field to keep the sediment and its biogeochemical layering intact. The conditions of the laboratory experiment were designed to mimic as closely as possible the conditions present in the field. The stirring in the chambers induced water percolation through the upper layers of the sediment as produced by bottom currents in the field. This percolation is critical for the natural metabolism of the permeable sublittoral sands because it dominates the solute exchange between the sediment and the overlying water column (Huettel et al. 1998, Huettel & Rusch 2000). The settings used for the laboratory experiment were conservative with respect to the pore water exchange caused by the chamber stirring, therefore the results on C fixation and N assimilation should be regarded as conservative as well. Tidal currents and waves can cause higher pressure gradients at the study site than the 0.2 Pa cm^{-1} chosen for the experiment, and Cook & Roy (2006) showed that enhanced pore water percolation can increase benthic photosynthesis in sublittoral sands due to the advective relief of CO_2 limitation in microphytobenthos. The light regime was similar to the average measured by Wenzhofer & Glud (2004) under similar settings, and the water temperature was adjusted to in-situ temperature. The very work intensive and time consuming processing of the samples and associated high costs of the different analyses did not permit replication of the experiment. However, the results of the different analyses confirm each other supporting the general conclusions of the study.

3.4.1 Sediment microbial community

Pigment analysis showed that MPB biomass was homogeneously distributed at least within the first 2 cm and did not vary over the duration of the experiment. This was confirmed by the PLFA data showing an even distribution down to 5 cm deep. These

results support previous studies which showed that large quantities of living MPB can be found as deep as 20 cm in sandy sediments (Steele & Baird 1968, Jenness & Duineveld 1985). In sandy sediments, living microalgae include not only BMA but also some phytoplankton (Steele & Baird 1968) which could explain the presence of haptophytes in the sediment. This hypothesis is supported by the fact that 19'-hexanoyloxyfucoxanthin was more abundant in the deeper layers than at the sediment surface. Although diatoms dominated the MPB biomass (accounting for ~60%), the sediment composition also revealed a large fraction of cyanobacteria, which represented ~30% of MPB biomass. While one might argue the accuracy of the method used to obtain these estimates (i.e. CHEMTAX analysis instead of cell counts), cyanobacteria presence was confirmed by microscopic observations. Moreover, these results also agreed with the relative average fractions of diadinoxanthin and zeaxanthin (~62 and ~38% respectively), accessory pigments abundant in diatoms and cyanobacteria, respectively. The rather large proportion of cyanobacteria was consistent with previous studies that reported abiotic factors like warm temperatures and coarse sediment to be favourable conditions for their development (Watermann et al. 1999).

Based on the often used C:Chl *a* ratio of 40 (de Jonge 1980), MPB biomass of the top cm would have been overestimated (~2600 mmol C m^{-2}). More recent estimates based on a broader range of phytoplankton cultures suggest a ratio of 34 (Dijkman N.A., pers. comm.). This value was estimated from the same cultures that were used to estimate MPB PLFA concentrations (Dijkman & Kromkamp 2006; Dijkman N.A., pers. comm.) and would imply a MPB biomass of about 2250 mmol C m^{-2}, still well above the 1411 mmol C m^{-2} estimated from PLFA. Both methods used to estimate the MPB biomass were based on conversion factors derived from cultures of planktonic microalgae and cyanobacteria (Brinch-Iversen & King 1990; Dijkman & Kromkamp 2006; Dijkman N.A., pers. comm.) and perhaps were not representative for natural communities and benthic primary producers. Nevertheless, we remain confident that the MPB biomass at our study site lies between those estimates, allowing us to infer diatoms and cyanobacteria biomasses of 931 – 1482 mmol C m^{-2} and 466 – 741 mmol C m^{-2}, respectively (for the 0 – 2 cm layer). These estimates for MPB in subtidal permeable sediment are high, but within the range of biomass found in sheltered silty intertidal areas (Sundback et al. 1991, Underwood & Kromkamp 1999, Middelburg et al. 2000, Cook et al. 2004a). They are, however, significantly higher than estimates reported for subtidal sandy sediment (Sundback et al. 1991). Finally, using Chl *a* as proxy would yield MPB biomass estimates similar to those reported by Cahoon (1999), provided these estimates were limited to the surface sediment (128±101 mg Chl *a* m^{-2}). Applying this value to the whole sediment depth documented for pigments (0-2 cm), the biomass in the present study would be six times higher.

3.4.2 Algal production, EPS exudation and transfer to bacteria

MPB production based on oxygen concentration changes in light-dark incubations and the ^{13}C incorporation in bulk sediment over the first day led to very similar results (1.68 and 1.66 mmol C m^{-2} h^{-1} respectively). MPB production in these subtidal sediments was similar to that reported for sandy and silty intertidal sediments for the same period of the year (Barranguet et al. 1998). However, considering a daily production with 12 hours daylight, MPB production was slightly lower than that measured in two shallow subtidal sediments investigated in the summer (Sundback et al. 1996) as well as that measured *in situ* in deeper sandy sediments sites on the South Atlantic Bight (Jahnke et al. 2000). Our results are also lower than the average value proposed by Cahoon (1999) from a compilation of data from various shallow subtidal locations, which showed extreme variability (bearing in mind that those data were extrapolated to yearly production).

Although the label added was flushed away after 9 hours, sediment ^{13}C and ^{15}N enrichments (Fig. 3.2A,B) and incorporations (Fig. 3.3A,B) continued increasing after day 1, and maxima were reached on day 2. Day 3 still showed higher enrichments and incorporations than day 1. A very heterogeneous distribution of microbial biomass in sandy sediments (and accordingly different microbial biomasses among cores) would be the simplest explanation for this continued incorporation. However, stable isotope enrichment (δ^E) is independent from C biomass, and bulk sediment incorporation was normalized to C biomass to remove effects of spatial heterogeneity. An alternative explanation may be the retention of ^{13}C-DIC in sediment pore-water till day 2 and subsequent use by MPB for photosynthesis on day 2. Assuming that all the 10% labelled DIC would have remained within the pore water of the top 5 cm of sediment after two times flushing of the overlying water and that this DIC would have been mixed in ~200 L of unlabelled water this would reduce ^{13}C-DIC to ~0.04% of the total DIC pool. If the primary production rates remained the same, this would have led to an incorporation of ~5 10^{-3} mmol ^{13}C m^{-2} on day 2, which is almost two orders of magnitude lower than the actual difference between $I_{day\ 1}$ and $I_{day\ 2}$. Another possible explanation for the delayed maximum in enrichment and incorporation may be that MPB concentrated inorganic carbon internally. Such an internal carbon pool would be advantageous for organisms experiencing carbon limitation during photosynthesis. This possibility of accumulating inorganic carbon internally is also consistent with the delayed incorporation of ^{13}C into PLFA (i.e. MPB biomass) with regard to bulk sediment ^{13}C enrichment (Fig. 3.3A,C). The accumulated DIC within the microalgal cells and cyanobacteria then was later used for biomass production.

Our data also revealed some insights into the complex dynamics of allocation of fixed C. While total carbon fixation was assessed by quantifying bulk sediment incorporation of

^{13}C, the part allocated to microbial biomass was estimated with two independent methods: via incorporation into PLFA and THAA. ^{13}C incorporation into THAA accounted for 12 ± 1% of the bulk sediment incorporation and 27 ± 3% of total microbial biomass incorporation estimated from PLFA. THAA are considered to contribute ~45% to microalgal biomass (Brown 1991, Cowie & Hedges 1992), and ~50% to bacterial biomass (Simon & Azam 1989, Cowie & Hedges 1992). Therefore, given their relative contribution to microbial biomass, microbial incorporation of ^{13}C was estimated using a $^{13}C_{THAA}$:$^{13}C_{Biomass}$ ratio of 0.46 (mol:mol). In addition to the ^{13}C incorporation by the microbial compartment, a fraction of C fixation was very quickly transferred from primary producers to meiofauna and macrofauna (Chapter 4). However, the fauna compartment will be neglected for the following interpretations as maximum meiofauna ^{13}C assimilation represented a maximum of ~15 µmol m^{-2} for the top cm of sediment. Moreover, total respired ^{13}C after one day estimated from measurements of ^{13}C-DIC releases (Chapter 4) was about 3% of what was fixed and all macrofauna was handpicked and pulled out of the sediment samples before analysis and therefore was not part of the C content.

Assuming that almost all fixed ^{13}C remained in the sediment, we estimate that 45±2% (based on PLFA measurements) or 26±2% (based on THAA measurements) of the carbon was allocated to growth of microbial biomass. In other words, between 55±2 and 74±2% of the carbon fixed did not result in biomass formation, but was diverted to exudation of EPS. These results should be considered approximate given the dependence on conversion factors all based on culture studies. Nevertheless, they stress that in our experiment, MPB allocated a major part of its production to the synthesis of EPS. Our findings confirm previous observations made in intertidal areas suggesting that 42 to 73% of C fixation can be excreted (Smith & Underwood 1998, Goto et al. 1999, Middelburg et al. 2000, de Brouwer & Stal 2001). Recently, Cook et al. (submitted) using similar permeable sediments in mesocosm experiments reported that 50% of the fixed carbon was exuded in both nutrient depleted as well as replete conditions. In our experiment, nitrate was added as a pulse allowing us to stay as close as possible to the natural conditions, which appeared nutrient depleted (Hedtkamp 2005). In natural conditions, MPB exudation of EPS is significantly higher than that of phytoplankton (Goto et al. 1999) and therefore can provide a substantial substrate for benthic bacteria (Middelburg et al. 2000).

Heterotrophic bacteria living in sandy sediments are attached to sand grains and many of them grow close to active MPB (Huettel et al. 2003). While the response of heterotrophic bacteria on phytodetritus has been studied in permeable sediments (Sundback et al. 1996, Huettel et al. 2003, Buhring et al. 2006), little is known about the links between MPB and heterotrophic bacteria. In our experiment, MPB biomass was high, there was no apparent sign of MPB detritus (absence of pheopigments), and about

50 to 76% of C fixation was directed to EPS. These conditions explain the immediate and continuous incorporation of ^{13}C into bacterial biomass at the sediment surface (Fig. 3.3D,E), and support the findings of previous studies which demonstrated the strong affinity of bacteria for labile organic carbon from colloidal sources (Middelburg et al. 2000, Goto et al. 2001, van Oevelen et al. 2006a). However, the amount of label transferred to bacteria paradoxically remained low considering the amount of EPS exuded. A first hypothesis for this limited transfer is that bacterial activity, with respect to the transfer of C fixed by MPB, shows seasonal variations, low in the summer and higher in the winter (Sundback et al. 1996). Another explanation could be that the isotope signal was too diluted because only 10% of newly excreted EPS was labelled and taken up into a large unlabelled EPS pool. For example on day 2, assuming that EPS incorporation was ~1 mmol ^{13}C m^{-2} (difference of incorporation between bulk sediment and microbial compartment estimated from PLFA), we derive a total EPS production of 10 mmol C m^{-2} (DIC pool was 10% labelled). The EPS standing stock estimated from the difference between sediment organic C content (2400 mmol C m^{-2}) and MPB biomass (700 mmol C m^{-2}, estimated from PLFA) was 1700 mmol C m^{-2}. Thus, the EPS ^{13}C-labelled fraction was 1/1710 = 5.8 10^{-4}. Considering that bacterial ^{13}C incorporation on day 2 was 14 10^{-3} mmol ^{13}C m^{-2} and if we assume that bacteria relied exclusively on EPS as their substrate, we obtain a bacterial secondary production of 14 10^{-3}/5.8 10^{-4} = 24.1 mmol C m^{-2}. Although this bacterial secondary production estimate should be considered approximate at best, it clearly highlights the high bacterial activity taking place in permeable sediments. Based on a bacterial biomass of 150 mmol C m^{-2} (estimated from PLFA) and the secondary production, we derive a turnover of ~0.16 d^{-1}, which is twice that found on an exposed sandy beach (Koop & Griffiths 1982), and within the range reported for muddy and sandy coastal sands in the Mediterranean (Luna et al. 2002). Similar calculations for day 1 would yield significantly lower turnover rates, perhaps reflecting a delay related to the activation of "dormant" bacteria (Luna et al. 2002). Contrary to the bacteria to MPB ratio of excess ^{13}C observed at the sediment surface (Fig. 3.3E), the deeper layer showed an opposite trend suggesting a different ^{13}C incorporation pathway.

3.4.3 Chemoautotrophy

The dark incubation showed significant incorporation of ^{13}C on day 4 with higher incorporation into the deeper layer (Fig. 3.3A), although this incorporation was relatively low compared to that found in the light incubations. Because no photosynthesis took place in the dark core, incorporation could either be fuelled by an exogenous source of labelled organic matter (contamination of the dark cores by labelled substrate originating from the light cores) or from direct incorporation of inorganic ^{13}C into the microbial compartment by anapleurotic C-fixation or chemoautotrophy. Transfer of labelled DOC was very

limited as demonstrated by Cook et al. (submitted). Anapleurotic carbon fixation might have occurred, but cannot explain the incorporation of ^{13}C in PLFA in the dark (Boschker and Middelburg, 2002), indicating that chemoautotrophy was involved.

This is supported by bulk sediment immediate incorporation of ^{13}C in the deeper layers well below the photic zone, which we could not attribute to ^{13}C assimilation from buried ^{13}C-labelled EPS but only to direct ^{13}C-DIC fixation through chemoautotrophy. This is supported by the PLFA data showing that most dark and subsurface ^{13}C enrichment occurred in bacterial specific biomarkers (Fig. 3.2D). Although EPS may be transported into deeper layers and consumed by bacteria very quickly, it is unlikely that the burial alone would be responsible for all the incorporation in the deeper layers. Chemoautotrophic carbon fixation is also consistent with the incorporation profile, showing more bacterial ^{13}C in the deeper layers (1 – 5 cm) than at the sediment surface (Fig. 3.3D), and also by the opposite trend of the bacteria to MPB ratio of excess ^{13}C for the 0-1 and 1-2 cm layers (Fig. 3.3E). Little is known on chemoautotrophy in permeable sediment and on its co-occurrence with heterotrophic bacterial activity, but the importance of the coupling between chemoautotrophy and heterotrophy has previously been shown for muddy sediments (Kepkay & Novitsky 1980). Nevertheless, chemoautotrophy remained relatively low compared to bacterial heterotrophy (~1% if we consider that chemoautotrophic bacteria were 10% labelled, as their substrate). Our findings support previous evidence of the importance of chemoautotrophy in subsurface sandy sediments (Thomsen & Kristensen 1997). Parallel experiments at the study site also showed a large HCl-extractable Fe^{2+} pool (presumably associated with reduced sulphides), which was rapidly re-oxidised when the sediments were exposed to O_2, supporting the hypothesised presence of an active chemoautotrophic community (Cook et al. 2007). Visual observation of white deposits at the sediment surface in the middle of the cores sampled on day 3 and 4 (both dark and light) provide additional support for chemoautotrophy. Although, no further analysis was performed to determine the nature of those white spots, they were likely caused by mass accumulations of *Beggiatoa* spp., filamentous sulphur oxidizing bacteria that grow at anoxic/oxic interfaces where sulphide and oxygen meet. The presence of *Beggiatoa* in the vicinity of our sampling site was reported by de Beer et al. (2005). The Beggiatoa spot formed in the centre of the core surface where anoxic porewater was released from the sediment staining the sand black, characteristic for reduced sediment. *Beggiatoa* spp.'s chemoautotrophic activity has been previously assessed in cultures (Nelson & Jannasch 1983) and found to enhance sulphide oxidation by 3 orders of magnitude compared to spontaneous chemical oxidation (Nelson et al. 1986).

3.4.4 C fixation vs. N assimilation

Although the bulk sediment showed similar patterns of ^{13}C and ^{15}N incorporations, both in dynamics and amplitude, one has to consider the labelled fraction in each pool. The DIC pool was 10% ^{13}C-DIC, while the N pool was ~100% labelled, because there was almost no dissolved N in the overlying water and in the first 5 cm of sediment (Hedtkamp 2005). The measurements of bulk sediment C and N incorporation revealed that the C:N ratio of assimilation increased from 12.7 to 14.9 between days 1 and 4, much larger than expected for microbial growth, hinting at a large fraction of EPS produced. If we attribute all N assimilation to MPB biomass (i.e. assuming that EPS does not contain N), and combine this with C fixation from the PLFA data (MPB carbon biomass), the C:N ratio of assimilation increased from 5.4 on day 1 to 6.8 on day 3, values close to Redfield ratio. Similar to the ^{13}C incorporation into THAA, ^{15}N incorporation into THAA was less than that of the bulk sediment incorporation but represented a higher relative contribution of 32 ± 4%. The difference between C and N behaviour is consistent with the fact that N_{THAA}:$N_{Biomass}$ is higher (0.62; Cowie & Hedges 1992). Finally, the assimilation C:N ratio based on the THAA did not vary and had an average value of 5.2. Both the PLFA and THAA based approaches are consistent with Redfield ratios for microbial growth and significant allocation of fixed carbon to EPS.

The most remarkable difference observed between C and N fixation was the incorporation in the dark. While ^{13}C fixation in the dark was limited to chemoautotrophy, total ^{15}N fixation in the dark was ~75% of the light incubation on day 4. Diurnal variations in carbon and nitrogen assimilation by planktonic communities were subject of numerous studies (Berges et al. 1995, Clark et al. 2002, Needoba & Harrison 2004, Holl & Montoya 2005). Phytoplankton fixes C during light exposure, but can assimilate nitrate in the lights as well as in the dark (Dugdale & Goering 1967). To our knowledge, dark assimilation of nitrate by MPB has only been suggested in the literature a few times (Sundback & Snoeijs 1991, Rysgaard et al. 1993, Cook et al. 2004b). Bacteria (with low C:N ratio) can also assimilate inorganic N to balance the high C:N ratio of their substrate (Goldman & Dennett 2000). Given that heterotrophic bacteria in these sediments rely primarily on EPS, one might expect significant bacterial N uptake (Kirchman & Wheeler 1998, Allen et al. 2001, Van den Meersche et al. 2004). The present study is the first to report such a high assimilation of nitrate in the dark through assessing the actual ^{15}N incorporation into biomass via THAA rather than bulk sediment. The next step would be to determine the relative contribution of diatoms and cyanobacteria to nitrate assimilation.

3.4.5 ^{13}C and ^{15}N incorporation in D-Ala

While THAA is informative of the biomass of all living organisms in the sediment, D-Ala provides a tool to assert the significance of the prokaryotes. D-Ala, contrary to L-Ala, which is found in all living organisms, is only present in the peptidoglycan of prokaryotes. Therefore, incorporation of label (^{13}C and ^{15}N) into D-Ala can be attributed to assimilation by heterotrophic bacteria (Hbac), chemoautotrophic bacteria (Cbac) or cyanobacteria. D-Ala is the only biomarker which allows following the fate of nitrogen through all microbial compartments.

The ^{13}C and ^{15}N D/L-Ala incorporation ratios (Fig. 3.4B) provide a direct indication of the contribution of prokaryotes/bacteria (i.e. Hbac, Cbac and cyanobacteria) to total microbial (i.e. prokaryotes/bacteria + BMA) ^{13}C and ^{15}N incorporation. For ^{13}C, the low incorporation into $PLFA_{bacsp}$, indicates that ^{13}C incorporation into microbial biomass was dominated by MPB. Therefore, the measured ^{13}C D/L-Ala ratios of incorporation illustrate the uptake of ^{13}C by BMA (corresponding to a D/L-Ala ratio around racemisation background, 0.017, as BMA do not contain D-Ala) and cyanobacteria (with a D/L-Ala ratio ~0.1, see update Veuger et al. 2007b). The average ^{13}C D/L-Ala ratio of incorporation of 0.046 indicates that cyanobacteria contributed ~35% to total ^{13}C incorporation, leaving a ~65% contribution for BMA. These contributions are in good agreement with their relative biomasses based on pigment analyses (Fig. 3.1).

Interpretation of the ^{15}N D/L-Ala ratios of incorporation is less straightforward as these values may also include a significant contribution by Hbac and/or Cbac (D/L-Ala ratio between 0.05 and 0.1, see update Veuger et al. 2007b). Therefore, we can only derive an estimate of the prokaryotic (cyanobacteria + Hbac + Cbac) contribution total ^{15}N incorporation (prokaryotes + BMA), which was 23-58%.

Chapter 4

The pivotal role of benthic primary producers in moderating carbon and nitrogen flows through food webs in sandy sediments

In preparation for Limnology and Oceanography

4.1 Introduction

Although bare and apparently unvegetated sediments might not be visually appealing, they nevertheless often harbour a very rich diversity of flora and fauna. The microphytobenthos (MPB), which comprises microscopic photosynthetic organisms (e.g. diatoms and cyanobacteria) living at the sediment surface, is highly dependent on light availability (Barranguet et al. 1998). Recently, Gattuso et al. (2006) reported that about 33% of the global shelf receives enough light to sustain a positive net community production (gross primary production > respiration) indicating that benthic primary production contributes significantly to global shelf production and thus to global marine production. Together with phytoplankton, MPB represents the principal direct or indirect source of food for various heterotrophic organisms living in sediments (Heip et al. 1995). On the one hand, it can directly sustain grazers, deposit feeders and, through predation, higher trophic levels, and after resuspension also suspension feeders (Miller et al. 1996, Sundback et al. 1996, Cahoon 1999, Herman et al. 2000, Middelburg et al. 2000), altogether constituting the classical benthic food web. On the other hand, MPB can allocate part of the photosynthesis to the production of carbohydrates (Smith & Underwood 1998, Goto et al. 1999) in different forms but mainly exopolymeric

substances (EPS) that will be available for deposit feeders, bacteria and organisms predating on them (Cahoon 1999, Goto et al. 2001).

Recognition of the important contribution of MPB to coastal primary production and its pivotal role in moderating carbon flow through food webs (Middelburg et al. 2000) has initiated much research, but there are still many unresolved issues. First, coastal food web studies have often been confined to accreting silty and muddy sediments (e.g. depositional shelf sediments, estuaries or coastal lagoons) and more specifically to the intertidal area (Reise 1979, Heip et al. 1995, Deegan & Garritt 1997, Middelburg et al. 2000, Carman & Fry 2002), leaving sandy, permeable sediments poorly documented (Sundback et al. 1996, Buhring et al. 2006). Studies of benthic respiration confirm this bias towards accreting sediments (Middelburg et al. 2005). Nevertheless, sandy sediments represent about 70% of the continental shelf (Emery 1968). In contrast with fine-grained sediments, where grazing and mineralization occurs within the top millimetres of sediment and solute transfer is diffusion-limited, grazing and mineralization in permeable sediments occurs much deeper and solute transfer is strongly enhanced trough pore water advection (Huettel & Rusch 2000, Rusch & Huettel 2000). A second observation is that most (benthic) food web studies are limited to one kingdom of life or to one specific taxon or size class while neglecting interactions with other size classes or taxa. The smaller, most abundant organisms at the base of the food web (i.e. the microbial domain) are either ignored or not resolved and lumped with detritus into the bulk sediment compartment. This is unfortunate since many studies have reported variable dependence of metazoans on algae, bacteria and detritus (Epstein & Shiaris 1992, Herman et al. 2000, Middelburg et al. 2000, Pinckney et al. 2003). This lack of resolution in the lower domains also hinders disentangling detritivorous, bacterivorous and herbivorous food chains in sediments (van Oevelen et al. 2006c). A third observation, following from the previous point, is that trophic interactions can be more complex than they appear. A powerful and straightforward way to address food web complexity, particularly for small organisms is to use stable isotope measurements. ^{13}C and ^{15}N stable isotopes allow inferring the carbon source and the trophic level respectively (Fry & Sherr 1984, Vander Zanden & Rasmussen 1999, Herman et al. 2000, Ponsard & Arditi 2000, Smit 2001). However, using ^{13}C as a proxy for the carbon food source, for example, without considering potential pitfalls can yield misinterpretations. Zoobenthos inhabiting sediments are not only sustained by MPB but also by deposition of phytoplankton. While ^{13}C natural abundance signatures of MPB and phytoplankton are often distinct (France, 1995), it can be difficult to clearly elucidate the feeding behaviour of some heterotrophic organisms as the MPB is a heterogeneous mixture of microalgae with different isotopic signatures. For example, benthic cyanobacteria and phytoplankton may not be easily distinguished as a food source, if they show similar ^{13}C-depleted isotopic signatures. This is particularly disadvantageous when

trying to unravel resource partitioning or ontogenetic shifts (Carman & Fry 2002, Pinckney et al. 2003).

An efficient way to overcome this problem of overlapping isotope signatures of potential food resources is to combine a natural abundance stable isotope investigation with a deliberate tracer stable isotope addition experiment (Herman et al. 2000, Carman & Fry 2002). Studies during the last decade have shown that it is possible to selectively label different food sources (phytodetritus: Blair et al. 1996, MPB: Middelburg et al. 2000, and bacteria: van Oevelen et al. 2006b) and follow their fate within the benthic food web. Moreover, through the use of compound specific isotope analysis of biomarkers, specific for (groups of) organisms, it is now possible to include the microbial compartments of food webs as well (Middelburg et al. 2000, Boschker & Middelburg 2002).

The present study, coupled to its companion paper (Chapter 3), unravels with an unprecedented resolution the different trophic interactions of the benthic community in a subtidal sandy sediment. We combine a natural abundance stable isotope approach with a dual stable isotope pulse-chase experiment. Freshly sampled cores of sandy sediment were incubated in the laboratory and its MPB was labelled with ^{13}C-bicarbonate and ^{15}N-nitrate. The fate of MPB was then assessed within all the different heterotrophic compartments of the benthos.

4.2 Materials and Methods

4.2.1 Study site and experimental setting

The study took place in List (Germany) on the island of Sylt in the Wadden Sea, in July 2004. The sampling site, called the Hausstrand was situated south of the harbour close to the site described earlier by de Beer et al. (2005). The area is exposed to the east and strongly influenced by North-South tidal currents parallel to the shore and wind driven waves. The sediment is a coarse silicate sand with a median grain size of 350 µm and a porosity of 0.42.

At low tide, 6 sediment cores (5 transparent and 1 black - used later as a dark control) containing approximately 15 cm of sediment and the overlying water were retrieved from the shallow subtidal area (~1.5 m deep) and brought back to the laboratory. The cores consisted of 19 x 33 cm (inner diameter x height, sediment surface equivalent to 28.35 10^{-3} m^{-2}) acrylic cylinders closed at both ends by PVC lids. The upper lid held a 15 cm diameter rotating disc, powered by a motor inserted on top and allowing circulation of the water column. The stirring disks were set to 40 rpm, providing an advective flux of

porewater into the sediment (Huettel & Gust 1992b). All the cores were immersed in sea water in a tank of ~ 200 L, maintained under *in situ* conditions of temperature (20°C) and constantly aerated. Chamber upper lids were elevated about 1 cm above the chambers edge to allow aerated water to circulate within the whole system. The circulation of water in the tank was enhanced using a submersible water pump placed at the bottom of the tank. The system was left to settle 24 h prior to the start of the experiment.

4.2.2 Labelling and incubations

On the following day, one of the transparent cores was immediately sampled to provide background values (T = 0) for the different analyses described hereafter. The remaining cores were closed and received a pulse of 0.85 mmol $NaH^{13}CO_3$ (~ 11 mg ^{13}C-DIC), equivalent to a 10% ^{13}C-labelling of the DIC pool (~1950 µmol L^{-1}), and 0.17 mmol $Na^{15}NO_3$ (~ 2.5 mg ^{15}N), corresponding to ~90% labelling of the DIN pool (< 5 µmol L^{-1}). The four remaining transparent cores were illuminated by artificial light providing a homogenous irradiance of 185 µmol quanta m^{-2} s^{-1} at the sediment surface, in the range of that observed in the field for that period. The MPB was labelled during a light exposure of 9 hours. After 9 hours, the light was switched off and the water column of the 5 cores was flushed twice to remove labelled bicarbonate and nitrate and replaced with seawater from the study site. Care was taken to avoid disruption at the sediment surface. When in the dark, the cores were always kept opened and immersed in the trough to allow aeration and mixing of the water within the whole system. A 9:15 light:dark cycle was maintained during the 4 days of the experiment.

Every day (T = 0, 1, 2, 3 and 4 d) total respired ^{13}C was estimated from changes in ^{13}C-DIC concentrations over a 4 hours period. Four hours prior to every illumination period, each core was closed and a small amount of its overlying water was sampled into a 12 mL headspace vial to which 12 µL HgCl was added to kill the sample; after 4 h incubation in the dark and just before turning on the light, another water sample was taken and the concentration difference between those two points allowed estimating ^{13}C respiration. Integrated over the whole time scale of the experiment it permitted estimation of total respired ^{13}C.

Immediately after each period of illumination, one core was taken out of the tank and sampled, until day 4, when both the last transparent and the black core were sampled together. The cores were sampled using 5 small subcores (3.56 cm inner diameter, ~ 10 cm^2). For C, N, and phospholipid-derived fatty acids (PLFA) analysis, 3 small subcores were sliced 0-1, 1-2, 2-3, 3-4, 4-5 and 5-10 cm, the same layers pooled together and freeze-dried. Another small core was sliced the same way and kept for meiofauna analysis. The last one was sliced 0-2, 2-4, 4-6, 6-8, 8-10 and 10-20 mm, freeze-dried and

used for analysis of pigments concentrations. All sediment samples were stored in a freezer until analyzed.

4.2.3 Fauna sampling

Macrofauna. On the same day as the field sampling of the 6 sediment cores, two extra cores with ~15 cm of sediment were pooled into a large bucket and the sediment sifted on a 1 mm mesh size sieve. Retained macrofauna was handpicked, pooled into a large container with sea water and gently brushed to remove mucus, faeces and particles. Animals were then sorted to species level into different glass vials and stored in the freezer. Macrofauna from the different labelled cores, sampled at day 1, 2, 3 and 4, was extracted the same way from the remaining sediment of each big core. Prior to analysis, animals were dried individually in a stove for 48 hours at 60°C to estimate their dry weight. For each core, macrofauna individuals belonging to the same species were pooled together and ground into a fine homogenous powder, for their analysis of organic C, N and isotopic signatures.

Meiofauna. Animals were neither stained nor fixed to avoid addition of exogenous C that might contaminate the samples with a different isotopic signature. Sediment samples were thawed at room temperature and thoroughly rinsed with distilled water on a 38 µm sieve. Meiofauna was extracted from sediment with colloidal silica (Ludox HS 40, DuPont) with a density of 1.31 g cm^{-3} following the protocol proposed by (Burgess 2001). In brief: the sediment sample is washed with Ludox® into a 50 mL disposable polypropylene centrifuge tube. The tube is capped and thoroughly mixed using a vortex at a gradually decreasing speed and finally centrifuged. The supernatant is rinsed again with distilled water in the sieve and finally poured into a Petri dish for counting and picking. The sediment pellets were set aside for verification of its remaining content.

All samples were treated successively to avoid degradation of material. The animals were sorted to major taxonomic levels and counted under a stereo-microscope. They were finally transferred to Sn cups for C and N analysis. Animals with a $CaCO_3$ shell were transferred to Ag cups and acidified to remove inorganic C. Due to the large amount of material needed for N analysis (e.g. > 100 individuals for nematodes, >200 for tardigrades), priority was given to C analysis thus only a few taxa could be analyzed for N.

4.2.4 Analyses and Data handling

Pigment samples were analyzed by reverse-phase high-performance liquid chromatography (Barranguet et al. 1998) and data expressed in mg m^{-2}. Relative

proportions of microphytobenthic taxa were estimated using the CHEMTAX program (Mackey et al. 1996).

Small fractions of the sediment samples were ground in agate mortar to obtain a homogeneous and fine powder. Organic carbon and nitrogen content and isotopic composition (^{13}C and ^{15}N) of sediment, macrofauna and meiofauna were measured using a Carlo Erba/Fisons/Interscience elemental analyser coupled on-line via a conflo interface to a Finnigan Delta S isotope ratio mass spectrometer.

PLFA for all the layers down to 5 cm deep were extracted from approximately 6 g of sediment per layer, following the method of (Boschker et al. 1999, Middelburg et al. 2000) and their concentrations were determined by gas chromatograph-flame ionization detection (GC-FID). PLFA carbon isotopic composition was determined using a gas-chromatograph combustion-interface isotope ratio mass spectrometer (GC-c-IRMS). Bacterial and MPB carbon content in the sediment were estimated from the PLFA concentrations. The bacterial carbon biomass (C_{bac}, expressed in mg C m^{-2}) was calculated from the bacterial PLFA ($PLFA_{bac}$) as $C_{bac} = PLFA_{bac}/a$ where a is the average PLFA concentration in bacteria (0.073 mg of carbon PLFA per mg of Cbac; Brinch-Iversen & King 1990). $PLFA_{bac}$ was estimated from the bacteria-specific PLFA ($PLFA_{bacsp}$: i14:0, i15:0, a15:0 and i16:0; specific of both heterotrophic and chemoautotrophic bacteria) as $PLFA_{bac} = \Sigma\, PLFA_{bacsp}/b$, where b is the average fraction-specific bacterial PLFA (0.14 mg of carbon bacterial-specific PLFA per mg of carbon bacterial PLFA; Moodley et al. 2000). MPB carbon biomass (C_{MPB}, expressed in mg C m^{-2}) was calculated from the difference between the sum of all PLFA and $PLFA_{bac}$ as $C_{MPB} = (\Sigma\, PLFA - PLFA_{bac})/c$ where c is the average PLFA concentration in MPB (0.053 mg of carbon PLFA per mg C_{MPB}). c was calculated as the weighted average PLFA concentrations in a mix of different phytoplankton monocultures similar to the MPB composition (Dijkman, N.A. pers. comm.).

Stable isotope data are expressed in the delta notation ($\delta^{13}C$ and $\delta^{15}N$) relative to the Vienna Pee Dee Belemnite (VPDB) and air standards, for carbon and nitrogen respectively and calculated from the stable isotope ration (R): $\delta X = (R/R_{std}-1)\times 1000$, where X is ^{13}C or ^{15}N and R the $^{13}C/^{12}C$ ratio measured in the sample and in the standard (for C, $R_{std} = R_{VPDB} = 0.0111797$ and for N, $R_{std} = R_{air} = 0.0036765$). Following, Maddi et al (2006) we use the enrichment (δ^E notation) as a measure of label enrichment. $X\delta^E = [(\delta X_s+1000)/(\delta X_b+1000)-1]\times 1000 = (R_s/R_b-1)\times 1000$, where R_b is the isotope ratio in the background and R_s in the sample. This enrichment notation δ^E can be compared directly with the previously used specific uptake ($\Delta\delta$ notation; $\Delta\delta = 1000\times(R_s-R_b)/R_{std}$), but has the advantage that it is exact and thus accurate for high levels of enrichment. The incorporation (I), i.e. the total uptake of label, as defined by Middelburg et al. (2000), is expressed in mg X m^{-2}, as a product of the atomic excess (E) and a quantity (organic C, N,

C-PLFA). E = F_s-F_b, is the difference between the fraction of the sample and the one of the background, with F = R/(R+1).

4.3 Results

4.3.1 Benthos composition

Fauna. Although meiofauna was sampled down to 15 cm deep in the sediment, we report only the first 3 cm layers due to a radical decrease of densities with depth, precluding isotope analyses (Fig. 4.1). Nematodes and copepods (harpacticoid), omnipresent in sediments, were the two most abundant taxa with about 640 and 400 10^3 ind m^{-2} respectively within the first cm layer. Tardigrades were the third most abundant taxon with approximately 100 10^3 ind m^{-2}. With the exception of nematodes, all taxa were mainly restricted to the first cm layer of sediment. Although adult bivalves and gastropods are part of the macrofauna, the juvenile forms (j) (the gastropod *Littorina littorea* and

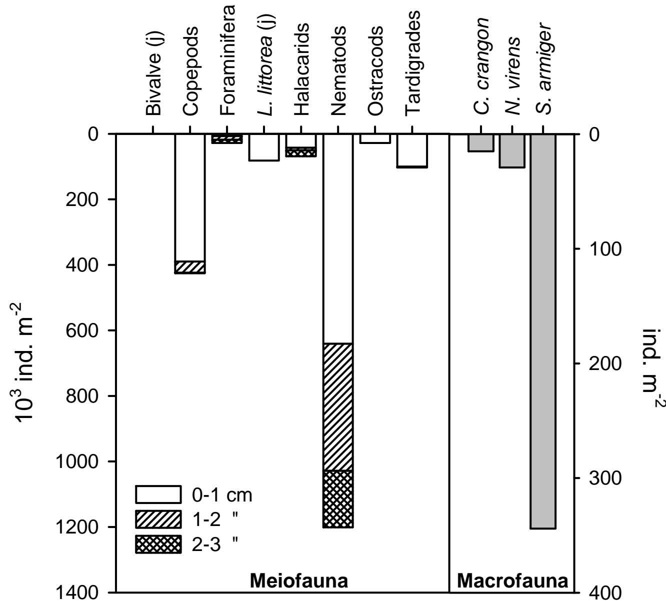

Figure 4.1. Meiofauna (left, j = juveniles) and macrofauna (right) vertical densities (mean, n = 6) for the top 3 cm and depth integrated 15 cm respectively. Standard deviations are not show for clarity.

unidentified bivalves) were included in the meiofauna compartment due to their microscopic size class.

The macrofauna composition was rather simple and consisted of two species of deposit feeding polychaetes and one decapod predator (Fig. 4.1). The polychaete *Scoloplos armiger* was the most abundant species with about 340 ind m^{-2}. The other polychaete present in the study, *Nereis virens*, was far less abundant with approximately 30 ind m^{-2}. Some young and small individuals of the brown shrimp *Crangon crangon* was observed in 3 cores out of 6 and had an average density of 17 ind m^{-2}. As *C. crangon* is a highly motile epifaunal species its estimated density should be taken with caution as it might have escaped during sampling.

The horizontal distribution of fauna was very patchy as reflected in macrofauna and meiofauna organic C and N biomasses for the top 3 cm of sediment (Table 4.1). The biomass of the fauna was calculated with the mean weight. However, to overcome high variability with individual weight, nematodes, copepods and *L. littorea* biomasses were calculated with their median weight. The standard deviation (SD) of the biomass was calculated with that of the densities so as to reflect patchiness. The coefficient of variation

Table 4.1. Fauna biomass in mg m^{-2} (mean or †median individual weight × mean ± SD of densities, n = 6) for the top 3 cm of sediment. (j): juveniles. *Scaled to 3 cm.

	Taxon	C_{org}	N
Macrofauna	*C. crangon*	17 ± 18	4 ± 4
	*N. virens**	41 ± 20	10 ± 5
	*S. armiger**	420 ± 163	93 ± 36
Meiofauna	Bivalves (j)	53 ± 72	
	Copepods†	91 ± 36	16 ± 6
	Foraminifera	7 ± 4	
	L. littorea (j)†	247 ± 157	56 ± 36
	Halacarids	21 ± 6	
	Nematodes†	258 ± 91	55 ± 19
	Ostracods	5 ± 2	
	Tardigrades	7 ± 3	

(sd/mean) was always higher than 25%, on average 60%. Altogether, the meiofauna accounted surprisingly for about 60% of the total metazoan biomass. Nematodes and juveniles of *L. littorea*, followed by copepods, contributed most to the total biomass of the meiofauna compartment. Other species represented only a very small fraction of the total faunal biomass.

Microbial compartment. MPB composition and biomass was assessed from pigment concentrations and confirmed with the PLFA composition of the bulk sediment (for further details, please see Chapter 3). Chl *a* concentration was 793 ± 70 mg m^{-2} for the top 2 cm of sediment and distributed rather uniformly with depth. Pigment analysis revealed a significant amount of fucoxanthin together with Chl *c* and diadinoxanthin, suggesting a major contribution of diatoms. Zeaxanthin accounted for a moderate fraction of the pigments, implying the presence of cyanobacteria. Green algae were not present as there was no Chl *b*. CHEMTAX analysis of pigment data revealed a relative contribution of diatoms, cyanobacteria and haptophytes of 60, 30 and 10% respectively. Haptophytes, that are supposedly part of the phytoplankton, may have settled prior to the sampling. Regarding their small contribution and to simplify the picture for further interpretation, we decided to neglect their presence and consider diatoms and cyanobacteria contributions of 66 and 33% respectively.

MPB composition was further confirmed through PLFA analysis. The algal-specific PLFA C20:5ω3 contributed significantly to the total PLFA present in the sediment. In addition, since the presence of green algae could not be detected (absence of Chl *b*), C18:3ω6 and C18:4ω3 PLFAs can be attributed to cyanobacteria and therefore used as a proxy for their isotopic signatures. MPB biomass was estimated from PLFA to about 17 g C_{org} m^{-2} for the top 2 cm of sediment. Bacterial biomass, estimated from the bacterial-specific PLFA (iC14:0, iC15:0, aiC15:0 and iC16:0), was 3.8 g C_{org} m^{-2} for the top 2 cm of sediment.

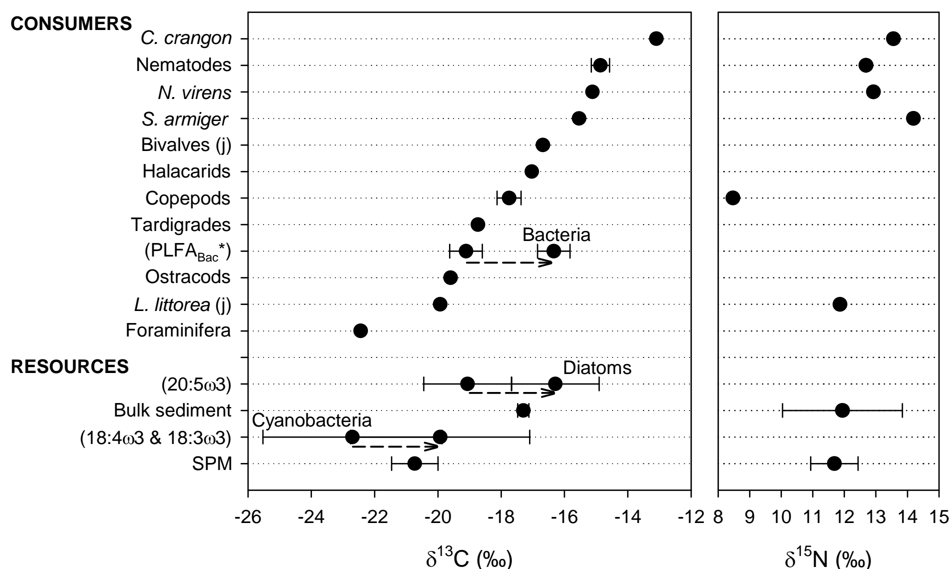

Figure 4.2. Stable isotopes background $\delta^{13}C$ and $\delta^{15}N$ values (where available) of the benthic components (mean ± SD, n = 3 if applicable). Arrows represent the shift of $\delta^{13}C$ values to account for fractionation between PLFA and biomass. * Bacterial PLFA: iC14:0, iC15:0, aC15:0 and iC16:0.

4.3.2 Natural abundance isotopic signatures

Microbial compartment. MPB (diatoms and cyanobacteria) and suspended particulate matter (SPM, i.e. mainly phytoplankton) were the two major resources for the benthic food web (Fig. 4.2). The added resolution obtained from PLFA analysis allowed us to characterize natural abundance $\delta^{13}C$ values of diatoms, cyanobacteria and bacteria. However, a correction factor has to be added to account for the carbon depletion of PLFA relative to that of the biomass (Boschker et al. 1999, Hayes 2001, Boschker et al. 2005). This constant (+2.77 ‰) was calculated assuming that $\delta^{13}C$ of the bulk sediment reflects that of the MPB and therefore, $\delta^{13}C_{Bulk\ Sediment} = 0.66\ \delta^{13}C_{Diatoms} + 0.33\ \delta^{13}C_{Cyanobacteria}$. This value is consistent with and supports previous estimates of a correction factor about +3 ‰ (Boschker et al. 1999, Hayes 2001, Boschker et al. 2005). Based on the weighted average of their specific PLFA, diatoms, cyanobacteria and heterotophic bacteria were estimated to have $\delta^{13}C$ values of -16.3 ± 1.4 ‰, -19.9 ± 2.8 ‰ and -16.3 ± 0.5 ‰, respectively. $\delta^{15}N$ of SPM and bulk sediment were similar with 11.7 ± 0.8 and 11.9 ± 1.9 ‰ respectively.

Unfortunately we lack biomarkers that would allow us to assess the $\delta^{15}N$ distinction between diatoms and cyanobacteria.

Meiofauna. Most taxa present in the study had their natural abundance $\delta^{13}C$ values within the range of the values of the different food sources, suggesting no clear dependency on either source but rather a heterogeneous diet (Fig. 4.2). However, foraminifera for instance were extremely ^{13}C-depleted, outside the range of the different food sources. Care should be taken on the interpretation of that extreme value because foraminifera were very scarce within the samples and their quality could not be asserted during extraction. Juveniles of *L. littorea* and ostracods were ^{13}C-depleted but within the range of the 3 food sources, suggesting a reliance on settled SPM and/or cyanobacteria. Juveniles of bivalves clearly showed however higher $\delta^{13}C$, more similar to that of diatoms. Nematodes showed $\delta^{13}C$ values more enriched than that of diatoms. With the exception of copepods that showed rather low $\delta^{15}N$ values, the $\delta^{15}N$ of nematodes and juveniles of *L. littorea* were similar to those of the bulk sediment or SPM.

Macrofauna. The polychaetes *S. armiger* and *N. virens* and the shrimp *C. crangon* were the heaviest with background $\delta^{13}C$ values ranging from -15.5 to -13.1 ‰, more or less similar to that of diatoms for the two polychaete deposit feeders and significantly higher for the predatory shrimp (Fig. 4.2). All macrofauna species showed background $\delta^{15}N$ values slightly higher than that of the main substrates.

4.3.3 Isotope labelling experiment

MPB isotopic enrichment was very rapid and reached its maximal value on day 2 (Fig. 4.3A). There was a pronounced difference between diatoms and cyanobacteria labelling. As estimated from PLFA data, cyanobacteria enrichment was almost two fold that of diatoms. Bacterial enrichment was rather low compared to that of MPB and reached its maximum at day 3, suggesting label transfer from MPB to bacteria. ^{13}C-δ^{E} in the dark was negligible for all microbial compartments. Total sediment enrichment (Fig. 4.3B) followed the MPB’s dynamics, reaching its maximum after 2 days, with about 70 ‰ for ^{13}C-δ^{E} and about 1200 ‰ for ^{15}N-δ^{E}. Dark ^{15}N-δ^{E} of the sediment was very important and similar to that of the incubation in the light, whilst dark ^{13}C-δ^{E} of the sediment was very low (~4‰).

Macrofauna ^{13}C and ^{15}N enrichment was clear. Unfortunately, due to the high spatial heterogeneity and low densities in these sandy sediments, we didn’t always recover enough animals and there were no data at day 1 (light incubation) and day 4 (dark incubation) for *C. crangon* (Fig. 4.3C) and at day 1 for *N. virens* (Fig. 4.3D). The predatory shrimp, *C. crangon* was the most enriched macrofauna species, although there

was high variability within the time series. *N. virens*, as an omnivorous surface deposit-feeder, was as expected much more enriched than *S. armiger* (Fig. 4.3E), a subsurface deposit-feeder. ^{13}C-δ^E in the dark was very low for all macrofauna species. Surprisingly, ^{15}N-δ^E of *S. armiger* was about twice as high in the dark as in the light incubation at day 4.

Meiofauna enrichment was significant and rapid for all taxa (Fig. 4.3F-K). Unfortunately, foraminifera and juveniles of bivalves were not in sufficient numbers to allow measurements of isotopic enrichment. With the exception of ostracods and copepods, all taxa reached maximum ^{13}C and ^{15}N enrichment within the 4 days of the experiment. Tardigrades and ostracods (Fig. 4.3K,J), the taxa with the smallest organisms, were the most enriched. Their limited biomass didn't allow ^{15}N-δ^E measurements. Copepods, juveniles of *L. littorea* and nematodes (Fig. 4.3F, G and H) also showed

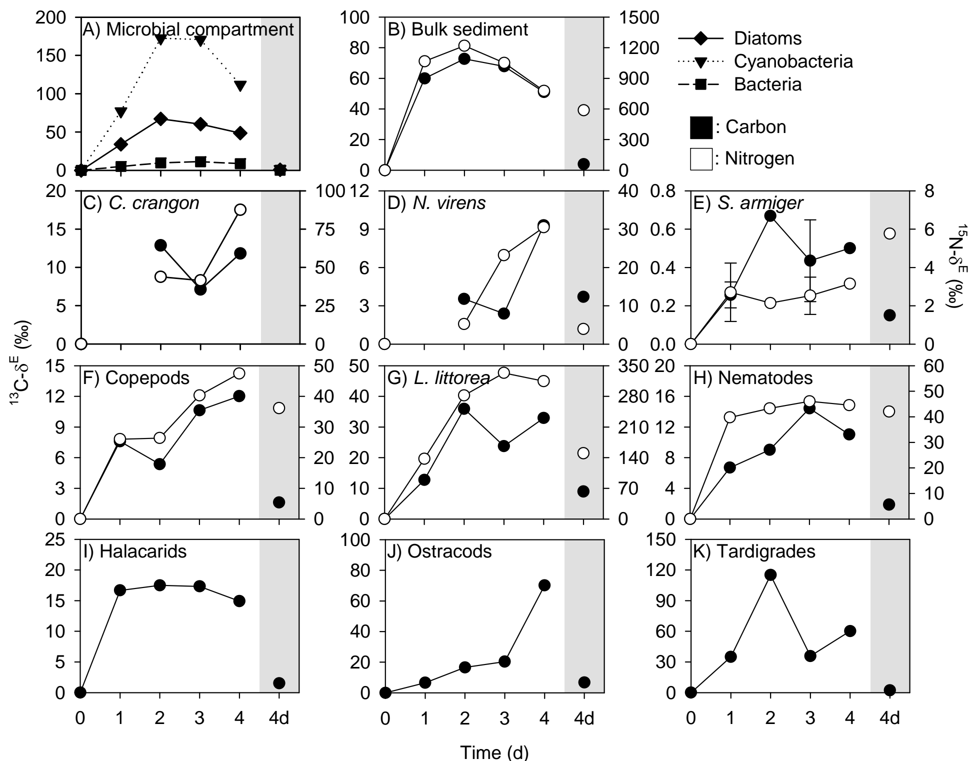

Figure 4.3. Benthic ^{13}C and ^{15}N enrichment (δ^E), respectively on the primary and secondary axis (where available), were estimated every day over a 4-days period from PLFA for the microbial compartment (A) and from bulk organic material for the sediment (B), the macrofauna (C – E) and the meiofauna (F – K). Enrichment in the dark was assessed on day 4 only (4d, greyed area). (E) day 1 and 3, mean ± SD (n = 4).

relatively high enrichment, especially compared to that of the polychaetes, due to their somewhat faster turnover. Compared to that of other meiofauna or macrofauna, juveniles of *L. littorea* showed high nitrogen incorporation relative to carbon.

4.3.4 Total C and N incorporation

Total organic C and N pools (bulk sediment) for the top cm layer were about 29 and 4 g m^{-2} respectively, with a molar C/N ratio of about 7.9 (Table 4.2). MPB and bacterial pools accounted for about 30 and 6% of the total carbon pool, respectively. Meiofauna and macrofauna (scaled to one cm) contributed 1.7 and 0.5% to the total carbon and ~2.3 (estimated only from the three main taxa) and 0.9% to the total nitrogen pool, respectively. The sum of all living compartment accounted for only about 37% of the sediment organic carbon pool indicating that detritus and extracellular polymeric substances (EPS) pools dominated total organic carbon.

MPB organic ^{13}C incorporation estimated from PLFA, was very important with a maximum of 10 mg ^{13}C m^{-2}. However, as the label incorporation of the bulk sediment was much higher, it seems that the majority of the fixed carbon went to the production of EPS (average of about 55%). Bulk sediment ^{15}N incorporation in the dark was significant with 9 mg N m^{-2} and is likely due to MPB uptake given the enhanced incorporation in the light and the high MPB to bacterial biomass ratio. Estimated molar C:N ratio of incorporation was very high (~14) and consistent with a high production of carbohydrates.

Bacterial ^{13}C incorporation, estimated from PLFA, was the highest among heterotrophic organisms, reaching 0.165 mg m^{-2} at day 4, followed by the entire meiofauna compartment (~ 0.125 mg m^{-2}). Juveniles of *L. littorea* accounted for the largest fraction of meiofauna ^{13}C incorporation, with 0.088 mg m^{-2} at day 4 and they also dominated the identifiable ^{15}N incorporation. Nematodes and copepods also contributed, though to a lower extent, to label incorporation. As observed from the very low $^{13}C/^{15}N$ ratios of incorporation, meiofauna preferentially assimilated N.

Macrofauna ^{13}C and ^{15}N incorporation was very limited, even when considering the whole 15 cm depth of sediment. Macrofauna incorporation for the whole 15 cm depth was about 25% that of the top cm for meiofauna. Contrarily to meiofauna, $^{13}C/^{15}N$ ratios of incorporation were very high implying that macrofauna preferentially assimilated C or ingested material with a high ^{13}C to ^{15}N ratio (i.e. EPS).

Table 4.2. Organic C and N biomass and label incorporation (mg m^{-2}) within the different compartments of the sediment, for the uppermost cm layer. *Polychaetes data relate to the whole depth integrated sediment layer (0-15 cm). Bulk sediment includes all the different components marked '+'. - : no data. † Calculated as the average ratio over the 4 days period in the light considering a 10% ^{13}C and a 100% ^{15}N labelling. Numbers with uncertainty due to missing data are in italic.

	C_{org} Biomass	$^{13}C_{org}$ Incorporation					N Biomass	^{15}N Incorporation					C/N of biomass (mol/mol)	$^{13}C/^{15}N$ of incorporation (mol/mol)†
		Day1	Day2	Day3	Day4	Day4 (d)		Day1	Day2	Day3	Day4	Day4 (d)		
= Bulk Sediment	28795	19	23	21	16	1	4239	17	19	16	12	9	7.9	14.04
+ MPB (from PLFA)	8377	8	10	10	7	0	-	-	-	-	-	-	-	-
+ Bac (from PLFA)	1757	0.096	0.182	0.213	0.165	0.016	-	-	-	-	-	-	-	-
+ Copepods	83	0.007	0.005	0.010	0.011	0.001	14	0.138	0.140	0.213	0.251	0.191	6.7	0.50
+ *L. littorea* (j)	247	0.034	0.096	0.063	0.088	0.024	56	2.840	5.839	6.897	6.510	3.103	5.1	0.15
+ Halacarids	13	0.002	0.002	-	0.002	0.000	-	-	-	-	-	-	-	-
+ Nematodes	131	0.010	0.013	0.020	0.016	0.003	27	0.397	0.432	0.460	0.445	0.420	5.7	0.39
+ Ostracods	5	0.000	0.001	0.001	0.004	0.000	-	-	-	-	-	-	-	-
+ Tardigrades	7	0.003	0.008	0.003	0.004	0.000	-	-	-	-	-	-	-	-
C. crangon	17	-	0.002	0.001	0.002	-	4	-	0.001	0.001	0.001	-	5.0	26.85
*N. virens**	205	-	0.034	0.005	0.021	0.008	52	-	0.001	0.004	0.006	0.001	4.6	61.32
*S. armiger**	2099	0.006	0.015	0.010	0.011	0.003	463	0.005	0.004	0.004	0.005	0.010	5.3	27.04
+ *Other*	18176	*11*	13	*11*	9	*1*	-	-	-	-	-	-	-	-

Total respired ^{13}C estimated from ^{13}C-DIC incorporation measurements integrated over the 4 days period was 3 mg m^{-2}. Considering that MPB was 10% labelled and that we can neglect MPB respiration, total respired C of the new production was 30 mg m^{-2}.

4.4 Discussion

This food web study clearly demonstrates the importance of MPB as a resource for benthic heterotrophic organisms in permeable, subtidal sediments. Before discussing in detail the different findings, it is important to reiterate the choices made regarding the experimental settings.

The deliberate tracer experiment was conducted in the laboratory rather than *in situ* so as to allow maximum control and recovery of different parameters investigated. Contrary to tidal flats where sampling plots can be easily delimited and sprayed with label during air exposure (Herman et al. 2000, Middelburg et al. 2000, Carman & Fry 2002), deliberate tracer additions in subtidal areas are more complex. The only way to perform a pulse-chase experiment *in situ*, in a subtidal area, would have been through the use of benthic chambers anchored in the sediment. The complete recovery of biological, sediment and water samples would have been put at risk in an environment often subject to rough wave conditions. Furthermore, deviations from natural conditions in benthic chambers employed in permeable sediments are usually larger than those in core incubations in the laboratory, where temperature, light regime and advective flow were set to closely mimic that of natural ones (Huettel & Rusch 2000, Cook et al. 2007). Our approach is based on balancing maximal resolution in food web compartments and closely mimicking natural flows conditions on the one hand and replication on the other hand. The time series over four days showed systematic patterns. Although replication at a single time point might have allowed us to apply standard traditional statistical analysis, we have chosen to follow the time dynamics because of the very low variance of the control (< 1 ‰) and the high δ^E values (Fig. 4.3), that represent the difference between control and treatment.

4.4.1 Sediment composition

PLFA analysis supported by pigment analysis clearly proved to be a very good approach in defining MPB composition. We found a small fraction of haptophytes to be present, which we attributed to a recent phytoplankton settlement and therefore neglected in interpretation of the carbon flows. MPB biomass, comprising 66% diatoms and 33% cyanobacteria, made up about half the organic content of the sediment, which is relatively high compared to that reported for other permeable sediments (Barranguet et al. 1998).

Heterotrophic bacteria made up the most important heterotrophic fraction of the sediment with 73% of the total heterotrophic biomass of the top first cm.

Our macro- and meiofauna census showed that taxonomic diversity, densities and biomass were much lower than those reported earlier for a contiguous site (Armonies & Reise 2000). The previous study, which to our knowledge represents the most complete interstitial fauna investigation, was carried out a number of times and along a transect. Some 652 species of meio-/microfauna grouped in 14 taxa were recorded, together with up to 23 macrofauna species. In contrast, in the present study we investigated the interstitial fauna only to higher taxonomic levels in one single subtidal sampling area and we did not attempt to quantify ciliate numbers. Nevertheless, it clearly showed a simpler fauna composition, with only 8 higher meiofauna taxa and few macrofauna species. Furthermore, whereas (Armonies & Reise 2000) found turbellaria to be the most abundant taxon, they were not observed from the sediment in our study at the time of sampling. In contrast, our study highlighted the predominance of nematodes, juvenile of the gastropod *L. littorea* and copepods both in terms of densities and biomass. However, the presence of *L. littorea* in the meiofauna should be regarded as a transient phenomenon as their presence as interstitial fauna is only momentary, possibly just following larval recruitment (Saier 2000). It is clear that permeable sediments are highly heterogeneous environments where drastic changes can occur in faunal assemblages. Nevertheless, our study supported earlier observations of a limited relative contribution of macrofauna to meiofauna (in terms of densities and biomass).

4.4.2 Trophic interactions

Although phytoplankton, MPB, macroalgae and other plants can all serve as a resource for benthic consumers, in most benthic food web studies a distinction is made between phytoplankton on the one hand and benthic primary producers on the other hand (Fry & Sherr 1988, Heip et al. 1995, Herman et al. 2000). This simple partitioning into pelagic and benthic resources has been popularized (e.g. France 1995) because of the clear distinction between the stable isotope signatures of SPM and that of surficial sediment (i.e. proxies for phytoplankton and MPB respectively). In this study isotope signatures could also be interpreted as being based on two distinct food sources: a pelagic ^{13}C-depleted one (~ -21‰) and a heavier benthic one (~ -17‰). However, this simple approach would have led to erroneous conclusions, because PLFA revealed a clear difference between the $\delta^{13}C$ values of cyanobacteria (~ -20‰) and diatoms (~ -16‰), both members of the MPB community.

Meiofauna and macrofauna stable isotope analysis exhibited a broad range, indicating variable dependence of consumers on available resources. Young individuals of *C.*

crangon are typical predators of meiofauna (Oh et al. 2001, Feller 2006). Their $\delta^{15}N$ value significantly was higher than that of copepods, supporting previous observations that small individuals of the brown shrimp feed preferentially on harpacticoid copepods, especially in sandy sediments (Gee 1987). However, the $\delta^{13}C$ signature of *C. crangon* is inconsistent with that assumption and suggests that this single measurement might be erroneous. The two macrofauna deposit feeders (*N. virens* and *S. armiger*) showed rather similar $\delta^{13}C$ values, slightly higher than that of the average of diatoms but within the range of the standard deviation. Nematodes, halacarids and copepods showed $\delta^{13}C$ values closer to that of diatoms (~ -16‰), or bulk sediment (~ -17‰), suggesting a direct dependence or a mixed diet mainly composed of diatoms. Tardigrades, ostracods and juveniles of *L. littorea* showed $\delta^{13}C$ values similar to that of cyanobacteria (~ -20‰), and/or SPM (phytoplankton, ~ -21‰). Juveniles of bivalves, which are surface suspension feeders showed a $\delta^{13}C$ value similar to that of diatoms. We speculate that contrary to diatoms, cyanobacteria might be more tightly associated to sand grains preventing them from being resuspended or directly ingested. Our findings support previous observations of the significant reliance of bivalves on MPB (Sauriau & Kang 2000, Rossi et al. 2004). Halacarid $\delta^{13}C$ signature also suggested a strong dependence on diatoms, in line with the brown spots in the guts of the animals during picking (own observations) and also previous studies related to their diet (Bartsch 2004). The biomarker approach, supported by labelling experiments, revealed that juveniles of *L. littorea*, ostracods and halacarids were selectively grazing on cyanobacteria, while the natural stable isotope signature of these consumers could have easily been wrongly interpreted as a strong reliance on phytoplankton.

4.4.3 C and N flows

The ^{13}C and ^{15}N pulse chase experiment successfully allowed us to evaluate C and N flows from the MPB to the various heterotrophic compartments of the sediment. Label incorporation by MPB was clear and rapid, occurring within the first day and within the range what can be observed in intertidal flats with fine sediments (Underwood & Kromkamp 1999, Middelburg et al. 2000, Cook et al. 2004a). Turnover time, calculated as the ratio of biomass to production (respectively 8377 and ~190 mg on the first day considering 10% ^{13}C labelling of the inorganic C pool; see Table 4.2), was 44 d. This is similar to what has already been observed in subtidal permeable sediments (Sundback et al. 1996). Approximately 55% of MPB production was allocated to EPS production (Table 4.2; for further details see Chapter 3) which is a significant food source for bacteria (Goto et al. 2001) and also for non-selective deposit feeders: this constituted the largest fraction of ^{13}C incorporation in the sediment. Still, ^{13}C labelling of the total organic C pool remained very low (~0.1%) compared to that of ^{15}N (~0.4%). These findings are important

to understand label enrichment into heterotrophic organisms. ^{13}C and ^{15}N (where data were available) enrichment into the heterotrophic compartments was rapid and occurred within all taxa, but to variable extents (Fig. 4.3). Considering the extremely high dilution of isotope labels in the global pool (MPB and EPS standing stocks), we need to take into account that heterotrophic organisms had variable interaction with freshly produced MPB. This is consistent with subsurface deposit feeders (*S. armiger* and possibly a fraction of the nematode community) that showed lower ^{13}C enrichment than surface deposit feeders (*N. virens* and possibly some nematodes). The higher ^{13}C enrichment of juveniles of *L. littorea*, ostracods and tardigrades compared to that of the other metazoans agreed very well with their preference for cyanobacteria. Surprisingly, juveniles of *L. littorea* also showed extremely high ^{15}N enrichments compared to that of other animals suggesting that cyanobacteria were possibly more ^{15}N-enriched than diatoms. Unfortunately, there are no ^{15}N-δ^{E} data for cyanobacteria or ostracods and tardigrades to support that assumption. Nevertheless, this might be clarified through a closer look at the total label incorporation in the different benthic compartments.

4.4.4 Relative contribution of bacteria, meiofauna and macrofauna

Incorporations reported in Table 4.2 show not only high variability due to spatial heterogeneity but also uncertainty from the use of conversion coefficients (e.g. from PLFA concentration to biomass) and they should therefore be interpreted carefully. They nevertheless provide important information. The first observation is that very little label was actually transferred to the different heterotrophic compartments for both C and N, with the exception of bacteria and juveniles of *L. littorea*. Most of the label was still in MPB and EPS at the end of the experiment, i.e. after 4 days. Second, enrichment dynamics showed that all compartments were significantly. In addition, it appeared that the amount of label transferred (bacteria ≈ meiofauna >> macrofauna) followed more or less the biomass patterns (bacteria > meiofauna > macrofauna). There are few studies to compare with our own results as most are qualitative or cover only one size-class. Middelburg et al. (2000) studied the fate of intertidal MPB carbon and reported that label transfer from MPB to consumers followed more or less biomass pattern in silty sediments (bacteria>macrofauna>meiofauna). Moodley et al. (2005) and Woulds et al. (2007) investigated the fate of phytodetritus in ocean margin sediments and reported that label transfer followed biomass patterns, although deviations were reported for individual species/taxa. Although Sundback et al. (1996) did not include the macrofauna in their study, they also found that heterotrophic bacteria and meiofauna contributed proportionally to MPB derived carbon processing.

Third, our labelling study revealed a high contribution of meiofauna to total carbon processing, similar to that of bacteria. This high contribution of meiofauna to carbon processing in sandy sediments has often been implied from their high densities and fast turnover (Fenchel 1969, Kuipers et al. 1981), but has hardly been quantified before because quantitative and comprehensive data on carbon flow in sandy sediment has been lacking so far. Such a high contribution of the meiofauna has been reported for sediment underlying low-oxygen bottom waters (Woulds et al. 2007) and deep-sea sediments (Moodley et al. 2002). However, in these studies foraminifera accounted for most of the meiofaunal uptake whereas metazoans appear to dominate in sandy coastal sediments.

While carbon transfer from MPB to meiofauna has now been studied in a number of tidal flats (Middelburg et al. 2000, Moens et al. 2002), there are very few studies on nitrogen transfer from benthic microbes to meiofauna (Carman & Fry 2002, Veuger et al. 2007a). Nitrogen incorporation also occurred in the dark, at a rate that attained 75% of that in the light at day 4 (Fig. 4.3B). Nitrogen incorporation can be attributed to MPB as well as heterotrophic bacteria, although their respective biomasses suggest that MPB likely dominated nitrogen uptake (Chapter 3). The C:N ratio of total incorporation was high (~14) which is consistent for a sediment in which a large fraction of the fixed carbon is exudated as EPS. The three meiofauna taxa for which data were available showed a preferential incorporation of N relative to C. Although we have no data on the C:N ratio of the microbes consumed by these meiofauna taxa, it is likely these had a much lower C:N ratio (4 to 7). Conversely, macrofauna showed high ratios consistent with their feeding behaviour (bulk deposit feeders and predator), i.e. they consumed ^{13}C rich, ^{15}N poor EPS as well. Surprisingly, juveniles of *L. littorea* showed extremely high amount of ^{15}N incorporation. Considering they exclusively grazed on cyanobacteria, this merely suggests that cyanobacteria incorporated more ^{15}N and were more enriched than diatoms. *L. littorea* are highly selective feeders with the ability to select their food after it's been ingested and spit the rest out (Norton et al. 1990). It has been shown that their juveniles have high requirement for N relative to C and that, in N-limited conditions, they will exclusively rely on cyanobacteria, with higher N content (Sommer 2001).

Sandy permeable sediments represent the most common habitat on continental shelves (Boudreau et al. 2001, Hall 2002) and about one third of the continental shelf area receives enough light for MPB growth. Through the use of stable isotopes at natural abundance, a dual-tracer enrichment experiment and a close look at microbial biomarkers, we have documented key aspects of the role of MPB in structuring benthic food webs in subtidal sandy sediments. We remain cautious however regarding any generalizations that could be made with our findings and further investigation on larger spatial and temporal scales should be considered. Nevertheless, the combination of the different methods together with the thorough identification and analysis of the different fauna components

have revealed that the hidden green garden of sandy, subtidal sediments is pivotal to metazoans, in particular meiofauna, living in these permeable, dynamic sediments.

Chapter 5
Relative significance of phytodetritus deposition and microphytobenthos for the heterotrophic community of a shallow subtidal sandy sediment.

In preparation for Marine Ecology Progress Series

5.1 Introduction

Sandy sediments, due to their low standing stocks of organic carbon (C_{org}), have long been falsely considered as biogeochemically inactive environments (Boudreau et al. 2001). Recent studies have yielded strong evidence regarding their high primary production (Billerbeck et al. 2007, Cook et al. submitted; Chapter 3) and that advective porewater transport enhances remineralization rates in these permeable sediments (Huettel & Gust 1992a, Rusch et al. 2001, Ehrenhauss et al. 2004a). Dynamic environments are usually characterized by sediment with coarse grain size and high permeability. Food resources and consumers are heterogeneously distributed in these dynamic sediments. The different heterotrophs inhabiting these sediments (i.e. heterotrophic bacteria, microfauna, meiofauna and macrofauna) undergo frequent reworking due to physical forces.

The relative importance of the heterotrophic compartments has been assessed and there are some generic distribution patterns related to grain size. Firstly, macrofauna biomass shows a decreasing trend towards coarser sediments (Heip et al. 1992), which has been attributed to higher influence of hydrodynamics as well as scarcity of resources. Secondly, there is a coupled decrease in densities and increase in diversities of meiofauna population with increasing sediment grain size (Vanaverbeke et al. 2000, Rodriguez et al.

2003, Gheskiere et al. 2005). While the preponderance of heterotrophic bacteria with regard to respiration and remineralization is widely accepted for silty sediments (Gerlach 1971, Koop & Griffiths 1982, Sundback et al. 1996, van Oevelen et al. 2006c), the few studies available for sandy sediments have suggested a higher significance of meiofauna, sometimes outcompeting bacteria (Chapter 4; Sundback et al. 1996).

Most of the benthic food web studies have been carried out in accreting silty and muddy sediments (e.g. depositional shelf sediments, estuaries or coastal lagoons) and more specifically in intertidal settings (Reise 1979, Heip et al. 1995, Deegan & Garritt 1997, Middelburg et al. 2000, Carman & Fry 2002), leaving sandy sediments poorly documented (Chapter 4; Sundback et al. 1996, Buhring et al. 2006). However, sandy sediment covers ~70% of the continental shelf. In contrast with fine sediments, where grazing and mineralization occur within the top millimetres of sediment and solute transfer is diffusion-limited, these processes can occur much deeper in permeable sediments and solute transfer is strongly enhanced through pore water advection (Huettel & Rusch 2000, Rusch & Huettel 2000). Considering that depth integrated microphytobenthos (MPB) production can significantly exceed that of intertidal muddy areas (Chapter 3) and that permeable sediments can act as particles traps actively filtering the overlying water through pore water advection (Huettel et al. 2007), food webs in sandy sediments might also contribute significantly to C flows at the scale of the global shelf.

Most benthic food web studies are limited to one compartment (i.e. microfauna, meiofauna or macrofauna) or to one specific taxon or size class while neglecting interactions with other size classes or taxa. For example nematodes, which are omnipresent in the meiofauna, have received greater attention than other smaller animals like ostracods and tardigrades. Also, microscopic juveniles of macrofauna (i.e. bivalves, gastropods), which can occur in dense cohorts and can contribute significantly to MPB grazing (Chapter 4), are often neglected. The smaller and most abundant organisms at the base of the food web (i.e. the microbial domain) are either ignored or not resolved and lumped with detritus into the bulk sediment compartment. This is unfortunate since many studies have reported variable dependence of metazoans on algae, bacteria and detritus (Sherr & Sherr 1987, Epstein et al. 1992, Epstein & Shiaris 1992). This lack of resolution in the lower trophic domain also hinders disentangling detritivory, bacterivory and grazing on MPB (van Oevelen et al. 2006c).

Stable isotopes are increasingly used to unravel trophic interactions in benthic communities. While stable isotopic analysis is a powerful method that allows addressing the complexity of food webs (Fry & Sherr 1988, Herman et al. 2000, Ponsard & Arditi 2000), it is important to consider some pitfalls that might yield misinterpretations. Evrard et al. (Chapter 4) have shown that, although it is widely accepted that ^{13}C natural

abundance of MPB and phytoplankton are often distinct (France 1995), ^{13}C-replete with high δ^{13}C values and ^{13}C-depleted with low ones respectively, consumers with low δ^{13}C values do not necessarily depend on SPM. For example, animals selectively feeding on cyanobacteria in a heterogeneous MPB community would get more ^{13}C-depleted compared to those feeding exclusively on diatoms with high δ^{13}C values or feeding indiscriminately. Combined to the study of natural abundance of stable isotopes, stable isotopes enrichment experiments offer a powerful way to circumvent the problem of overlapping of different food sources (Herman et al. 2000, Carman & Fry 2002). Studies during the last decade have also shown that it is possible to selectively label different food sources (phytodetritus: Blair et al. 1996, MPB: Middelburg et al. 2000, and bacteria: van Oevelen et al. 2006b) and follow their fate within the benthic food web. Although most studies using enrichment experiments offer only a qualitative approach, isotopic enrichment also allows quantifying C fluxes and assessing the fate of organic matter in a quantitative sense.

In the present study, we investigate the trophic interactions in an organic carbon-poor subtidal, sandy sediment. We integrate the results of a natural abundance approach with results of two deliberate tracer experimental approaches. In a first experiment, sediment cores received a pulse of freeze-dried ^{13}C-labelled diatoms and the fate of that enriched material was quantified in detail in the different compartments of the benthic food web. In a second experiment, sediment cores received a pulse of ^{13}C-bicarbonate to label the MPB and the fate of labelled MPB was followed. The combined information from these three approaches allowed us to constrain resource partitioning among heterotrophic organisms living in coastal sands.

5.2 Materials and methods

5.2.1 Study site

The research was carried out in Hel (Hel peninsula, Poland), situated in the north-western part of the gulf of Gdansk in the Baltic. The sampling site was ~50 m off the shore, in shallow water (~1.5 m) and consisted of coarse and well sorted quartz sand, with a median grain size of 210 µm. The tidal amplitude is negligible and the water is brackish with a salinity of 7. The water temperature at the time of the study was 20°C.

5.2.2 Experimental settings

5.2.2.1 Experiment 1: ^{13}C-labelled phytodetritus

Ten transparent acrylic cylinders (19 cm inner diameter x 33 cm high) were used to sample the sediment with overlying water from the study site on the 14th of August 2003. The cores, with approximately 15 cm of sediment and 18 cm of overlying water, were immediately transferred to the laboratory and closed by acrylic lids. Each lid carried an electric motor that propelled a 15 cm diameter horizontal rotating disc that stirred the water column at 15 cm above the sediment water interface. The motor maintained an electronically controlled angular velocity set to 40 rpm which generated a pressure gradient of 1.9 Pa between the circumference and the centre of the sediment surface (~0.2 Pa cm^{-1}). This pressure gradient corresponds to gradients produced by slow bottom currents (~10 cm s^{-1}) interacting with sediment ripple topography (15 mm ripple amplitude) as were typical for the study site during our investigations. Such pressure gradients force bottom water to enter the sediment in the ripple troughs and to emerge at the ripple crests. Similarly, the simulated pressure gradients in the chamber forced water to enter the sediment near the chamber wall and to emerge from the centre of the core. The functioning and deployment of these chambers has been described in detail by Huettel & Gust (1992b), Huettel et al. (1996), Janssen et al. (2005a) and Janssen et al. (2005b).

The set of chambers was immersed in a trough, filled with water from the sampling site, maintained under *in situ* temperature (20°C) and constantly aerated. Each chamber's upper lid with stirrer was elevated about 1 cm above the chamber's edge, allowing circulation of aerated water within the whole system. The whole circulation of water was enhanced by a water pump placed at the bottom of the trough. The system was left to settle 24 h before labelling.

An axenic clone of the benthic diatom *Amphora coffeaeformis* (UTCC 58) was cultured beforehand at 16°C under 32 W incandescent lights. The artificial seawater (F2 medium) contained 50% ^{13}C-enriched bicarbonate (98% ^{13}C, Isotech) to label the diatoms. After 3 weeks, the labelled diatoms were concentrated by centrifugation, washed several times to remove adhering ^{13}C-bicarbonate and subsequently freeze-dried. The final product was 11% ^{13}C-labelled. The axenic state of the diatom culture was verified microscopically.

After the acclimation period of 24 h, one core was taken out of the system and sampled (see below) to provide background values for the different parameters investigated. Prior to labelling, the freeze-dried diatoms were resuspended and gently homogenised in 50 mL 0.2 µm filtered sea water from the trough. The nine remaining

cores were closed and 5 mL of the phytodetritus solution was injected in their overlying water, with stirrers off, and let to settle. The phytodetritus addition was equivalent to a pulse of 377 mg C_{org} m^{-2}, including 41.5 mg $^{13}C_{org}$ m^{-2}. After one hour, another core was taken out of the system and sampled so as to provide a T_0 data point (considered as the beginning of the incubation period), and the stirring of the eight remaining cores were switched on again. At each sampling time (t = 12, 24, 48 and 72 h), duplicate cores were taken out of the system and sampled as described below.

5.2.2.2 Experiment 2: ^{13}C pulse-chase labelling

On the 22nd of August 2003, eight days after the first experiment, one opaque and five transparent cylinders were used to sample the sediment with overlying water at the same sampling site. The sediment cores were transferred to the laboratory and handled similarly as in experiment 1. After 24 hours of acclimation, one core was taken out of the system so as to provide background data. The remaining cores, including the opaque one, were hermetically closed and received each a pulse of 25 mg ^{13}C-bicarbonate in their overlying water, equivalent to 6.7% labelling of the DIC pool (1.2 mmol L^{-1}). All the cores (except the opaque one) were illuminated during 8 h by artificial light providing an irradiance of 150 µmol quanta m^{-2} s^{-1} at the sediment surface. After the light exposure, one transparent core was taken out of the system and sampled so as to provide the end of labelling data point (T_1 = 8 h). The overlying water of the remaining cores was flushed to remove ^{13}C-bicarbonate and carefully replaced with unlabelled water from the sampling site. This was performed twice to maximize dissolved label removal. When in the dark, the cores were always kept opened and immersed in the trough to allow aeration and mixing of the water within the whole system. A 8:16 h light:dark cycle was maintained during the four days of the experiment. At t = 24, 48 and 96 h, after each illumination period, one core was taken out of the system and sampled as described below. The opaque core (referred to as 96d) was sampled at t = 96 h.

5.2.3 Sampling and analysis

5.2.3.1 Chamber sampling

All sediment cores were subsampled using 4 smaller cores (3.56 cm inner diameter, ~10 cm^2). For bulk sediment organic carbon (C_{org}) and its stable isotope analysis and, phospholipid-derived fatty acid (PLFA) analysis, 2 small cores were sliced in three layers: 0-1, 1-2, and 2-3 cm. The different layers were pooled horizontally together to obtain enough material for PLFA analysis, and subsequently freeze-dried. Another small core was sliced the same way and kept for meiofauna analysis. The last small core was sliced

in six thin layers (0-2, 2-4, 4-6, 6-8, 8-10 and 10-20 mm), freeze-dried and used for analysis of pigments concentrations. Prior to the experiments, 4 L water from the field was sampled. 2 L were filtered onto glass-fibre filters n°6 for pigments analysis (Schleicher & Schuell) and the remaining 2 L were filtered onto GF/F filters for SPM stable isotope analysis. All samples were stored in a freezer until analyzed. Sediment and SPM samples used for pigment analysis were stored in a -80°C freezer.

Total respired ^{13}C was estimated from the variation in ^{13}C-DIC concentration. Each day, during a period of 4 hours, cores were closed and a small amount of water from its overlying water was sampled at the start and at the end. Samples were drawn into a 12 mL headspace vial to which 12 µL HgCl was added to kill the sample. ^{13}C respiration rate, based on the increase in ^{13}C-DIC over 4 hours, were extrapolated to the 24 hours-period preceding each measurement and integrated over the whole time scale of the experiment (96 hours) to estimate total respired ^{13}C in mg m^{-2}.

5.2.3.2 Fauna extraction

Remaining sediment from each core was sieved on a 1 mm mesh and the macrofauna handpicked and pooled into a large container filled with sea water. Macrofauna found in slices from the small cores for chemical analysis were added to the container as well. The entire chamber was thus covered (28.35 10^{-3} m^2). The animals were gently brushed to remove mucus, faeces and particles and then sorted to species level, pooled into different glass vials and stored in the freezer until analysed. Prior to analysis, animals were thawed and successively dried in a stove for 48 h at 60°C to estimate their dry weight. Finally, they were ground into a fine homogenous powder.

Meiofauna was neither stained nor fixed to avoid any addition of exogenous C that might contaminate the samples with a different isotopic signature. Sediment layers were thawed at room temperature and thoroughly rinsed with distilled water on a 38 µm sieve. Meiofauna was extracted from the sediment with colloidal silica (Ludox® HS 40, DuPont) with a density of 1.31 g cm^{-3} following the protocol proposed by Burgess (2001). Briefly, sediments are washed from the sieve with Ludox® into a 50 mL disposable polypropylene centrifuge tube. The tube is capped and thoroughly mixed using a vortex at a gradually decreasing speed and finally centrifuged. The supernatant is rinsed again with distilled water on the sieve and finally poured into a Petri dish for counting and picking. The sediment pellets were set aside for verification of its remaining content. All samples were treated successively to avoid degradation of material. The animals were sorted to higher taxonomic levels or for some to the genus or species level and counted under a microscope. They were finally transferred to Sn cups for C analysis. Animals with $CaCO_3$ shells were transferred to Ag cups and then acidified to remove inorganic C.

5.2.3.3 Analyses and data handling

Pigment samples were analysed by reverse-phase high-performance liquid chromatography (Barranguet et al. 1998). The relative proportions of phytoplanktonic and microphytobenthic taxa were estimate using the CHEMTAX program (Mackey et al. 1996).

Small fractions of the sediment samples were ground in agate mortar to obtain a homogeneous and fine powder. Organic carbon content and isotopic composition ($\delta^{13}C$) of sediment, macrofauna and meiofauna were measured using a Carlo Erba/Fisons/Interscience elemental analyser coupled on-line via a conflo interface to a Finnigan Delta S isotope ratio mass spectrometer.

PLFA for the top cm were extracted from approximately 6 g of sediment, following the method of Boschker et al. (1999) and Middelburg et al. (2000) and, their concentrations were determined by gas chromatograph-flame ionization detection (GC-FID, Carlo Erba HRGC mega 2 GC). PLFA carbon isotopic composition was determined using a gas-chromatograph combustion-interface isotope ratio mass spectrometer (GC-c-IRMS, Hewlett Packard 6890 GC coupled via a Thermo combustion interface III to a Thermo Delta Plus isotope ratio mass spectrometer). Bacterial carbon content in the sediment was estimated from the PLFA concentrations. The bacterial carbon biomass (C_{bac}, expressed in mg C m^{-2}) was calculated from bacterial PLFA ($PLFA_{bac}$) as $C_{bac} = PLFA_{bac}/a$ where a is the average PLFA concentration in bacteria (0.073 mg of PLFA carbon per mg of C_{bac} in oxidised sediment; Brinch-Iversen and King 1990). $PLFA_{bac}$ was estimated from the bacteria-specific PLFA ($PLFA_{bacsp}$: i14:0, i15:0, a15:0 i16:0 and 18:1ω7c; the latter was not included for the second experiment because benthic diatoms might have 18:1ω7c) as $PLFA_{bac} = \Sigma\, PLFA_{bacsp}/b$, where b is the average fraction-specific bacterial PLFA (0.28 or 0.14 mg of carbon bacterial-specific PLFA per mg of carbon bacterial PLFA whether 18:1ω7c is included or not; Moodley et al. 2000).

Stable isotope data are expressed in the delta notation ($\delta^{13}C$, per mille ‰) relative to Vienna Pee Dee Belemnite standard (VPDB) and calculated from the stable isotope ratio (R): $\delta^{13}C = (R/R_{VPDB}-1)\times 1000$, where R is the $^{13}C/^{12}C$ ratio measured in the sample and R_{VPDB} in the standard ($R_{VPDB} = 0.0111797$). Following, Maddi et al (2006) we use the enrichment (δ^{E} notation), as a measure of label enrichment. $^{13}C\ \delta^{E} = [(\delta^{13}C_s+1000)/(\delta^{13}C_b+1000)-1]\times 1000 = (R_s/R_b-1)\times 1000$, where R_b is the ratio in the background and Rs in the sample. The enrichment, δ^{E}, can be compared directly with the previously used specific uptake ($\Delta\delta$ notation; $\Delta\delta = 1000\times(R_s-R_b)/R_{VPDB}$), but has the advantage that it is exact and thus valid for high levels of enrichment. The incorporation (I), i.e. the total uptake of label, as defined by Middelburg et al. (2000), is expressed in mg $^{13}C\ m^{-2}$, as a product of the atomic excess (E) and a quantity (C_{org} or C_{PLFA}). $E = F_s-F_b$, is

the difference between the fraction of the sample and the one of the background, with F = R/(R+1).

5.3 Results

5.3.1 Benthos composition

Bulk sediment C_{org} contents were low (~0.05% C) and similar for Exp1 (~9.3 g C m^{-2} and 21.6 g C m^{-2} for the uppermost cm and the first 3 cm respectively) and Exp2 (~10 g C m^{-2} and 22.4 g C m^{-2} for the uppermost and first 3 cm, respectively). Chl *a* profiles also showed similar concentrations for Exp1 and Exp2 (94 and 95.1 mg Chl *a* m^{-2} in the top cm, respectively). However, analysis of minor pigments revealed that microphytobenthos (MPB) composition differed. Based on CHEMTAX analysis the uppermost cm of sediment for Exp1 was composed of 69% diatoms, 23% cyanobacteria and 8% green algae. In Exp2, the composition was 84%, 12% and 4% for the same taxa respectively (Fig. 5.1). Moreover, MPB taxonomic vertical profile in Exp1 was somewhat stratified

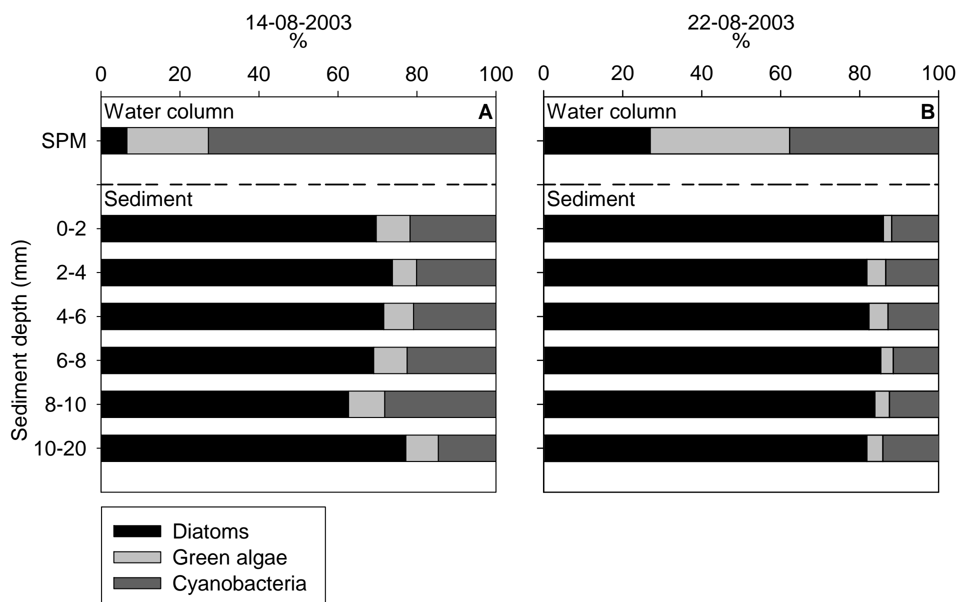

Figure 5.1. Phytoplankton and MPB composition (vertical profile in mm) on (A) the 14-08-2003 and (B) the 22-08-2003, estimated from the CHEMTAX analysis of the pigment data.

compared to that of Exp2. Using C:Chl *a* ratios of 32 and 30 for Exp1 and Exp2 respectively, calculated from the weighted average of C:Chl *a* ratios of microalgae making up the ambient MPB community (Dijkman & Kromkamp 2006, Dijkman N.A., pers. comm.), we derived MPB biomasses for the top cm of sediment of ~3006 and ~2851 mg C m^{-2} for Exp1 and Exp2 respectively. In contrast to the relative uniformity observed in the sediment, phytoplankton composition was very different for the two sampling dates. Phytoplankton was dominated by cyanobacteria on the 14th of August (Fig. 5.1A) while the composition was more evenly distributed between diatoms, cyanobacteria and green algae on the 22nd of August (Fig. 5.1B).

Benthic fauna was diverse, with 13 meiofauna taxa and 3 macrofauna species sampled, but assemblages were different at the two sampling dates. In the first experiment, meiofauna was surprisingly dominated by the harpacticoid copepod *Paraleptastacus spinicauda* and by ostracods at the sediment surface, with ~53×10^3 ind m^{-2} and ~48×10^3 ind m^{-2} respectively, although the latter showed high patchiness (Table 5.1). Although the harpacticoid copepod community appeared to be heterogeneous (microscopic observations), only data from *P. spinicauda* and another copepods species, *Huntemania jadensis*, were obtained. Other unidentified individuals were present erratically and were

Table 5.1. Experiment 1. Meiofauna and macrofauna biomass.

	Median weight (μg C_{org} ind^{-1})	Densities (10^3 ind m^{-2}) 0-1 cm	1-2 cm	2-3 cm
Amphipods (j)	13.73	0.60 ± 0.70	0.50 ± 0.71	0.10 ± 0.32
Chironomids	1.93	13.44 ± 6.42	0.10 ± 0.32	0.20 ± 0.63
H. ulvae (j)	3.76	11.90 ± 8.96	2.90 ± 2.56	1.50 ± 2.07
H. jadensis	0.81	28.14 ± 17.19	18.00 ± 9.30	16.33 ± 9.02
Halacarides	N.D.	2.40 ± 2.01	1.00 ± 1.05	3.30 ± 3.16
M. arenaria (j)	1.84	14.78 ± 12.73	5.00 ± 2.12	5.11 ± 6.09
Mytilus sp. (j)	0.79	11.22 ± 11.08	12.22 ± 7.60	15.78 ± 10.43
Nauplii	N.D.	21.00 ± N.D.	7.00 ± N.D.	4.00 ± 2.00
Nematodes	0.17	43.90 ± 16.31	76.60 ± 25.46	89.50 ± 53.76
Oligochaetes	3.78	1.80 ± 2.25	9.50 ± 10.21	9.50 ± 7.25
Ostracods	0.13	46.80 ± 93.36	4.90 ± 12.83	0.80 ± 2.53
P. spinicauda	0.10	52.86 ± 30.98	27.50 ± 19.62	37.67 ± 41.59
Tardigrades	0.02	13.00 ± 16.00	38.80 ± 58.10	63.80 ± 105.76
Turbellaria	0.18	6.75 ± 4.27	5.00 ± 2.98	2.38 ± 3.81
	Mean weight (mg C_{org} ind^{-1})	**Densities (ind m^{-2}) 0-15 cm**		
B. pilosa	0.28 ± 0.15	167.55 ± 107.07		
N. diversicolor	3.26 ± 1.07	156.77 ± 78.79		

scarce, precluding isotope analysis. Nematodes showed the third highest densities at the sediment surface with ~44×10^3 ind m^{-2}. However, their numbers increased with depth to 90×10^3 ind m^{-2} for the 2-3 cm layer. Other significant contributors to the meiobenthic community were chironomid larvae, and juveniles of *Mytilus* sp., *Mya arenaria* and amphipods. Macrofauna was limited to large individuals of the suspension feeder *Nereis diversicolor* (~157 ind m^{-2}) and the amphipod *Bathyporeia pilosa* (~168 ind m^{-2}) present only at the sediment surface. For the second experiment, fauna distribution was different with fewer taxonomic groups (Table 5.2). Chironomid larvae were not present anymore; nematodes were the most important taxon (~122×10^3 ind m^{-2}) and showed a clear vertical separation, with small individuals at the sediment surface and increasingly bigger ones in the deeper layers. Low densities of juveniles of *M. arenaria* and *Mytilus* sp. didn't allow their separation for further stable isotope analysis and were pooled into a "bivalves" group. The macrofauna compartment was different compared to Exp1 with significantly smaller individuals of *N. diversicolor* and high densities of *Gammarus* sp. (~423 ind m^{-2}) that were not present before.

The sediment contained also a fraction of heterotrophic bacteria whose biomass was estimated from PLFA concentrations. Both in Exp1 and Exp2, the biomass of heterotrophic bacteria accounted for the largest fraction of the heterotrophic organisms with 672 mg C m^{-2} and 380 mg C m^{-2} respectively (Table 5.3).

Table 5.2. Experiment 2. Meiofauna and macrofauna biomass. *Individual weight of nematodes is provided for all layers (0-1, 1-2 and 2-3 cm respectively).

	Median weight (μg C_{org} ind^{-1})	Densities (10^3 ind m^{-2}) 0-1 cm	1-2 cm	2-3 cm
Amphipods (j)	7.32	1.50 ± 0.71		
Bivalves (j)	2.71	3.83 ± 1.17	0.17 ± 0.41	1.00 ± 1.55
H. ulvae (j)	7.54	1.60 ± 0.89		
H. jadensis	0.53	12.00 ± 3.67		
Nematodes	0.15, 0.19, 0.29*	121.83 ± 77.97	127.33 ± 70.92	35.00 ± 25.47
P. spinicauda	0.08	26.00 ± 17.17		
Tardigrades	N.D.	30.40 ± 14.94	6.17 ± 9.60	
	Mean weight (mg C_{org} ind^{-1})	**Densities (ind m^{-2}) 0-15 cm**		
B. pilosa	0.22 ± 0.02	105.82 ± 127.04		
Gammarus sp.	0.17 ± 0.05	423.28 ± 236.50		
N. diversicolor	0.38 ± 0.30	261.02 ± 124.21		

5.3.2 Stable carbon isotope signatures

Natural stable isotopic signatures of macrofauna and meiofauna showed a broad range of $\delta^{13}C$ values ranging from -13.5‰ for *H. jadensis* to -20.4‰ for juveniles of bivalves (Fig. 5.2). Traditionally, in organic-poor sediments, $\delta^{13}C$ values of lumped compartments such as bulk sediments and SPM are considered proxy measures for the $\delta^{13}C$ of the potential food sources MPB and phytoplankton, respectively. The $\delta^{13}C$ values of bulk sediment and SPM were -16.5 and -19.0‰, respectively. However, most of the isotopic signatures of the taxonomic groups analysed were outside this range, but for juveniles of the gastropod *Hydrobia ulvae* and ostracods. PLFA analysis offered additional resolution which allowed us to estimate $\delta^{13}C$ values of the diatoms, the dominant groups within the MPB. Unfortunately, the resolution of the chromatograms didn't allow distinguishing the peaks of the PLFA C18:3ω3 and C18:4ω3 which are characteristic of cyanobacteria and green algae. Using a correction of 2-3‰ to account for the carbon depletion of PLFA relative to that of their biomass (Boschker et al. 1999, Hayes 2001, Boschker et al. 2005, Evrard et

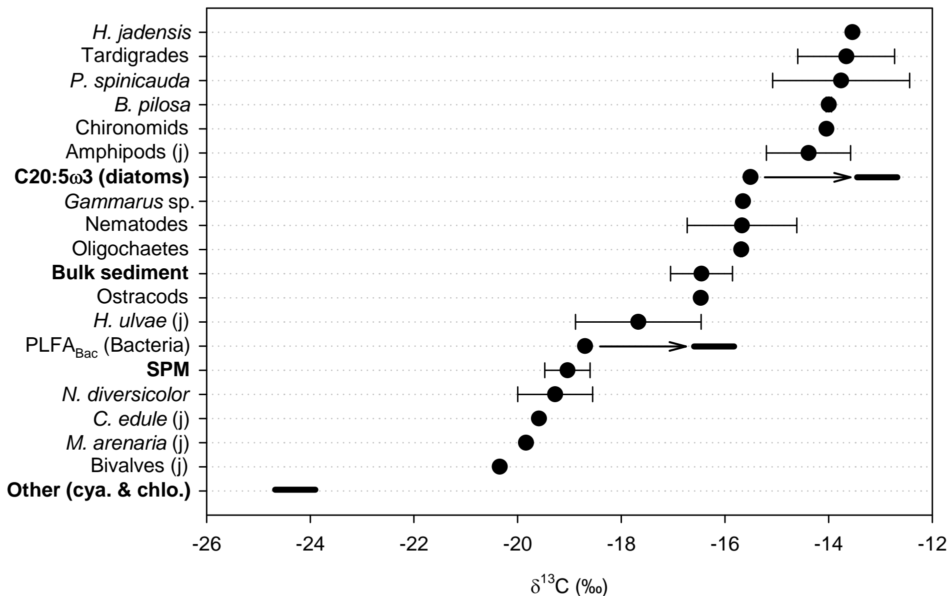

Figure 5.2. $\delta^{13}C$ signatures of consumers and food resources (mean ± SD, n = 2 if applicable). Potential food sources are in bold. Arrows represent the shift of microbial $\delta^{13}C$ values to account for fractionation between PLFA and biomass. The isotopic signature of the lumped cyanobacteria and green algae compartment (other) has been calculated from mass balance (see text). j: juveniles.

al. in revision), we could derive, from the isotopic signature of the diatom specific PLFA (C20:5ω3), a $\delta^{13}C$ value for diatoms ranging from -13.5 to -12.5‰. That value is consistent with the highest value found for the consumers. Similarly, we also estimated the bacteria ^{13}C natural abundance from the weighted average bacterial-specific PLFA $\delta^{13}C$ (-18.7 ‰ for i14:0, i15:0, a15:0 i16:0 and 18:1ω7c) and derived a $\delta^{13}C$ value comprised between -16.7 and -15.7‰.

Based on these $\delta^{13}C$ values for benthic diatoms and using a mass balance, based on the assumption that bulk organic matter is mostly made up of MPB or MPB-derived material, we can approximate the $\delta^{13}C$ values of the other microalgal groups (cyanobacteria + green algae) contributing to MPB. Considering that the sediment contains 69% of diatoms, 31% of another microalgal group (i.e. 23% cyanobacteria and 8% green algae) and a negligible fraction of phytoplankton and bacteria, we can write $\delta^{13}C_{Bulk\ sediment} = 0.69 \times \delta^{13}C_{Diatoms} + 0.31 \times \delta^{13}C_{Group}$, where $\delta^{13}C_{Diatoms} = \delta^{13}C_{C20:5\omega3}$ +2.5‰. We have estimated a $\delta^{13}C$ value of ~-24.8 to -23.8 ‰ for this microbial group (cyanobacteria and green algae). The low $\delta^{13}C$

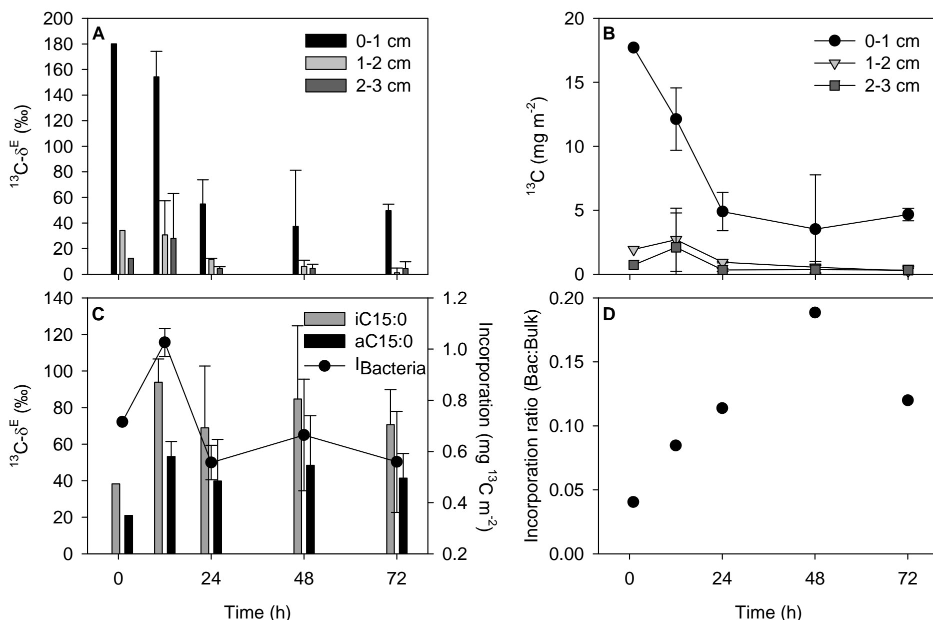

Figure 5.3. Phytodetritus addition experiment. (A) Enrichment (δ^E) and (B) incorporation (mg ^{13}C m^{-2}) for the different layers of the bulk sediment. Bacterial-specific PLFA enrichment and bacterial incorporation ($I_{Bacteria}$) for the top centimetre layer (C). All but t = 1h data points are mean ± SD with n = 2. Bacterial to bulk sediment incorporation ratio reflecting the gradual transfer o label to bacteria (D).

of SPM, composed mainly of cyanobacteria and green algae also support our finding that these two group are significantly ^{13}C-depleted relative to diatoms. These more depleted end-members are consistent with the $\delta^{13}C$ values of the group of suspension feeders (juveniles of bivalves and *N. diversicolor*) that were lower than that of SPM. Altogether these estimates permitted to visualise three different groups of fauna: a first one of deposit feeders depending mainly on benthic diatoms, with $\delta^{13}C$ values higher than ~-15‰; a second one composed of suspension feeders depending mainly on phytoplankton and/or a fraction of the MPB (cyanobacteria and green algae), with $\delta^{13}C$ values lower than ~19‰; and finally, a group of non-selective deposit feeders with intermediate $\delta^{13}C$ values relying on multiple resources.

5.3.3 Experiment 1

Sediment organic carbon ^{13}C enrichment was very rapid (Fig. 5.3A) with a maximum δ^E of ~180‰ at the sediment surface after settlement of the labelled phytodetritus, at the start of the experiment (t = 1 h). This surface enrichment significantly decreased from t = 12 h to reach a value of ~55‰ at t = 24 h, which then decreased very slowly until t = 72 h. Penetration of labelled phytodetritus into the sediment was very rapid as maximum enrichment for the 2 subsurface layers took place at the beginning of the experiment and decreased with time. Bulk sediment incorporation of ^{13}C showed dynamics similar to that of the enrichment (Fig. 5.3B). Surface sediment received a pulse of 17.71 mg ^{13}C m^{-2} and incorporation initially decreased rapidly until t = 24 h and then more slowly until t = 72 h. ^{13}C incorporation in deeper layers showed maximal values at t = 12 h for both the 1-2 and 2-3 cm layers.

Bacterial-specific PLFA in the layer 0-1 cm, illustrated here with iC15:0 and aC15:0, were rapidly enriched from the start of the experiment (Fig. 5.3C). The enrichment increased up to 93.87‰ at t = 12 h and then decreased over time. The dynamics of bacterial ^{13}C incorporation, projected on the secondary y axis, was consistent with that of the enrichment with a maximum of 1.03 mg ^{13}C m^{-2} incorporated at t = 12 h, followed by a sharp decrease to 0.56 mg ^{13}C m^{-2} at t = 24 h. Although both bacterial and bulk sediment incorporations showed an overall decreasing trend, there was a clear transfer of label to bacteria illustrated by the bacteria:bulk sediment incorporation ratio (Fig. 5.3D) increased over time to ~0.19 at t = 48h suggesting that about 20% of the added phytodetritus was transferred to bacteria at that time.

Meiofauna was rapidly labelled as illustrated by the levels of enrichment measured (Fig. 5.4A,B). Chironomid larvae were the most enriched taxon with a maximum δ^E value of ~267‰ at t = 48 h (Fig. 5.4A). Their high enrichment was consistent with the typical green colour of their gut content as observed under the microscope at the time of sorting

and picking. Juveniles of the gastropod *H. ulvae* and the copepod *P. spinicauda* were also significantly enriched, reaching maximal δ^E values of ~57‰ and ~73‰ respectively at t = 72 h. Juveniles of the bivalves *M. arenaria* and *Mytilus* sp. were also clearly enriched. Remaining meiofauna taxa were only slightly enriched (Fig. 5.4B). But note that patchy distribution of scarcely distributed taxa didn't always allow measuring their enrichment: data for ostracods were only available at t = 1, 48 and 72 h; tardigrades at t = 24 h; and juveniles of amphipods at t = 1, 12 and 24 h. Enrichment of the large copepod *H. jadensis* was estimated at every sampling but labelling was very limited and only detected at t = 1 and 12 h. Considering the large standard errors associated with these measurements, this could be due to adhesion of particles on the body of the animal. Finally, nematodes showed gradually increasing but limited enrichment (~4‰ at t = 72 h).

Contrary to the meiofauna, macrofauna showed labelling patterns similar to that of the bulk sediment with highest δ^E values right after the pulse of labelled phytodetritus (Fig. 5.4C). The suspension feeder *N. diversicolor* was the most enriched species with values above 100‰ at t = 1 and 12 h and between 25 and 30‰ from t = 24 h onwards. The

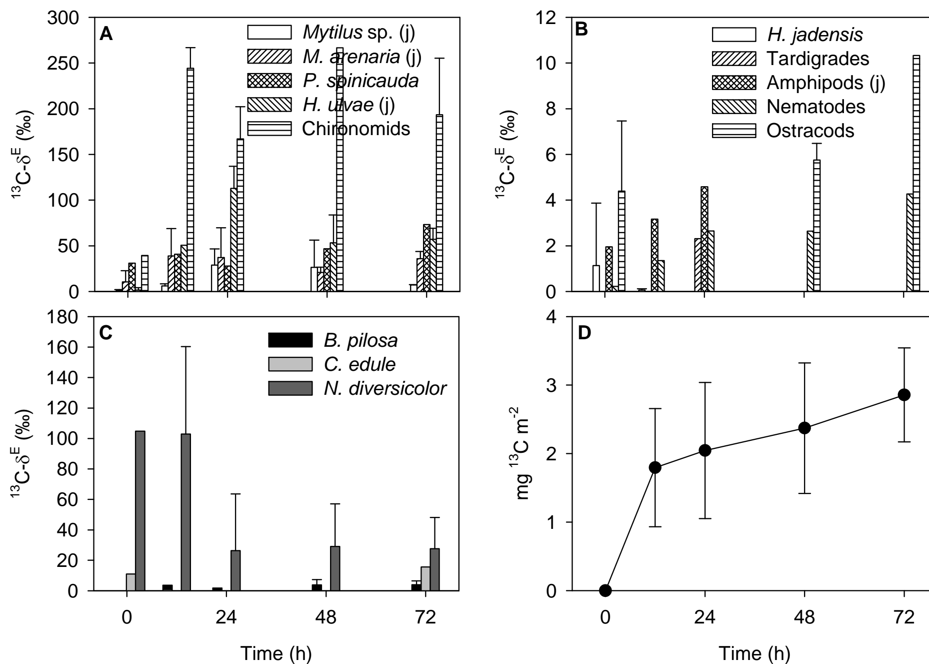

Figure 5.4. Phytodetritus addition experiment. Meiofauna (A, B) and macrofauna (C) enrichment (δ^E : mean ± SD with n = 2 if applicable) in the top centimetre layer. Total ^{13}C respired by the heterotrophic benthic community (D).

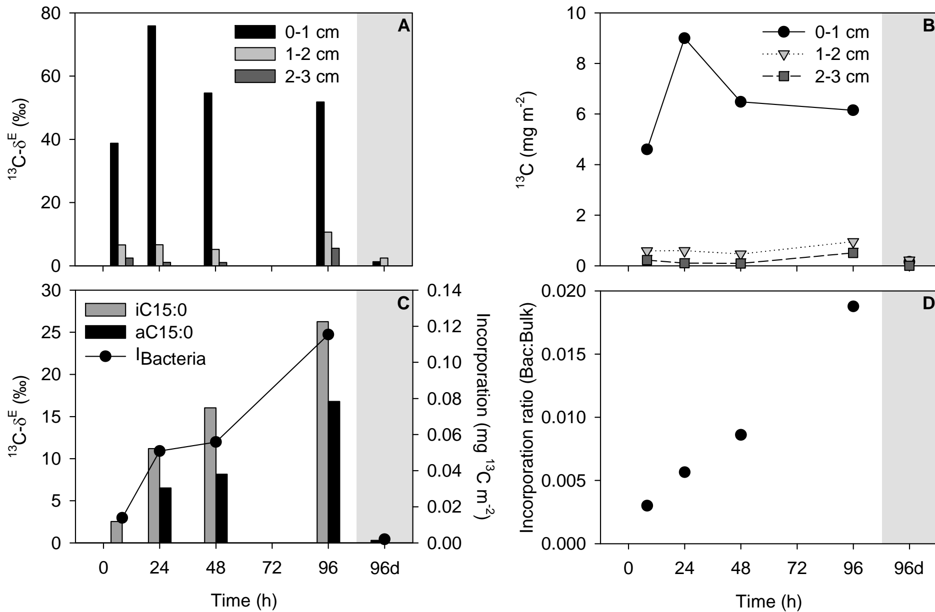

Figure 5.5. MPB addition experiment. Enrichment (δ^{E}) (A) and incorporation (mg ^{13}C m^{-2}) (B) for the different layers of the bulk sediment. Bacterial-specific PLFA enrichment and bacterial incorporation ($I_{Bacteria}$) for the top centimetre layer (C). Bacterial to bulk sediment incorporation ratio reflecting the gradual transfer of label to bacteria (D).

amphipod *B. pilosa* showed very little enrichment with a maximum of ~4‰ at t = 72 h. In addition, the erratic distribution of the bivalve *Cerastoderma edule* allowed us to measure its enrichment at t = 1 and 72 h only (11‰ and 15.7‰ respectively). Finally, ^{13}C-DIC incubations allowed us to estimate that 2.86 mg ^{13}C m^{-2} had been released from respiration at t = 72 h (Fig. 5.4D), which represents about 16% of the label present at the start of the experiment. This corresponds to a respiration rate of 6% of the added organic matter per day.

5.3.4 Experiment 2

Fixation of ^{13}C-DIC by the MPB was rapid and significant, as observed from bulk sediment enrichment (Fig. 5.5A). Although all cores were flushed twice after the labelling period of 8 h, sediment enrichment increased until t = 24 h to reach a maximum δ^{E} value of 75.9‰. Enrichment was lower at t = 48 h and remained rather constant until t = 96 h with a δ^{E} value of 51.8‰. Enrichment in the deeper layers was visible from the start and

slightly increased with time suggesting slow burial below 3 cm of labelled material. There was a very small though detectable enrichment in the dark after 96 h (2.4‰) that appeared more important in the subsurface layer (1-2 cm). Total sediment ^{13}C incorporation in the different layers followed the same pattern as for the enrichment (Fig. 5.5B). Maximum incorporation was reached at t = 24 h with a total of 9 mg ^{13}C m^{-2} and further decreased to 6.15 mg ^{13}C m^{-2} at t = 96 h. Incorporation in the dark was negligible.

Contrary to Exp1, enrichment of bacterial-specific PLFA showed a steady linear increase from the start of the experiment until t = 96 h, clearly suggesting a direct dependence of bacteria on recently fixed carbon (Fig. 5.5C). Consistent with Exp1, iC15:0 showed higher enrichment than aC15:0. Bacterial enrichment in the dark at t = 96 h was detectable but negligible compared to that in the light at the same time. Consistently with the enrichment of their specific PLFA, bacterial ^{13}C incorporation was steady and linear (~0.03 mg ^{13}C m^{-2} d^{-1}) during the whole course of the experiment and reached ~0.12 mg ^{13}C m^{-2}. Transfer of label from MPB to bacteria was confirmed from the bacteria to bulk sediment ^{13}C incorporation ratio (Fig. 5.5D), which showed a linear increase during the whole experiment. At t = 96 h, about 1.9% of label present in the sediment was incorporated into bacteria.

Besides the fact that the meiofauna community was very different compared to that of Exp1, i.e. density and diversity were generally lower, taxonomic groups also showed different labelling patterns (Fig. 5.6A,B). *H. ulvae* was most highly labelled with maximum δ^E value of ~129‰ at t = 96 h (Fig. 5.6A). Juveniles of amphipods for which enrichment could only be assessed at t = 24 and 96 h (dark) were also significantly labelled in the light incubation (~122‰). Juveniles of bivalves were also gradually enriched however to a lower extent. *H. jadensis* showed very contrasting response to label enrichment compared to Exp1, with high δ^E values straight from the beginning of the experiment (~79‰). In contrast, the other copepod species *P. spinicauda* which could only be measured at t = 24, 48 and 96 h showed relatively lower enrichment with maximum δ^E value of ~16‰ at t = 48 h. Nematodes, represented in a separate plot (Fig. 5.6B), were in sufficient numbers to get a vertical resolution of label enrichment. Smaller individuals from the upper centimetre of sediment showed higher enrichment than bigger individuals from the deeper layers (1-2 and 2-3 cm). However, their enrichment remained limited compared to other taxa, as illustrated earlier (Exp1), with δ^E values increasing to a maximum of ~12‰ at the sediment surface and ~8‰ in the deeper layers.

Macrofauna enrichment also showed striking difference with that of Exp2. *Gammarus* sp. which were not present in Exp1 showed the highest enrichments with maximum at t = 48 h (73‰). The other amphipod, *B. pilosa*, was slightly more enriched than in Exp1 (~13‰ at t = 96 h). Small individuals of *N. diversicolor* were enriched gradually, reaching a maximum δ^E value of 53‰ at t = 96 h. *C. edule* for which data were only available at t =

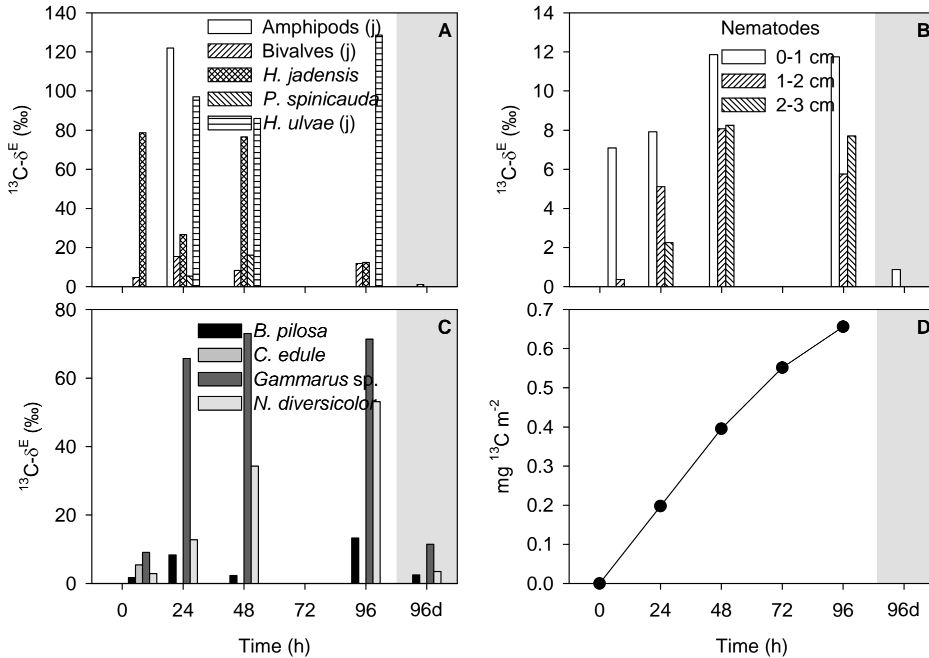

Figure 5.6. MPB addition experiment. Meiofauna (A), nematodes (B) and macrofauna (C) enrichment (δ^E) in the top centimetre layer. Total ^{13}C respired by the heterotrophic benthic community (D).

8 h showed a very small enrichment. Benthic fauna and bacteria contributed to the mineralisation of about 0.66 mg ^{13}C m^{-2} as estimated from labelled DIC incubations, which correspond to ~ 7.3% of the label fixed considering maximum bulk sediment incorporation (at t = 24 h).

5.3.5 Carbon budget

C_{org} inventories and $^{13}C_{org}$ incorporation of the different compartments are reported in Table 5.3. Bulk sediment C_{org} and MPB C_{org} estimated from Chl *a* were similar for the two experiments. Remaining identified compartments which accounted for most of the C_{org} contained in the sediment were bacteria with 672 and 380 mg C_{org} m^{-2} for Exp1 and Exp2 respectively. Meiofauna represented a very small fraction of C_{org} in both experiments. Total macrofauna biomass, which was not included in the bulk sediment, was more important than that of meiofauna and slightly lower than that of bacteria. The major

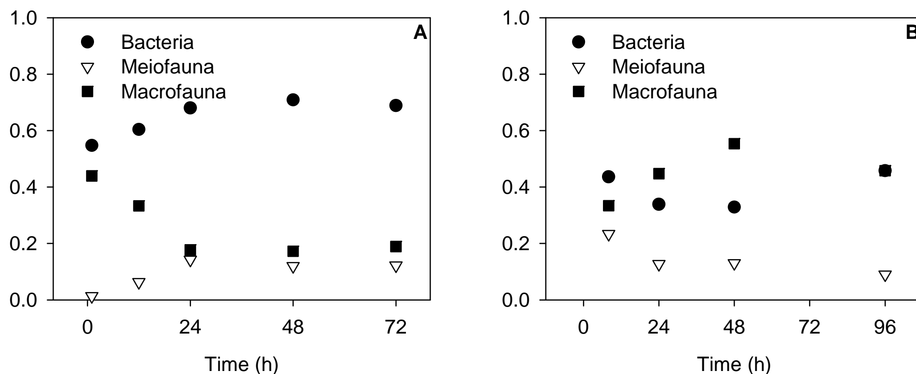

Figure 5.7. Relative incorporation of label into the different heterotrophic compartments in the phytodetritus addition experiment 1 (A) and MPB labelling experiment 2 (B).

fraction of C_{org} was not directly identified and accounted for 58.5% (5513 mg C m^{-2}) and 67% (6697 mg C m^{-2}) of total C_{org} in Exp1 and Exp2 respectively.

In both experiments, relative ^{13}C incorporation among the different heterotrophic compartments reflected that of their biomass. Typically, incorporation was highest in bacteria followed by macrofauna and meiofauna incorporation was somewhat limited (Table 5.3). In Exp1, relative label incorporation from phytodetritus by microscopic organisms like bacteria and meiofauna showed a rapid increase within the first 24 h, after which relative incorporation didn't significantly change (Fig. 5.7A). However, the larger organisms present in the macrofauna showed a rapid decrease followed by a steady relative incorporation. In Exp2, the patterns of relative incorporations were different (Fig. 5.7B). While the contributions of macrofauna and bacteria to label incorporation were relatively constant straight from the beginning of the experiment, meiofauna which contributed the least, showed a decreasing relative incorporation.

Table 5.3. Organic carbon stock and ^{13}C incorporation (I) of the different compartments of the benthos within the top centimetre of sediment (unless specified). Single zeros correspond to no incorporation.

		Experiment 1						Experiment 2					
		Biomass	I (mg $^{13}C_{org}$ m^{-2})					Biomass	I (mg $^{13}C_{org}$ m^{-2})				
		(mg C_{org} m^{-2})	1	12	24	48	72	(mg C_{org} m^{-2})	8	24	48	96	96d
	Bullk sediment 0-1 cm	9254.8	17.711	12.121	4.890	3.522	4.662	9988.1	4.599	8.999	6.483	6.149	0.149
	Bullk sediment 1-2 cm	6999.8	1.927	2.693	0.924	0.542	0.251	6118.9	0.587	0.594	0.462	0.951	0.217
	Bullk sediment 2-3 cm	5322.8	0.717	2.098	0.330	0.353	0.309	6341.5	0.220	0.098	0.091	0.507	0
	Bacteria	672.0	0.715	1.026	0.557	0.664	0.559	380.4	0.014	0.051	0.056	0.115	0.002
Meiofauna	Amphipods (j)	8.2	0.000	0.000	0.000	-	-	11.0	-	0.014	-	-	0
	Bivalves (j)	-	-	-	-	-	-	10.4	0.001	0.002	0.001	0.001	0.000
	Chironomids	25.9	0.011	0.068	0.047	0.074	0.054	-	-	-	-	-	-
	H. ulvae (j)	44.8	0.001	0.024	0.054	0.026	0.027	12.1	0	0.013	0.011	0.017	0
	H. jadensis	22.9	0.000	0.000	0	0	0	6.4	0.005	0.002	0.005	0.001	0
	M. arenaria (j)	27.2	0.003	0.011	0.011	0.006	0.010	-	-	-	-	-	-
	Mytilus sp. (j)	8.8	0.000	0.001	0.003	0.002	0.001	-	-	-	-	-	-
	Nematodes 0-1 cm	7.5	0.000	0.000	0.000	0.000	0.000	18.3	0.001	0.002	0.002	0.002	0.000
	Nematodes 1-2 cm	-	-	-	-	-	-	24.2	0.000	0.001	0.002	0.002	0
	Nematodes 2-3 cm	-	-	-	-	-	-	10.2	0	0.000	0.001	0.001	0
	Oligochaetes	6.8	0	0.001	0	0.000	0.002	-	-	-	-	-	-
	Ostracods	5.9	0.000	-	-	0.000	0.001	-	-	-	-	-	-
	P. spinicauda	5.1	0.002	0.002	0.002	0.003	0.004	2.0	-	0.000	0.000	0	-
	Tardigrades	0.3	-	-	0.000	-	-	-	-	-	-	-	-
Macrofauna	*B. pilosa*	47.6	0	0.002	0.001	0.002	0.002	23.7	0.000	0.002	0.001	0.003	0.001
	Gammarus sp.	-	-	-	-	-	-	72.9	0.007	0.052	0.057	0.056	0.009
	N. diversicolor	511.0	0.574	0.564	0.144	0.159	0.152	98.4	0.003	0.013	0.036	0.056	0.004

5.4 Discussion

The present study combines a natural abundance stable isotope approach with dedicated experiments in which either phytodetritus or microphytobenthos have been labelled with ^{13}C. This allowed identifying the various trophic interactions in a C_{org}-poor subtidal sandy sediment. Before discussing in detail the results, it is important to address the different methodological choices made.

Although the labelled phytodetritus addition experiment (Exp1) as well as the pulse-chase microphytobenthos labelling experiment (Exp2) could have been carried out *in situ*, they were realised in the laboratory so as to minimise risks of technical problems such as loss of material and disruption of the experimental setting by currents and waves, and to facilitate periodical sampling. The conditions of the laboratory experiments were set to mimic as closely as possible the conditions in the field. The stirring in the chambers induced water percolation through the upper layers of the sediment as produced by bottom currents in the field. This percolation is critical for the natural metabolism of the permeable sublittoral sands because it dominates the solute exchange between the sediment and the overlying water column (Huettel et al. 1998, Huettel & Rusch 2000). Replicate incubations were used for Exp1 but that was logistically not possible for Exp2. Our approach was based on balancing maximal resolution in food web compartments and reproducing natural flow conditions on the one hand and replication on the other hand. Although Exp2, was not replicated in the traditional sense, the time series over four days showed systematic patterns and allowed capturing most of the dynamics of the processes involved.

It is also important to mention that for logistic and technical reasons, the two experiments could not be carried out simultaneously. Sediment characteristics, in terms of faunal composition and density, differed significantly due to a storm that reworked the whole sediment bed in between the two experiments.

5.4.1 Sediment composition

With 0.06 ± 0.02% C for the uppermost cm, the C_{org} content of Hel sediment (~9300 mg C m^{-2} and ~10000 mg C m^{-2} for Exp1 and Exp2 respectively) was typical of C_{org}-poor sandy subtidal sediments (Dauwe et al. 1998). This characteristic was not affected by the storm between Exp1 and Exp2 nor linked to seasonal conditions as similar observations were made during the following spring, with values of 0.05 ± 0.01% DW (our unpublished data). These values were significantly lower than those found in intertidal mudflat areas

(Herman et al. 2000) and also lower than those of other shallow sandy subtidal areas (Chapter 4, Sundback et al. 1996).

MPB, comprising a heterogeneous mixture of diatoms, cyanobacteria and green algae, made up ~30% of total C_{org} present in the sediment with ~3000 mg C m^{-2}. These values were estimated from C:Chl *a* ratios calculated from an extensive set of pure microalgal cultures (Dijkman N.A., pers. comm.), complementing the widely used ratio of 40 proposed earlier by de Jonge(1980). Consistently with the lower values found for total C_{org} found in the sediment, MPB biomass data were significantly lower than those found in sheltered silty intertidal areas (Sundback et al. 1991, Underwood & Kromkamp 1999, Middelburg et al. 2000, Cook et al. 2004a) and other studies in subtidal sandy sediments (Sundback et al. 1991; Chapter 3). Nevertheless, MPB constitutes an important resource to benthic consumers.

The largest fraction of C_{org} in the sediment could not be specifically identified. It consisted of the C_{org} comprised in the bulk sediment minus all carbon in living organisms and was ~4850 mg C_{org} m^{-2} and ~6500 mg C_{org} m^{-2} in Exp1 and Exp2 respectively. This unidentified C_{org} pool comprised EPS and detrital organic matter from various sources. This is consistent with previous reports for subtidal sandy sediments in which relative stock fractions were also found to be roughly similar to their production (Chapter 3), implying that MPB diverted between 29.9% to 35.7% of its primary production to biomass and therefore 64.3% to 70.1% to exudation of EPS (averages of 32.8% and 67.2% respectively). These estimates fall within the range found in the literature for intertidal areas, reporting that 42 to 73% of C fixation can be excreted (Smith & Underwood 1998, Goto et al. 1999, Middelburg et al. 2000, de Brouwer & Stal 2001). In natural conditions, MPB exudation of EPS is significantly higher than that of phytoplankton (Goto et al. 1999) and therefore can provide a substantial substrate for benthic bacteria (Middelburg et al. 2000).

Contrary to the MPB which usually recovers rapidly from reworking of the sediment, the whole heterotrophic compartment was significantly affected by the storm event that separated the two experiments. Among heterotrophic compartments, bacteria showed the most significant biomass with 672 mg C m^{-2} and 380 mg C m^{-2} in Exp1 and Exp2 respectively, representing 48% and 59% of the heterotrophic biomass. These values are lower than those reported earlier for sandy sediment (Chapter 4) due to (1) lower organic carbon resources available and (2) a higher contribution of macrofauna in these sediments. Although only three species were sampled, macrofauna was the second most significant compartment in terms of biomass and represented about 40% and 31% of the heterotrophic biomass in Exp1 and Exp2 respectively. Meiofauna, which showed the highest diversity, represented the smallest compartment with ~10% of the biomass. The overall picture of the relative fractions of the heterotrophic compartments presented here

is not common, as most studies have emphasised the dominance of meiofauna over macrofauna in sandy sediments (Gerlach 1971, Koop & Griffiths 1982, Heip et al. 1992, Urban-Malinga & Moens 2006).

Taxonomic investigation of the different compartments revealed interesting points. Macrofauna was dominated by amphipods (*B. pilosa* and *Gammarus* sp.) in Exp1 and Exp2 respectively. These animals are typical of highly dynamic environments. In addition, the facultative filter-feeding polychaete *N. diversicolor* showed the highest biomass. This species is known to be able to sustain its growth with both phytoplankton and MPB (Vedel & Riisgard 1993, Smith et al. 1996). In Exp1, the meiofauna compartment was clearly dominated by juvenile forms of macrofauna species (i.e. *H. ulvae*, *M. arenaria*, *Mytilus* sp. and chironomid larvae). Surprisingly, harpacticoid copepods that were represented by two main species (*H. jadensis* and *P. spinicauda*) showed higher densities and biomass than nematodes, which are often regarded as the most dominant taxon in terms of densities and biomass, regardless of the ecosystem (Heip et al. 1985, Huys et al. 1992). Although nematode assemblages in sandy sediments would deserve a quantitative study at the species level because of their high diversity (Gheskiere et al. 2005), this was not possible because of low densities.

5.4.2 Trophic interactions

MPB clearly plays a central role in the benthic food web (Fig. 5.2). Indeed, bulk sediment C_{org} $\delta^{13}C$ values reflect that of the MPB. The usual approach in benthic food web studies implies considering benthic food sources on the one hand (e.g. MPB, macroalgae, marine macrophytes) and pelagic sources (phytoplankton) on the other hand (Fry & Sherr 1988, Heip & Craeymeersch 1995, Herman et al. 2000, Carman & Fry 2002, Maddi et al. 2006). This partitioning between benthic and pelagic sources in food web studies has emerged because often a clear distinction can be made between the stable isotope signature of SPM and that of the surficial sediments (i.e. MPB and phytoplankton, France 1995). In the present study, the different taxonomic groups showed $\delta^{13}C$ values distributed within a range centred on the bulk sediment but only *H. ulvae* and ostracods were comprised within the bulk sediment and SPM boundaries. However, a closer look at the $\delta^{13}C$ of the diatom-specific PLFA (20:5ω3) revealed a higher value for benthic diatoms, likely ranging between -13.5‰ to -12.5‰ (bearing in mind a fractionation of 2-3‰ to account for the carbon depletion of PLFA relative to that of the biomass). This enlarged range comprises all taxonomic groups with isotopic signatures heavier than that of the bulk sediment indicating that benthic diatoms contribute significantly to the diets of many heterotrophs.

However, several taxa were outside the boundaries defined by the diatoms and SPM (most ^{13}C depleted potential source), with values lower than this range. We attribute these depleted values to a reliance of these consumers on other members of the MPB (cyanobacteria and green algae). Unfortunately, isotopic analysis of PLFA specific of cyanobacteria and green algae was not possible in these organic poor sandy sediments. As a proxy we have derived a δ^{13}C value for the combined pool of cyanobacteria and green algae. This estimated δ^{13}C value (-23.8 to -24.8‰) allowed including the rest of the taxonomic groups. Taxonomic groups with extremely high δ^{13}C values suggested high dependence or selectivity for diatoms, i.e. *H. jadensis*, tardigrades, *P. spinicauda*, *B. pilosa*, chironomids and juveniles of amphipods. Intermediate δ^{13}C values of a few taxa (*Gammarus* sp., nematodes, oligochaetes, ostracods and *H. ulvae*) suggest that they are not feeding selectively; rather their diet is composed of heterogeneous MPB or MPB-derived material. Finally, the remaining taxa, that all belong to the suspension-feeders, consistently showed δ^{13}C values similar to that of SPM. However, all these values were more ^{13}C-depleted than that of SPM, which we could also ascribe to a contribution of ^{13}C-depleted cyanobacteria and green algae to their diet.

In addition, the weighted average δ^{13}C value of bacterial-specific PLFA isotopic signatures (data not shown) varied between -16.7‰ and -15.7‰. This was very similar to the isotopic signature of diatom-specific PLFAs (Fig. 5.2) implying a clear reliance of bacteria on MPB or derived material. This combined isotope-biomarker approach, already illustrated in a similar study by Evrard et al. (Chapter 4), allowed providing further details on the potential trophic interactions and avoiding the pitfall of rejecting potential resources because δ^{13}C values of heterogeneous resources don't correspond to those of consumers.

5.4.3 Phytodetritus and MPB as food sources

We suppose that in C_{org}-poor sediments, heterotrophs would depend equally on phytodetritus inputs (settling from the water column) and MPB production in the sediment. Before comparing the relative significance of these two pathways, the dual experimental approach, together with the ^{13}C natural abundance study, will be discussed to highlight various trophic relationships. The benthic copepods *H. jadensis* and *P. spinicauda* were both rich in ^{13}C at natural abundance, indicating that they derive substantial resources from benthic diatoms. However, MPB grazing is often species-specific and density dependent (De Troch et al. 2005), thus in favour of resource partitioning strategies. This is particularly important for dominant species. Our dual experimental approach allowed elucidating this hypothesis as both copepods responded very differently in the two labelling experiments. While *P. spinicauda* showed high

affinity for the labelled phytodetritus, *H. jadensis* was not significantly enriched in the first experiment. Contrastingly, in the second experiment *H. jadensis* was significantly more labelled than *P. spinicauda*. To our knowledge, this is one of the few studies documenting resource partitioning between co-occurring species of benthic copepods (Carman & Thistle 1985, Pace & Carman 1996). However this is the first study to illustrate resource partitioning between *H. jadensis* and *P. spinicauda* and these findings are consistent with the very low C_{org} content in the sediment likely implying food limitation. Chironomid larvae were the most labelled taxon of the meiofauna and also showed the highest total incorporation while they were not the most abundant. On the basis of 11% ^{13}C-labelling of the phytodetritus pool, we can derive from the maximum incorporation at t = 48h (0.074 mg ^{13}C m^{-2}) a total uptake of 0.672 mg C_{org} m^{-2} of phytodetritus in two days, which represents ~3% of their biomass. Unfortunately, no chironomids were present in the second experiment and we cannot compare both treatments. However, it is well known that they are significant MPB consumers (sometimes the most important grazer among meiofauna, Pinckney et al. 2003) and they can have a top-down effect on the resource (Goldfinch & Carman 2000).

^{13}C natural abundance of juveniles of *H. ulvae* was intermediate, indicative of a non selective diet on MPB, consistent with knowledge on the feeding preferences of adult forms (Fenchel et al. 1975). In addition, Exp1 gave evidence that phytodetritus might also significantly contribute to its diet which to our knowledge has never been shown before. Juveniles of bivalves (i.e. *Mytilus* sp. and *M. arenaria*) showed natural abundance ^{13}C values typical of suspension feeders. This was confirmed by the important uptake of labelled phytodetritus in Exp1. However, the bivalves were also enriched in Exp2 suggesting that MPB, either directly or indirectly following resuspension, is also an important part of their diet. Sauriau & Kang (2000) also found that more than 70% of the cockle growth (*Cerastoderma edule*) was derived from MPB, with highest proportions for juveniles.

Although ubiquitous, nematodes were present only in very limited amount in our study, contrasting with previous observations of increased diversities and densities in coarser sediments (Gheskiere et al. 2005). Nematodes showed very limited enrichment in Exp1 and a slightly higher enrichment in Exp2 (Figs. 5.4 and 5.6). Most nematodes are non selective deposit-feeders (Gheskiere et al. 2004) using labile organic matter (Moens et al. 2002). Our results suggest that phytodetritus or MPB was not readily assimilated and that nematodes might depend on microbenthic organisms that rely on MPB or phytodetritus. Their very low but steady increasing levels of enrichment were consistent with such a higher trophic position. It is also important to notice that during the second experiment, there was an apparent size-class stratification of the nematode community distribution, with small individuals living at the sediment surface and large individuals in

the deeper layers. Specific observation at the same sampling site and at the same period by Urban-Malinga et al. (2006) revealed a mix community of non-selective deposit feeders and predators. The nematode community of this low C_{org} sandy sediment was highly contrasting in terms of biomass, densities and carbon uptake with that of C_{org}-rich North Sea sandy sediment studied by Evrard et al. (Chapter 4) and Urban-Malinga et al. (2006).

The macrofauna community was mainly dominated by the amphipods *B. pilosa* and *Gammarus* sp., although the latter was present only in Exp2. Their enrichments were generally lower than those of the meiofauna but, due to their significant biomass, their contribution to ^{13}C incorporation and remineralisation was important. *N. diversicolor* was highly enriched in ^{13}C in both experiments, suggesting that this suspension feeder is not limited to phytoplankton or particulate detrital resources and that MPB can constitute an important fraction of its diet as well. Altogether these results emphasize the pivotal importance of MPB for benthic macrofauna.

Bacterial tracer enrichment was significant and ^{13}C incorporation by bacteria after 24 h accounted for 69% of total heterotrophic community uptake in Exp1 and 38 % in Exp2, second only to macrofauna. Our results for Exp 1 are in good agreement with Buhring et al. (2006) who found, in a similar labelled phytodetritus addition experiment, that up to 62% was incorporated into bacteria. In the first experiment, where we administered labelled phytodetritus, at t = 48h, almost 20% of total label fixation in bulk sediment was actually present in the bacterial compartment. The same ratio in the second experiment, in which we labelled MPB, was smaller by one order of magnitude. However, assuming that bacteria relied principally on EPS (Middelburg et al. 2000), we need to take into account an extreme dilution of the label in their substrate. If EPS production is proportional with the fraction (67.2%) found in the stock (MPB + EPS = bulk sediment - bacteria - meiofauna), we can estimate that newly produced ^{13}C-EPS at t = 24h is 0.672x9=6.048 mg ^{13}C m^{-2}. In addition, DIC labelling was 6.7%, which implies that total EPS production at t = 24h was 6.048/0.067 = 90 mg C_{org} m^{-2}. If EPS standing stock was 6697 mg C_{org} m^{-2}, then final dilution of ^{13}C in the EPS is 6.048/(6697+90) = 0.0009 (0.09%). We can estimate that total C assimilation at t = 96h by bacteria was 0.115/0.0009 = 127.78 mg C_{org} m^{-2} (~32 mg C_{org} m^{-2} d^{-1}), which is close to the lower limit found in an intertidal sand flat in the North Sea (Rusch et al. 2001). This suggests that about 24% of the MPB daily production (~134 mg C m^{-2} d^{-1}) is assimilated by bacteria. In comparison, somewhat less than 20% of the phytodetritus deposition was assimilated by bacteria after 72 h. These findings confirm the central role of MPB production for bacterial growth particularly in C_{org}-poor sandy sediments. This pivotal role of MPB production for bacterial growth, based on deliberate tracer experiments, is supported by the similarity of natural abundance $\delta^{13}C$ of bacterial and diatom PLFA, indicating that bacteria obtain most of their carbon from MPB.

In both tracer experiments, most of the ^{13}C was recovered in the DIC pool indicating that respiration was the largest sink for labelled carbon, consistent with observations of Moodley et al. (2000, 2005). The overall benthic community growth efficiency, i.e. the ratio of the total ^{13}C incorporation by the heterotrophs/(^{13}C incorporation by heterotrophs + respired ^{13}C), was ~ 26% for both experiments, consistent with observations by Moodley et al. (2002)

Finally, we can estimate the relative significance of phytodetritus and MPB as food sources for heterotrophs in sandy subtidal sediments. Assuming a ^{13}C dilution percentage calculated similarly to that of the EPS (dilution of fixed ^{13}C within the standing stock of MPB), we estimate that about 0.1% of the MPB was ^{13}C labelled. This then allows estimating total C_{org} assimilated by the different heterotrophic compartments. These results normalized to the biomass of each compartment were compared to those calculated the same way as in Exp1. We calculated the relative dependence at t = 24, 48 and 72h (we linearly interpolated 72h for Exp2) as the normalised C_{org} assimilation Exp2/(normalised C_{org} assimilation Exp1+normalised C_{org} assimilation Exp2). The dependence on MPB for the global heterotrophic community was 98.2±0.3%. The dependencies were 95.4±1.3%, 98±0.3% and 99.5±0.1% for bacteria, meiofauna and macrofauna respectively. This study confirms the prominent role of MPB in sandy sediments both from an ecological point of view as it represents the main food source and governs trophic relationships, and in terms of biogeochemical processes as it governs C flows.

References

Agawin NSR, Duarte GM (2002) Evidence of direct particle trapping by a tropical seagrass meadow. Estuaries 25:1205-1209

Allen AE, Booth MG, Frischer ME, Verity PG, Zehr JP, Zani S (2001) Diversity and detection of nitrate assimilation genes in marine bacteria. Appl Environ Microbiol 67:5343-5348

Aller RC, Aller JY (1998) The effect of biogenic irrigation intensity and solute exchange on diagenetic reaction rates in marine sediments. J Mar Res 56:905-936

Anderson WT, Fourqurean JW (2003) Intra- and interannual variability in seagrass carbon and nitrogen stable isotopes from south Florida, a preliminary study. Org Geochem 34:185-194

Armonies W, Reise K (2000) Faunal diversity across a sandy shore. Mar Ecol Prog Ser 196:49-57

Barranguet C, Kromkamp J, Peene J (1998) Factors controlling primary production and photosynthetic characteristics of intertidal microphytobenthos. Mar Ecol Prog Ser 173:117-126

Bartsch I (2004) Geographical and ecological distribution of marine halacarid genera and species (Acari : Halacaridae). Exp Appl Acarol 34:37-58

Ben-David M, Flynn RW, Schell DM (1997) Annual and seasonal changes in diets of martens: evidence from stable isotope analysis. Oecologia 111:280-291

Berges JA, Cochlan WP, Harrison PJ (1995) Laboratory and Field Responses of Algal Nitrate Reductase to Diel Periodicity in Irradiance, Nitrate Exhaustion, and the Presence of Ammonium. Mar Ecol Prog Ser 124:259-269

Bertness MD, Leonard GH (1997) The role of positive interactions in communities: Lessons from intertidal habitats. Ecology 78:1976-1989

Billerbeck M, Roy H, Bosselmann K, Huettel M (2007) Benthic photosynthesis in submerged Wadden Sea intertidal flats. Estuarine, Coastal and Shelf Science 71:704-716

Bird KT, Johnson JR, Jewett-Smith J (1998) In vitro culture of the seagrass Halophila decipiens. Aquat Bot 60:377-387

Blaabjerg V, Mouritsen KN, Finster K (1998) Diel cycles of sulphate reduction rates in sediments of a Zostera marina bed (Denmark). Aquat Microb Ecol 15:97-102

Blair NE, Levin LA, DeMaster DJ, Plaia G (1996) The short-term fate of fresh algal carbon in continental slope sediments. Limnol Oceanogr 41:1208-1219

Boschker HTS, de Brouwer JFC, Cappenberg TE (1999) The contribution of macrophyte-derived organic matter to microbial biomass in salt-marsh sediments: Stable carbon isotope analysis of microbial biomarkers. Limnol Oceanogr 44:309-319

Boschker HTS, Kromkamp JC, Middelburg JJ (2005) Biomarker and carbon isotopic constraints on bacterial and algal community structure and functioning in a turbid, tidal estuary. Limnol Oceanogr 50:70-80

Boschker HTS, Middelburg JJ (2002) Stable isotopes and biomarkers in microbial ecology. Fems Microbiol Ecol 40:85-95

Boschker HTS, Wielemaker A, Schaub BEM, Holmer M (2000) Limited coupling of macrophyte production and bacterial carbon cycling in the sediments of *Zostera* spp. meadows. Mar Ecol Prog Ser 203:181-189

Boudreau BP, Huettel M, Forster S, Jahnke RA, McLachlan A, Middelburg JJ, Nielsen P, Sansone F, Taghon G, Van Raaphorst W (2001) Permeable marine sediments: overturning an old paradigm. EOS 82:133-136

Brinch-Iversen J, King GM (1990) Effects Of Substrate Concentration, Growth-State, And Oxygen Availability On Relationships Among Bacterial Carbon, Nitrogen And Phospholipid Phosphorus-Content. Fems Microbiol Ecol 74:345-355

Brown MR (1991) The amino-acid and sugar composition of 16 species of microalgae used in mariculture. J Exp Mar Biol Ecol 145:79-99

Buhring SI, Ehrenhauss S, Kamp A, Moodley L, Witte U (2006) Enhanced benthic activity in sandy sublittoral sediments: Evidence from C-13 tracer experiments. Mar Biol Res 2:120-129

Burgess B (2001) An improved protocol for separating meiofauna from sediments using colloidal silica sols. Mar Ecol Prog Ser 214:161-165

Cahoon LB (1999) The role of benthic microalgae in neritic ecosystems. In: Ansell AD, Gibson RN, Barnes M (eds) Oceanogr Mar Biol Ann Rev, Vol 37. Taylor & Francis, London, p 47-86

Carignan R, Kalff J (1980) Phosphorus Sources For Aquatic Weeds - Water Or Sediments. Science 207:987-989

Carman KR, Fry B (2002) Small-sample methods for delta C-13 and delta N-15 analysis of the diets of marsh meiofaunal species using natural-abundance and tracer-addition isotope techniques. Mar Ecol Prog Ser 240:85-92

Carman KR, Thistle D (1985) Microbial food partitioning by three species of benthic copepods. Mar Biol 88:143-148

Clark DR, Flynn KJ, Owens NJP (2002) The large capacity for dark nitrate-assimilation in diatoms may overcome nitrate limitation of growth. New Phytol 155:101-108

Cook PLM, Butler ECV, Eyre BD (2004a) Carbon and nitrogen cycling on intertidal mudflats of a temperate Australian estuary. I. Benthic metabolism. Mar Ecol Prog Ser 280:25-38

Cook PLM, Revill AT, Butler ECV, Eyre BD (2004b) Carbon and nitrogen cycling on intertidal mudflats of a temperate Australian estuary. II. Nitrogen cycling. Mar Ecol Prog Ser 280:39-54

Cook PLM, Roy H (2006) Advective relief of CO2 limitation in microphytobenthos in highly productive sandy sediments. Limnol Oceanogr 51:1594-1601

Cook PLM, Veuger B, Boer S, Middelburg JJ (submitted) Effect of nutrient availability on carbon and nitrogen incorporation and flows through benthic algae and bacteria in nearshore sediment

Cook PLM, Wenzhöfer F, Glud RN, Janssen F, Huettel M (2007) Benthic solute exchange and carbon mineralization in two shallow subtidal sandy sediments: Impact of advective porewater exchange. Limnol Oceanogr 52:XXXX-XXXX

Costanza R, dArge R, deGroot R, Farber S, Grasso M, Hannon B, Limburg K, Naeem S, Oneill RV, Paruelo J, Raskin RG, Sutton P, vandenBelt M (1997) The value of the world's ecosystem services and natural capital. Nature 387:253-260

Coull BC (1999) Role of meiofauna in estuarine soft-bottom habitats. Aust J Ecol 24:327-343

Coull BC, Chandler GT (1992) Pollution and meiofauna: field, laboratory, and mesocosm studies. Oceanogr Mar Biol Ann Rev 30:191-271

Cowie GL, Hedges JI (1992) Sources and Reactivities of Amino-Acids in a Coastal Marine-Environment. Limnol Oceanogr 37:703-724

Dale NG (1974) Bacteria in intertidal sediments: Factors related to their distribution. Limnol Oceanogr 19:509-518

Dauwe B, Herman PMJ, Heip CHR (1998) Community structure and bioturbation potential of macrofauna at four North Sea stations with contrasting food supply. Mar Ecol Prog Ser 173:67-83

Dauwe B, Middelburg JJ (1998) Amino acids and hexosamines as indicators of organic matter degradation state in North Sea sediments. Limnol Oceanogr 43:782-798

Dauwe B, Middelburg JJ, Herman PMJ (2001) Effect of oxygen on the degradability of organic matter in subtidal and intertidal sediments of the North Sea area. Mar Ecol Prog Ser 215:13-22

Dawson TE, Mambelli S, Plamboeck AH, Templer PH, Tu KP (2002) Stable isotopes in plant ecology. Annu Rev Ecol Syst 33:507-559

de Beer D, Wenzhofer F, Ferdelman TG, Boehme SE, Huettel M, van Beusekom JEE, Bottcher ME, Musat N, Dubilier N (2005) Transport and mineralization rates in North Sea sandy intertidal sediments, Sylt-Romo Basin, Wadden Sea. Limnol Oceanogr 50:113-127

de Brouwer JFC, Stal LJ (2001) Short-term dynamics in microphytobenthos distribution and associated extracellular carbohydrates in surface sediments of an intertidal mudflat. Mar Ecol Prog Ser 218:33-44

de Jonge VN (1980) Fluctuations in the organic carbon to chlorophyll a ratios for estuarine benthic diatom populations. Mar Ecol Prog Ser 2:345-353

de Jonge VN, Vanbeusekom JEE (1995) Wind- and tide-induced resuspension of sediment and microphytobenthos from tidal flats in the Ems estuary. Limnol Oceanogr 40:766-778

De Troch M, Steinarsdottir MB, Chepurnov V, Olafsson E (2005) Grazing on diatoms by harpacticoid copepods: species-specific density-dependent uptake and microbial gardening. Aquat Microb Ecol 39:135-144

Deegan LA, Garritt RH (1997) Evidence for spatial variability in estuarine food webs. Mar Ecol Prog Ser 147:31-47

Deniro MJ, Epstein S (1981) Influence of Diet on the Distribution of Nitrogen Isotopes in Animals. Geochim Cosmochim Acta 45:341-351

Dexter DM (1992) Sandy Beach Community Structure - the Role of Exposure and Latitude. J Biogeogr 19:59-66

Dijkman NA, Kromkamp JC (2006) Phospholipid-derived fatty acids as chemotaxonomic markers for phytoplankton: application for inferring phytoplankton composition. Mar Ecol Prog Ser 324:113-125

Duarte CM, Cebrian J (1996) The fate of marine autotrophic production. Limnol Oceanogr 41:1758-1766

Duarte CM, Middelburg JJ, Caraco N (2005) Major role of marine vegetation on the oceanic carbon cycle. Biogeosciences 1:173-180

Dugdale RC, Goering JJ (1967) Uptake of New and Regenerated Forms of Nitrogen in Primary Productivity. Limnol Oceanogr 12:196-206

Edgar GJ, Shaw C (1995a) The production and trophic ecology of shallow-water fish assemblages in southern Australia .2. Diets of fishes and trophic relationships between fishes and benthos at Western Port, Victoria. J Exp Mar Biol Ecol 194:83-106

Edgar GJ, Shaw C (1995b) The production and trophic ecology of shallow-water fish assemblages in southern Australia .3. General relationships between sediments, seagrasses, invertebrates and fishes. J Exp Mar Biol Ecol 194:107-131

Ehrenhauss S, Witte U, Bühring SI, Huettel M (2004a) Effect of advective pore water transport on distribution and degradation of diatoms in permeable North Sea sediments. Mar Ecol Prog Ser 271:99-111

Ehrenhauss S, Witte U, Janssen F, Huettel M (2004b) Decomposition of diatoms and nutrient dynamics in permeable North Sea sediments. Cont Shelf Res 24:721-737

Emery KO (1968) Relict sediments on continental shelves of world. AAPG Bulletin 52:445-464

Epstein SS, Burkovsky IV, Shiaris MP (1992) Ciliate grazing on bacteria, flagellates, and microalgae in a temperate zone sandy tidal flat: ingestion rates and food niche partitioning. J Exp Mar Biol Ecol 165:103-123

Epstein SS, Shiaris MP (1992) Rates of microbenthic and meiobenthic bacterivory in a temperate muddy tidal flat. Appl Environ Microbiol 58:2426-2431

Erftemeijer PLA, Middelburg JJ (1995) Mass balance constraints on nutrient cycling in tropical seagrass beds. Aquat Bot 50:21-36

Evrard V, Cook PLM, Veuger B, Huettel M, Middelburg JJ (in revision) Tracing incorporation and pathways of carbon and nitrogen in microbial communities of photic subtidal sands. Aquat Microb Ecol

Evrard V, Kiswara W, Bouma TJ, Middelburg JJ (2005) Nutrient dynamics of seagrass ecosystems: ^{15}N evidence for the importance of particulate organic matter and root systems. Mar Ecol Prog Ser 295:49-55

Feller RJ (2006) Weak meiofaunal trophic linkages in Crangon crangon and Carcinus maenus. J Exp Mar Biol Ecol 330:274-283

Fenchel T (1969) The ecology of marine microbenthos: 4. Structure and function of the benthic ecosystem, its chemical and physical factors and the microfauna communities with special reference to the ciliated protozoa. Ophelia 6:1-182

Fenchel T, Kofoed LH, Lappalainen A (1975) Particle size-selection of two deposit feeders: the amphipod *Corophium volutator* and the prosobranch *Hydrobia ulvae*. Mar Biol 30:119-128

Fenchel TM (1978) Ecology of Microbenthos and Meiobenthos. Annual Review of Ecology and Systematics 9:99-121

Field CB, Behrenfeld MJ, Randerson JT, Falkowski P (1998) Primary production of the biosphere: Integrating terrestrial and oceanic components. Science 281:237-240

France RL (1995) Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. Mar Ecol Prog Ser 124:307-312

Franke U, Polerecky L, Precht E, Huettel M (2006) Wave tank study of particulate organic matter degradation in permeable sediments. Limnol Oceanogr 51:1084-1096

Fry B (2006) Stable Isotope Ecology, Vol. Springer, New York

Fry B, Sherr EB (1984) Delta-C-13 Measurements as Indicators of Carbon Flow in Marine and Fresh-Water Ecosystems. Contributions in Marine Science 27:13-47

Fry B, Sherr EB (1988) δ^{13}C Measurements as Indicators of Carbon Flow in Marine and Freshwater Ecosystems. In: Rundel PW, Ehleringer JR, Nagy KA (eds) Sable Isotopes in Ecological Research. Springer-Verlag, New York, p 196-229

Gacia E, Duarte CM (2001) Sediment retention by a mediterranean *Posidonia oceanica* meadow: The balance between deposition and resuspension. Estuar Coast Shelf Sci 52:505-514

Gacia E, Duarte CM, Marba N, Terrados J, Kennedy H, Fortes MD, Tri NH (2003) Sediment deposition and production in SE-Asia seagrass meadows. Estuar Coast Shelf Sci 56:909-919

Gacia E, Duarte CM, Middelburg JJ (2002) Carbon and nutrient deposition in a Mediterranean seagrass (*Posidonia oceanica*) meadow. Limnol Oceanogr 47:23-32

Gattuso JP, Gentili B, Duarte CM, Kleypas JA, Middelburg JJ, Antoine D (2006) Light availability in the coastal ocean: impact on the distribution of benthic photosynthetic organisms and their contribution to primary production. Biogeosciences 3:489-513

Gee JM (1987) Impact of Epibenthic Predation on Estuarine Intertidal Harpacticoid Copepod Populations. Mar Biol 96:497-510

Gerlach SA (1971) Importance of Marine Meiofauna for Benthos Communities. Oecologia 6:176-&

Gerlach SA (1978) Food-Chain Relationships in Subtidal Silty Sand Marine-Sediments and Role of Meiofauna in Stimulating Bacterial Productivity. Oecologia 33:55-69

Gheskiere T, Hoste E, Vanaverbeke J, Vincx M, Degraer S (2004) Horizontal zonation patterns and feeding structure of marine nematode assemblages on a macrotidal, ultra-dissipative sandy beach (De Panne, Belgium). J Sea Res 52:211-226

Gheskiere T, Vincx M, Urban-Malinga B, Rossano C, Scapini F, Degraer S (2005) Nematodes from wave-dominated sandy beaches: diversity, zonation patterns and testing of the isocommunities concept. Estuar Coast Shelf Sci 62:365-375

Glud RN, Kuhl M, Wenzhofer F, Rysgaard S (2002) Benthic diatoms of a high Arctic fjord (Young Sound, NE Greenland): importance for ecosystem primary production. Mar Ecol Prog Ser 238:15-29

Goldfinch AC, Carman KR (2000) Chironomid grazing on benthic microalgae in a Louisiana salt marsh. Estuaries 23:536-547

Goldman JC, Dennett MR (2000) Growth of marine bacteria in batch and continuous culture under carbon and nitrogen limitation. Limnol Oceanogr 45:789-800

Goto N, Kawamura T, Mitamura O, Terai H (1999) Importance of extracellular organic carbon production in the total primary production by tidal-flat diatoms in comparison to phytoplankton. Mar Ecol Prog Ser 190:289-295

Goto N, Mitamura O, Terai H (2001) Biodegradation of photosynthetically produced extracellular organic carbon from intertidal benthic algae. J Exp Mar Biol Ecol 257:73-86

Granata TC, Serra T, Colomer J, Casamitjana X, Duarte CM, Gacia E (2001) Flow and particle distributions in a nearshore seagrass meadow before and after a storm. Mar Ecol Prog Ser 218:95-106

Grasshoff K, Kremling K, Ehrhardt M (1999) Methods of Seawater Analysis, Vol. Wiley-VCH, Weinheim

Hall SJ (2002) The continental shelf benthic ecosystem: current status, agents for change and future prospects. Environ Conserv 29:350-374

Hayes JM (2001) Fractionation of carbon and hydrogen isotopes in biosynthetic processes. In: Stable Isotope Geochemistry, Vol 43. Mineralogical Soc America, Washington, p 225-277

Hedtkamp S (2005) Shallow subtidal sand: permeability, nutrient dynamics, microphytobenthos and organic matter. Christian-Albrechts-Universität

Heip C, Basford D, Craeymeersch JA, Dewarumez JM, Dorjes J, Dewilde P, Duineveld G, Eleftheriou A, Herman PMJ, Niermann U, Kingston P, Kunitzer A, Rachor E, Rumohr H, Soetaert K, Soltwedel T (1992) Trends in Biomass, Density and Diversity of North-Sea Macrofauna. Ices J Mar Sci 49:13-22

Heip C, Craeymeersch JA (1995) Benthic Community Structures in the North-Sea. Helgol Meeresunters 49:313-328

Heip C, Vincx M, Vranken G (1985) The Ecology Of Marine Nematodes. In: Barnes M (ed) Oceanogr Mar Biol Ann Rev, Vol 23. Aberdeen University Press, Aberdeen, p 399-489

Heip CHR, Goosen NK, Herman PMJ, Kromkamp J, Middelburg JJ, Soetaert K (1995) Production and consumption of biological particles in temperate tidal estuaries. In: Ansell AD, Gibson RN, Barnes M (eds) Oceanogr Mar Biol Ann Rev, Vol 33. UCL Ltd, London, p 1-149

Hemminga MA (1998) The root/rhizome system of seagrasses: an asset and a burden. J Sea Res 39:183-196

Hemminga MA, Marba N, Stapel J (1999) Leaf nutrient resorption, leaf lifespan and the retention of nutrients in seagrass systems. Aquat Bot 65:141-158

Hentschel BT (1998) Intraspecific variations in delta C-13 indicate ontogenetic diet changes in deposit-feeding polychaetes. Ecology 79:1357-1370

Herman PMJ, Middelburg JJ, Van de Koppel J, Heip CHR (1999) Ecology of estuarine macrobenthos. In: Advances in Ecological Research, Vol 29, Vol 29. Academic Press Inc, San Diego, p 195-240

Herman PMJ, Middelburg JJ, Widdows J, Lucas CH, Heip CHR (2000) Stable isotopes as trophic tracers: combining field sampling and manipulative labelling of food resources for macrobenthos. Mar Ecol Prog Ser 204:79-92

Holl CM, Montoya JP (2005) Interactions between nitrate uptake and nitrogen fixation in continuous cultures of the marine diazotroph Trichodesmium (Cyanobacteria). J Phycol 41:1178-1183

Holmer M, Andersen FO, Holmboe N, Kristensen E, Thongtham N (1999) Transformation and exchange processes in the Bangrong mangrove forest-seagrass bed system, Thailand. Seasonal and spatial variations in benthic metabolism and sulfur biogeochemistry. Aquat Microb Ecol 20:203-212

Holmer M, Andersen FO, Nielsen SL, Boschker HTS (2001) The importance of mineralization based on sulfate reduction for nutrient regeneration in tropical seagrass sediments. Aquat Bot 71:1-17

Huettel M, Cook P, Janssen F, Lavik G, Middelburg JJ (2007) Transport and degradation of a dinoflagellate bloom in permeable sublittoral sediment. Mar Ecol Prog Ser 340:139-153

Huettel M, Gust G (1992a) Impact of bioroughness on interfacial solute exchange in permeable sediments. Mar Ecol Prog Ser 89:253-267

Huettel M, Gust G (1992b) Solute release mechanisms from confined sediment cores in stirred benthic chambers and flume flows. Mar Ecol Prog Ser 82:187-197

Huettel M, Roy H, Precht E, Ehrenhauss S (2003) Hydrodynamical impact on biogeochemical processes in aquatic sediments. Hydrobiologia 494:231-236

Huettel M, Rusch A (2000) Transport and degradation of phytoplankton in permeable sediment. Limnol Oceanogr 45:534-549

Huettel M, Ziebis W, Forster S (1996) Flow-induced uptake of particulate matter in permeable sediments. Limnol Oceanogr 41:309-322

Huettel M, Ziebis W, Forster S, Luther GW (1998) Advective transport affecting metal and nutrient distributions and interfacial fluxes in permeable sediments. Geochim Cosmochim Acta 62:613-631

Hughes JE, Deegan LA, Peterson BJ, Holmes RM, Fry B (2000) Nitrogen flow through the food web in the oligohaline zone of a new England estuary. Ecology 81:433-452

Huys R, Herman PMJ, Heip CHR, Soetaert K (1992) The Meiobenthos of the North-Sea - Density, Biomass Trends and Distribution of Copepod Communities. Ices J Mar Sci 49:23-44

Iizumi H, Hattori A (1982) Growth and organic production of eelgrass (*Zostera marina* L.) in temperate waters of the Pacific coast of Japan. III. The kinetics of nitrogen uptake. Aquat Bot 12:245-256

Jahnke RA, Nelson JR, Marinelli RL, Eckman JE (2000) Benthic flux of biogenic elements on the Southeastern US continental shelf: influence of pore water advective transport and benthic microalgae. Cont Shelf Res 20:109-127

Janssen F, Faerber P, Huettel M, Meyer V, Witte U (2005a) Pore-water advection and solute fluxes in permeable marine sediments (I): Calibration and performance of the novel benthic chamber system Sandy. Limnol Oceanogr 50:768-778

Janssen F, Huettel M, Witte U (2005b) Pore-water advection and solute fluxes in permeable marine sediments (II): Benthic respiration at three sandy sites with different permeabilities (German Bight, North Sea). Limnol Oceanogr 50:779-792

Jenness MI, Duineveld GCA (1985) Effects of Tidal Currents on Chlorophyll a Content of Sandy Sediments in the Southern North-Sea. Mar Ecol Prog Ser 21:283-287

Jones WB, Cifuentes LA, Kaldy JE (2003) Stable carbon isotope evidence for coupling between sedimentary bacteria and seagrasses in a sub-tropical lagoon. Mar Ecol Prog Ser 255:15-25

Kepkay PE, Novitsky JA (1980) Microbial Control of Organic-Carbon in Marine-Sediments - Coupled Chemoautotrophy and Heterotrophy. Mar Biol 55:261-266

Kirchman DL, Wheeler PA (1998) Uptake of ammonium and nitrate by heterotrophic bacteria and phytoplankton in the sub-Arctic Pacific. Deep-Sea Res Part I-Oceanogr Res Pap 45:347-365

Koch EM (2001) Beyond light: physical, geological, and geochemical parameters as possible submersed aquatic vegetation habitat requirements. Estuaries 24:1-17

Koch EW (1999) Sediment resuspension in a shallow *Thalassia testudinum* banks ex Konig bed. Aquat Bot 65:269-280

Koop K, Griffiths CL (1982) The Relative Significance of Bacteria, Meiofauna and Macrofauna on an Exposed Sandy Beach. Mar Biol 66:295-300

Kristensen E, Hansen K (1995) Decay of plant detritus in organic-poor marine sediment: Production rates and stoichiometry of dissolved C and N compounds. J Mar Res 53:675-702

Kuipers BR, Dewilde PAWJ, Creutzberg F (1981) Energy flow in a tidal flat ecosystem. Mar Ecol Prog Ser 5:215-221

Lee KS, Dunton KH (1999) Inorganic nitrogen acquisition in the seagrass *Thalassia testudinum*: development of a whole-plant nitrogen budget. Limnol Oceanogr 44:1204-1215

Luna GM, Manini E, Danovaro R (2002) Large fraction of dead and inactive bacteria in coastal marine sediments: Comparison of protocols for determination and ecological significance. Appl Environ Microbiol 68:3509-3513

MacIntyre HL, Geider RJ, Miller DC (1996) Microphytobenthos: The ecological role of the "secret garden" of unvegetated, shallow-water marine habitats .1. Distribution, abundance and primary production. Estuaries 19:186-201

Mackey MD, Mackey DJ, Higgins HW, Wright SW (1996) CHEMTAX - A program for estimating class abundances from chemical markers: Application to HPLC measurements of phytoplankton. Mar Ecol Prog Ser 144:265-283

Maddi P, Carman KR, Fry B, Wissel B (2006) Use of primary production by harpacticoid copepods in a Louisiana salt-marsh food web. In: Kromkamp JC, de Brouwer JFC, Blanchard GF, Forster RM, Créach V (eds) Functioning of microphytobenthos in estuaries. Royal Netherlands Academy of Arts and Sciences, Amsterdam, p 65-81

Mann KH (1988) Production and use of detritus in various freshwater, estuarine, and coastal marine ecosystems. Limnol Oceanogr 33:910-930

Marba N, Hemminga MA, Mateo MA, Duarte CM, Mass YEM, Terrados J, Gacia E (2002) Carbon and nitrogen translocation between seagrass ramets. Mar Ecol Prog Ser 226:287-300

McRoy CP, Goering JJ (1974) Nutrient Transfer Between Seagrass Zostera-Marina And Its Epiphytes. Nature 248:173-174

Middelburg JJ, Barranguet C, Boschker HTS, Herman PMJ, Moens T, Heip CHR (2000) The fate of intertidal microphytobenthos carbon: An in situ ^{13}C-labeling study. Limnol Oceanogr 45:1224-1234

Middelburg JJ, Duarte CM, Gattuso J-P (2005) Respiration in coastal benthic communities. In: del Giorgio PA, Williams PJlB (eds) Respiration in Aquatic Ecosystems. Oxford University Press, Oxford, p 206-224

Middelburg JJ, Soetaert K (2005) The role of sediments in shelf ecosystem dynamics. In: Robinson AR, Brink KH (eds) The Global Coastal Ocean: Multiscale Interdisciplinary Processes, Vol 13. Harvard University Press, 2005., Cambridge [etc.], p 1033 p.

Miller DC, Geider RJ, MacIntyre HL (1996) Microphytobenthos: The ecological role of the "secret garden" of unvegetated, shallow-water marine habitats .2. Role in sediment stability and shallow-water food webs. Estuaries 19:202-212

Minagawa M, Wada E (1984) Stepwise enrichment of 15N along food chains: further evidence and the relation between 15N and animal age. Geochim Cosmochim Acta 48:1135-1140

Moens T, Luyten C, Middelburg JJ, Herman PMJ, Vincx M (2002) Tracing organic matter sources of estuarine tidal flat nematodes with stable carbon isotopes. Mar Ecol Prog Ser 234:127-137

Moodley L, Boschker HTS, Middelburg JJ, Pel R, Herman PMJ, de Deckere E, Heip CHR (2000) Ecological significance of benthic foraminifera: ^{13}C labelling experiments. Mar Ecol Prog Ser 202:289-295

Moodley L, Middelburg JJ, Boschker HTS, Duineveld GCA, Pel R, Herman PMJ, Heip CHR (2002) Bacteria and Foraminifera: key players in a short-term deep-sea benthic response to phytodetritus. Mar Ecol Prog Ser 236:23-29

Moodley L, Middelburg JJ, Soetaert K, Boschker HTS, Herman PMJ, Heip CHR (2005) Similar rapid response to phytodetritus deposition in shallow and deep-sea sediments. J Mar Res 63:457-469

Needoba JA, Harrison PJ (2004) Influence of low light and a light: Dark cycle on NO3-uptake, intracellular NO3-, and nitrogen isotope fractionation by marine phytoplankton. J Phycol 40:505-516

Nelson DC, Jannasch HW (1983) Chemoautotrophic Growth of a Marine Beggiatoa in Sulfide-Gradient Cultures. Arch Microbiol 136:262-269

Nelson DC, Jorgensen BB, Revsbech NP (1986) Growth-Pattern and Yield of a Chemoautotrophic Beggiatoa Sp in Oxygen-Sulfide Microgradients. Appl Environ Microbiol 52:225-233

Norton TA, Hawkins SJ, Manley NL, Williams GA, Watson DC (1990) Scraping a Living - a Review of Littorinid Grazing. Hydrobiologia 193:117-138

Oh CW, Hartnoll RG, Nash RDM (2001) Feeding ecology of the common shrimp Crangon crangon in Port Erin Bay, Isle of Man, Irish Sea. Mar Ecol Prog Ser 214:211-223

Pace MC, Carman KR (1996) Interspecific differences among meiobenthic copepods in the use of microalgal food resources. Mar Ecol Prog Ser 143:77-86

Pedersen MF, Borum J (1992) Nitrogen dynamics of eelgrass *Zostera Marina* during a late summer period of high growth and low nutrient availability. Mar Ecol Prog Ser 80:65-73

Pedersen MF, Borum J (1993) An annual nitrogen budget for a seagrass *Zostera Marina* population. Mar Ecol Prog Ser 101:169-177

Peterson BJ, Fry B (1987) Stable Isotopes in Ecosystem Studies. Annual Review of Ecology and Systematics 18:293-320

Peterson BJ, Howarth RW (1987) Sulfur, Carbon, and Nitrogen Isotopes Used to Trace Organic-Matter Flow in the Salt-Marsh Estuaries of Sapelo Island, Georgia. Limnol Oceanogr 32:1195-1213

Phillips DL (2001) Mixing models in analyses of diet using multiple stable isotopes: a critique. Oecologia 127:166-170

Phillips DL, Gregg JW (2003) Source partitioning using stable isotopes: coping with too many sources. Oecologia 136:261-269

Pinckney JL, Carman KR, Lumsden SE, Hymel SN (2003) Microalgal-meiofaunal trophic relationships in muddy intertidal estuarine sediments. Aquat Microb Ecol 31:99-108

Ponsard S, Arditi R (2000) What can stable isotopes (delta N-15 and delta C-13) tell about the food web of soil macro-invertebrates? Ecology 81:852-864

Post DM (2002) Using stable isotopes to estimate trophic position: Models, methods, and assumptions. Ecology 83:703-718

Precht E, Huettel M (2003) Advective pore-water exchange driven by surface gravity waves and its ecological implications. Limnol Oceanogr 48:1674-1684

Reise K (1977) Predator Exclusion Experiments in an Intertidal Mud Flat. Helgolander Wissenschaftliche Meeresuntersuchungen 30:263-271

Reise K (1979) Moderate Predation on Meiofauna by the Macrobenthos of the Wadden Sea. Helgolander Wissenschaftliche Meeresuntersuchungen 32:453-465

Riedl RJ, Machan R, Huang N (1972) Subtidal Pump - Mechanism of Interstitial Water Exchange by Wave Action. Mar Biol 13:210-221

Rodriguez JG, Lastra M, Lopez J (2003) Meiofauna distribution along a gradient of sandy beaches in northern Spain. Estuar Coast Shelf Sci 58:63-69

Rossi F, Herman PMJ, Middelburg JJ (2004) Interspecific and intraspecific variation of delta C-13 and delta N-15 in deposit- and suspension-feeding bivalves (Macoma balthica and Cerastoderma edule): Evidence of ontogenetic changes in feeding mode of Macoma balthica. Limnol Oceanogr 49:408-414

Rusch A, Forster S, Huettel M (2001) Bacteria, diatoms and detritus in an intertidal sandflat subject to advective transport across the water-sediment interface. Biogeochemistry 55:1-27

Rusch A, Huettel M (2000) Advective particle transport into permeable sediments - evidence from experiments in an intertidal sandflat. Limnol Oceanogr 45:525-533

Rusch A, Huettel M, Reimers CE, Taghon GL, Fuller CM (2003) Activity and distribution of bacterial populations in Middle Atlantic Bight shelf sands. Fems Microbiol Ecol 44:89-100

Rysgaard S, Risgaard-Petersen N, Nielsen LP, Revsbech NP (1993) Nitrification and Denitrification in Lake and Estuarine Sediments Measured by the 15N Dilution Technique and Isotope Pairing. Appl Environ Microbiol 59:2093-2098

Saier B (2000) Age-dependent zonation of the periwinkle Littorina littorea (L.) in the Wadden Sea. Helgoland Mar Res 54:224-229

Sauriau PG, Kang CK (2000) Stable isotope evidence of benthic microalgae-based growth and secondary production in the suspension feeder Cerastoderma edule (Mollusca, Bivalvia) in the Marennes-Oleron Bay. Hydrobiologia 440:317-329

Schwinghamer P (1981) Characteristic size distributions of integral benthic communities. Canadian Journal of Fisheries and Aquatic Sciences 38:1255-1263

Sherr EB, Sherr BF (1987) High rates of consumption of bacteria by pelagic ciliates. Nature 325:710-711

Short FT, McRoy CP (1984) Nitrogen Uptake By Leaves And Roots Of The Seagrass Zostera-Marina L. Bot Mar 27:547-555

Shum KT, Sundby B (1996) Organic matter processing in continental shelf sediments - The subtidal pump revisited. Mar Chem 53:81-87

Simon M, Azam F (1989) Protein-Content and Protein-Synthesis Rates of Planktonic Marine-Bacteria. Mar Ecol Prog Ser 51:201-213

Smit AJ (2001) Source Identification in Marine Ecosystems: Food Web Studies using $\delta^{13}C$ and $\delta^{15}N$. In: Unkovich M, Pate J, McNeil A, Gibbs DJ (eds) Stable Isotope Techniques in the Study of Biological Processes and Functioning of Ecosystems, Vol 40. Luwer Academic Publishers, Dordrecht, p 219-245

Smith D, Hughes RG, Cox EJ (1996) Predation of epipelic diatoms by the amphipod Corophium volutator and the polychaete Nereis diversicolor. Mar Ecol Prog Ser 145:53-61

Smith DJ, Underwood GJC (1998) Exopolymer production by intertidal epipelic diatoms. Limnol Oceanogr 43:1578-1591

Sommer U (2001) Reversal of density dependence of juvenile Littorina littorea (Gastropoda) growth in response to periphyton nutrient status. J Sea Res 45:95-103

Stapel J, Aarts TL, vanDuynhoven BHM, deGroot JD, vandenHoogen PHW, Hemminga MA (1996) Nutrient uptake by leaves and roots of the seagrass *Thalassia hemprichii* in the Spermonde Archipelago, Indonesia. Mar Ecol Prog Ser 134:195-206

Stapel J, Hemminga MA (1997) Nutrient resorption from seagrass leaves. Mar Biol 128:197-206

Steele JH, Baird IE (1968) Production Ecology of a Sandy Beach. Limnol Oceanogr 13:14-25

Sundback K, Enoksson V, Graneli W, Pettersson K (1991) Influence of Sublittoral Microphytobenthos on the Oxygen and Nutrient Flux between Sediment and Water - a Laboratory Continuous-Flow Study. Mar Ecol Prog Ser 74:263-279

Sundback K, Nilsson P, Nilsson C, Jonsson B (1996) Balance between autotrophic and heterotrophic components and processes in microbenthic communities of sandy sediments: A field study. Estuar Coast Shelf Sci 43:689-706

Sundback K, Snoeijs P (1991) Effects of Nutrient Enrichment on Microalgal Community Composition in a Coastal Shallow-Water Sediment System - an Experimental-Study. Bot Mar 34:341-358

Terrados J, Duarte CM (2000) Experimental evidence of reduced particle resuspension within a seagrass (*Posidonia oceanica* L.) meadow. J Exp Mar Biol Ecol 243:45-53

Terrados J, Williams SL (1997) Leaf versus root nitrogen uptake by the surfgrass *Phyllospadix torreyi*. Mar Ecol Prog Ser 149:267-277

Thomsen U, Kristensen E (1997) Dynamics of Sigma CO2 in a surficial sandy marine sediment: The role of chemoautotrophy. Aquat Microb Ecol 12:165-176

Udy JW, Dennison WC, Long WJL, McKenzie LJ (1999) Responses of seagrass to nutrients in the Great Barrier Reef, Australia. Mar Ecol Prog Ser 185:257-271

Underwood GJC, Kromkamp J (1999) Primary production by phytoplankton and microphytobenthos in estuaries. In: Advances in Ecological Research, Vol 29. Academic Press Inc, San Diego, p 93-153

Urban-Malinga B, Hedtkamp SIC, van Beusekom JEE, Wiktor J, Weslawski JM (2006) Comparison of nematode communities in Baltic and North Sea sublittoral, permeable sands - Diversity and environmental control. Estuar Coast Shelf Sci 70:224-238

Urban-Malinga B, Moens T (2006) Fate of organic matter in Arctic intertidal sediments: Is utilisation by meiofauna important? J Sea Res 56:239-248

Van den Meersche K, Middelburg JJ, Soetaert K, van Rijswijk P, Boschker HTS, Heip CHR (2004) Carbon-nitrogen coupling and algal-bacterial interactions during an experimental bloom: Modeling a C-13 tracer experiment. Limnol Oceanogr 49:862-878

van Oevelen D, Middelburg JJ, Soetaert K, Moodley L (2006a) The fate of bacterial carbon in an intertidal sediment: Modeling an in situ isotope tracer experiment. Limnol Oceanogr 51:1302-1314

van Oevelen D, Moodley L, Soetaert K, Middelburg JJ (2006b) The trophic significance of bacterial carbon in a marine intertidal sediment: Results of an in situ stable isotope labeling study. Limnol Oceanogr 51:2349-2359

van Oevelen D, Soetaert K, Middelburg JJ, Herman PMJ, Moodley L, Hamels I, Moens T, Heip CHR (2006c) Carbon flows through a benthic food web: Integrating biomass, isotope and tracer data. J Mar Res 64:453-482

Vanaverbeke J, Gheskiere T, Vincx M (2000) The meiobenthos of subtidal sandbanks on the Belgian Continental Shelf (Southern Bight of the North Sea). Estuar Coast Shelf Sci 51:637-649

Vander Zanden MJ, Rasmussen JB (1999) Primary consumer delta C-13 and delta N-15 and the trophic position of aquatic consumers. Ecology 80:1395-1404

Vedel A, Riisgard HU (1993) Filter-feeding in the polychaete *Nereis diversicolor*: growth and bioenergetics. Mar Ecol Prog Ser 100:145-152

Veuger B, Eyre BD, Maher D, Middelburg JJ (2007a) Nitrogen incorporation and retention by bacteria, algae, and fauna in a subtropical intertidal sediment: An in situ 15N-labeling study. Limnol Oceanogr 52:XXXX-XXXX

Veuger B, Middelburg JJ, Boschker HTS, Houtekamer M (2005) Analysis of 15N incorporation into D-alanine: A new method for tracing nitrogen uptake by bacteria. Limnol Oceanogr Methods 3:230-240

Veuger B, Middelburg JJ, Boschker HTS, Houtekamer M (2007b) Update of “Analysis of 15N incorporation into D-alanine: A new method for tracing nitrogen uptake by bacteria” (Veuger et al. 2005, L&O: Methods 3: 230-240). Limnol Oceanogr Methods 5:192-194

Warwick RM, Clarke KR (1998) Taxonomic distinctness and environmental assessment. J Appl Ecol 35:532-543

Watermann F, Hillebrand H, Gerdes G, Krumbein WE, Sommer U (1999) Competition between benthic cyanobacteria and diatoms as influenced by different grain sizes and temperatures. Mar Ecol Prog Ser 187:77-87

Webb DG (1996) Response of macro- and meiobenthos from a carbon-poor sand to phytodetrital sedimentation. J Exp Mar Biol Ecol 203:259-271

Wenzhofer F, Glud RN (2004) Small-scale spatial and temporal variability in coastal benthic O-2 dynamics: Effects of fauna activity. Limnol Oceanogr 49:1471-1481

Wild C, Huettel M, Klueter A, Kremb SG, Rasheed MYM, Jorgensen BB (2004) Coral mucus functions as an energy carrier and particle trap in the reef ecosystem. Nature 428:66-70

Witte U, Wenzhofer F, Sommer S, Boetius A, Heinz P, Aberle N, Sand M, Cremer A, Abraham WR, Jorgensen BB, Pfannkuche O (2003) In situ experimental evidence of the fate of a phytodetritus pulse at the abyssal sea floor. Nature 424:763-766

Woulds C, Cowie GL, Levin LA, Andersson JH, Middelburg JJ, Vandewiele S, Lamont PA, Larkin KE, Gooday AJ, Schumacher S, Whitcraft C, Jeffreys RM, Schwartz M (2007) Oxygen as a control on seafloor biological communities and their roles in sedimentary carbon cycling. Limnol Oceanogr 52:1698-1709

Yamamuro M, Kayanne H, Yamano H (2003) delta N-15 of seagrass leaves for monitoring anthropogenic nutrient increases in coral reef ecosystems. Mar Pollut Bull 46:452-458

Summary

Carbon and nitrogen flows through sandy and other coarse, subtidal sediments constitute an important part of their global cycles. An assessment of the various processes involved and the quantification of the relative contribution of the different compartments is a prerequisite for a better understanding of the biogeochemical cycles at the global scale. Our knowledge of marine sandy sediments remains limited due to their heterogeneity and relative inaccessibility. Gaining an understanding of this environment requires the use and development of novel approaches and methodology. Through the use of stable isotopes and microbial biomarker techniques, this thesis, relying on several experimental approaches, attempts to unravel the structural, ecological and biogeochemical interactions within various benthic environments of the coastal ocean.

Chapter 2 illustrates the coupling between the bacterial compartment and primary producers. The availability of nitrogen in sediment phytodetritus for seagrass plants was investigated in 5 tropical species (*Thalassia hemprichii*, *Halodule uninervis*, *Halodule pinifolia*, *Halophila ovalis/ovata* and *Syringodium isoetifolium*) from Indonesia. In an *in situ* experiment, ^{15}N-labelled phytodetritus was injected into the sediment and the appearance of ^{15}N in the roots, the rhizomes and the leaves of the plants was measured after 1, 2, 4 and 8 d. The transfer of ^{15}N from sediment phytodetritus to the plant tissues was very rapid (within 1 d) and continued over the 8 d period indicating an efficient retention of the phytodetritus and a net mineralization of nitrogen rather than an immobilisation by bacteria. Relative enrichment of roots versus leaves indicated that ^{15}N was taken up by the roots and then transferred to the leaves. The combined efficiency of seagrass canopy-induced trapping and retention of sestonic particles and root-uptake, results in the acquisition of nutrients released upon mineralization of particulate organic matter (POM), giving them a competitive advantage over other primary producers in oligotrophic environments.

Chapter 3 provides a detailed snapshot of the microbial compartment of a coastal permeable sandy sediment in the photic zone, where the pathways and the fate of C and N

were studied in a pulse-chase experiment. ^{13}C-bicarbonate and ^{15}N-nitrate were added to the water column of 5 sediment cores incubated in 4 transparent and 1 opaque laboratory chambers. After 9 h of labelling in the light and dark, respectively, stable isotope incorporation by microphytobenthos (MPB) and bacteria was quantified over a period of 4 days through the analysis of phospholipid-derived fatty acids (PLFA) and hydrolysable amino acids (HAA). In the light ^{13}C was fixed by MPB and more than 50% was directed to the production of extracellular polymeric substance (EPS). MPB ^{15}N incorporations in the dark and in the light were similar. Bacterial activity appeared to have two effects that depended on sediment depth: at the sediment surface there was a steady increase of label in the bacterial PLFA suggesting the consumption of label-containing EPS; in the subsurface layers, uniform enrichment starting immediately after the labelling procedure indicated continuous incorporation of inorganic C by chemoautotrophic bacteria (CBac). This experiment demonstrates the efficient transfer of inorganic carbon and nitrogen to the benthic community through the activities of photo- and chemoautotrophic microorganisms, and the role of EPS as a carrier for energy to the benthic microbial food web.

Chapter 4 focuses on the benthic food web and provides a complementary picture to chapter 3. Carbon and nitrogen flows within the food web of a subtidal sandy sediment were studied using a dual stable isotope approach. First, the natural abundance δ^{13}C and δ^{15}N values of consumers and potential benthic and pelagic resources suggested a tripartite trophic network. The ^{13}C isotopic signatures of some benthic fauna were similar to those of benthic diatoms (~−16‰). Some other fauna species or taxa were ^{13}C-depleted, similar to benthic cyanobacteria (~−20‰) or suspended particulate matter (~−21‰). Another group of animals showed intermediate δ^{13}C values, suggesting a mixed diet. Second, the microphytobenthos (MPB) of sediment cores was labelled through a pulse-chase experiment with ^{13}C-bicarbonate and ^{15}N-nitrate. The fate of the MPB was followed in the different heterotrophic compartments. Transfer of ^{13}C and ^{15}N to consumers was fast. Heterotrophic bacteria contributed most to the total heterotrophic incorporation of ^{13}C, followed by meiofauna and macrofauna, consistent with the heterotrophic biomass distribution (bacteria>meiofauna>macrofauna). Significant labelling of metazoans allowed us distinguishing between utilization of MPB and phytoplankton derived carbon. The combined natural abundance and deliberate tracer addition approaches thus provided complementary information on the key role of MPB in structuring benthic communities in sandy sediments. Because this study was carried out in an organic carbon-replete sediment, the conclusion raises the question of whether MPB also plays a central role in the food web of organic carbon-poor sediments.

Chapter 5 addresses the remaining question, raised in chapter 4. The relative significance of phytodetritus deposition and autochthonous microphytobenthos (MPB)

production for benthic consumers in an organic carbon-poor sandy sediment was assessed in two consecutive tracer experiments. In a first experiment, sediment cores received a pulse of ^{13}C-labelled phytodetritus and the fate of that organic matter was followed in the benthic food web (bacteria, meiofauna and macrofauna) over a period of 72 h. In a second experiment, the MPB was labelled with ^{13}C-bicarbonate and its fate was followed as in the first experiment, over a period of 96h. Coupled to a detailed survey of the natural abundance of ^{13}C in the different benthic organisms, this study revealed complex trophic interactions among benthic consumers. In terms of carbon transfer, bacteria contributed most significantly to total phytodetritus assimilation (69%) while macrofauna was more significant for MPB assimilation (49%). Although meiofauna specific enrichment was highest, their contribution to total C assimilation was low in both experiments. Relative dependence of consumers on phytodetritus and MPB was estimated and revealed that benthic heterotrophs in these sandy, permeable sediments rely more than 95% on MPB.

Samenvatting

Koolstof- en stikstofstromen (resp. C en N) door zandige en andere grove, subtidale sedimenten maken een belangrijk deel uit van de mondiale kringlopen. Om tot een beter begrip te komen van deze biogeochemische cycli op globale schaal, is het bepalen van de verschillende betrokken processen en de kwantificering van de relatieve bijdrage van de verschillende compartimenten noodzakelijk. Onze kennis van mariene zandige sedimenten blijft beperkt door hun heterogeniteit en soms moeilijke bereikbaarheid. Om de kennis van deze omgeving te verbeteren dienen nieuwe benaderingen en nieuwe technologieën te worden gebruikt en ontwikkeld. Via het gebruik van stabiele isotopen en microbiële biomarkertechnieken, probeert deze thesis de structurele, ecologische en biogeochemische interacties te ontrafelen binnen verscheidene benthische milieus van kustgebieden.

Hoofdstuk 2 illustreert de koppeling tussen het bacteriële compartiment en primaire producenten. De beschikbaarheid van stikstof in sedimentair fytodetritus werd onderzocht voor 5 tropische Indonesische zeegrassoorten (*Thalassia hemprichii, Halodule uninervis, Halodule pinifolia, Halophila ovalis/ovata* and *Syringodium isoetifolium*). In een *in situ* experiment werd ^{15}N gelabeld fytodetritus geïnjecteerd in het sediment waarna het voorkomen van ^{15}N werd gemeten in de wortels, de rizomen en de bladeren van de planten na respectievelijk 1, 2, 4 en 8 dagen. De transfer van ^{15}N van het sedimentair fytodetritus naar het plantenweefsel gebeurde zeer snel (binnen 1 dag) en ging door gedurende de 8 dagen van het experiment. Dit wijst op een efficiënte retentie van het fytodetritus en een netto mineralisatie van N, eerder dan een N-immobilisatie door bacteriën. De relatief sterkere verrijking van wortels ten opzichte van bladeren, wees erop dat ^{15}N werd opgenomen door de wortels en dan pas werd getransporteerd naar de bladeren. De gecombineerde efficiëntie van wortelopname en het vangen en vasthouden van sestonpartikels door het zeegras-bladerdek, resulteert in de verwerving van nutriënten welke zijn vrijgekomen tijdens de mineralisatie van particulair organisch materiaal (POM). Dit geeft de zeegrasplanten een competitief voordeel ten opzichte van andere primaire producenten in oligotrofe omgevingen.

Hoofdstuk 3 geeft een gedetailleerde momentopname van het microbiële compartiment van een permeabel zandig kustsediment, waar het traject wat C en N ondergaan, werden bestudeerd aan de hand van een *pulse-chase* experiment. ^{13}C-bicarbonaat en ^{15}N-nitraat werden toegevoegd aan de waterkolom van vijf sedimentcores. Vier cores werden geïncubeerd in transparante laboratoriumkamers en één in een opake kamer. Na een incubatie van 9 uur in lichte, respectievelijk donkere omstandigheden werd de incorporatie van stabiele isotopen in microfytobenthos (MPB) en bacteriën gedurende 4 dagen gekwantificeerd. Hiertoe werden fosfolipidenvetzuren (PLFA) en hydrolyseerbare aminozuren (HAA) geanalyseerd. In het licht werd ^{13}C gefixeerd door het MPB, waar meer dan 50% werd gebruikt voor de productie van extracellulaire polymere substanties (EPS). De incorporatie van ^{15}N door het MPB was gelijkaardig in zowel licht- als donkercondities. Afhankelijk van de sedimentdiepte leek de bacteriële activiteit twee effecten te vertonen: aan het sedimentoppervlak was er een gestage stijging van de hoeveelheid label in bacteriële PLFA, wijzend op de consumptie van gelabelde EPS; in de diepere sedimentlagen wees de uniforme verrijking, reeds direct na het begin van de labelingsprocedure, op een continue incorporatie van anorganisch C door chemo-autotrofe bacteriën (CBac). Dit experiment toont de efficiënte transfer van anorganisch C en N naar de benthische gemeenschap aan, via de activiteiten van foto- en chemo-autotrofe micro-organismen, met de rol van EPS als drager van energie naar het benthische microbiële voedselweb.

Hoofdstuk 4 spitst zich toe op het benthische voedselweb en geeft een complementair beeld aan hoofdstuk 3. Koolstof- en stikstofstromen in het voedselweb van een subtidaal sediment werden met behulp van een dubbele isotoopbenadering bestudeerd. Ten eerste werden de waarden voor het natuurlijke voorkomen van $\delta^{13}C$ en $\delta^{15}N$ van consumenten en potentiële benthische en pelagische voedselbronnen bepaald, welke een driedelig trofisch netwerk suggereerden. De ^{13}C waarden van sommige benthische fauna waren gelijkwaardig aan die van benthische diatomeeën (~-16‰). Sommige andere diersoorten en –taxa waren ^{13}C-arm, net zoals benthische cyanobacteriën (~-20‰) of gesuspendeerd particulair materiaal (~-21‰). Weer een andere groep fauna had intermediaire waarden, wijzend op een gemengd dieet. Ten tweede werd het microfytobenthos (MPB) van sedimentcores gelabeld met ^{13}C-bicarbonaat en ^{15}N-nitraat via een *pulse-chase* experiment. Het traject wat werd afgelegd door het MPB werd gevolgd in de verschillende heterotrofe compartimenten. De transfer van ^{13}C en ^{15}N naar de consumenten gebeurde snel. Heterotrofe bacteriën droegen het meeste bij tot de totale heterotrofe incorporatie van ^{13}C, gevolgd door de meio- en macrofauna, wat consistent is met de heterotrofe biomassaverdeling (bacteriën>meiofauna>macrofauna). Het feit dat metazoa significante hoeveelheden gelabeld materiaal bevatten stelde ons in staat onderscheid te maken tussen het gebruik van C afkomstig van MPB en fytoplankton. Het gecombineerde gebruik van het natuurlijke voorkomen en de expliciete toediening van een tracer, verschafte zo

complementaire informatie over de sleutelrol welke MPB speelt in het structureren van benthische gemeenschappen in zandige sedimenten. Omdat deze studie werd uitgevoerd in een sediment rijk aan organisch C, doet de conclusie de vraag rijzen of MPB ook een centrale rol speelt in het voedselweb van organisch C-arme sedimenten.

Hoofdstuk 5 behandelt de overgebleven vraag, zoals gesteld in hoofdstuk 4. Het relatieve belang van de depositie van fytodetritus en de autochtone microfytobenthos (MPB) productie voor benthische consumenten in een zandig sediment, arm aan organisch C, werd bepaald in twee opeenvolgende tracer-experimenten. In een eerste experiment kregen sedimentcores een puls van ^{13}C gelabeld fytodetritus en het lot van dit organisch materiaal werd gedurende een periode van 72 uur gevolgd in het benthisch voedselweb (bacteriën, meiofauna en macrofauna). In een tweede experiment werd het MPB gelabeld met ^{13}C-bicarbonaat en werd het traject gevolgd zoals in het eerste experiment, maar over een periode van 96 uur. Samen met een gedetailleerde monitoring van het natuurlijke voorkomen van ^{13}C in de verschillende benthische organismen, onthulde deze studie complexe trofische interacties tussen benthische consumenten. Wat betreft koolstoftransfer droegen bacteriën het meest significant bij tot de totale assimilatie van fytodetritus (69%), terwijl macrofauna meer significant de assimilatie van MPB (49%) bepaalde. Hoewel de specifieke verrijking van het meiofauna het hoogst was, was de bijdrage tot de totale C-assimilatie in beide experimenten laag. De relatieve afhankelijkheid van consumenten van fytodetritus en MPB werd geschat en onthulde dat benthische heterotrofen in deze zandige, permeabele sedimenten, voor meer dan 95% afhankelijk zijn van MPB.

Acknowledgments

I would like to express my gratitude to my supervisors, Jack Middelburg and Carlo Heip, who have trusted me and given me freedom to carry out my research. With Karline Soetaert, who acted as my co-supervisor, the supervising trio provided excellent guidance and precious feedback along the long and winding road of the PhD thesis.

I warmly thank Markus Huettel, Riks Laanbroek, Gert de Lange, Tom Moens and Philippe Van Cappellen for accepting to be in my thesis committee.

My stay at the NIOO in Yerseke has been a very interesting and enriching experience both at the professional and social levels. I am very grateful to the many people whose kindness and generosity have contributed to making me feel at home at the NIOO. I would like to thank particularly Joke van Houte, Elly van Hulsteijn, Elly de Bruijn, Theo Davidse, Maarten Pronk, Jan Thijs Jonkman, Laura Wisse, Jan Megens and Marjan van Damme.

The high quality of the research at the NIOO is unequivocal and, beside the high research expertise, the technical staff provides a crucial support. I thank Peter van Breugel (very good and patient IRMS teacher), Cobie Kleppe, Yvonne van der Maas, Jan Sinke and Marco Houtekamer for their precious help and excellent analytical work. Among the people who contributed most to the success of my work, I especially want to thank Pieter van Rijswijk for providing such a good guidance and being so patient with me in my early days of experimental research, and also for processing such a big amount of samples I collected during my field work.

During these 4 years at the NIOO, I was given the opportunity to collaborate with other groups of scientists from the Max Plank Institute in Bremen, the Alfred-Wegener Institut (Wadden Sea Station Sylt), the University of Copenhagen (Marine Biological Laboratory) and the Institute of Oceanology of the Polish Academy of Sciences (Sopot). I have learnt a lot and I have had a very good time with all the Cosa project members. I thank Justus van Beusekom, Anni Glud, Ronnie Glud, Stefani Hedtkamp, Felix Janssen,

Tomek Kirzeniewski, Lech Kotwicki, Radek Koza, Tina Loebl, Susanne Menger, Basia, Urban-Malinga, Frank Wenzhofer and Jan Marcin Weslawski for sharing their experience and bringing such a good atmosphere to the long days of field work. I want to thank Perran Cook for being such a good lad, for chaperoning me during my experiments, listening to me complaining when things were not going according to the plans and being such a dynamic researcher with "heaps" of good ideas and a good digger experience. I want to particularly thank Markus Huettel for coordinating so well such a nice group, for being so easily available, for providing guidance for my work and finally for digging so much sand for me.

I also want to thank Iin Inayat Al Hakim, Achmad Arifin, Muchtar Muswerry, Marieke van Katwijk, Jan Roelofs, Leon Lamers, Marlies van der Welle, Esther Lucassen and Arie Vonk for their precious help in Derawan during the field work of the East Kalimantan Project. I thank Tjeerd Bouma for offering me to join him on the trip, for his help and for his constructive discussions. I am so grateful to Wawan Kiswara for his support throughout my stay and work in Indonesia and for introducing me to his family, his friends and his culture. Wawan, "terima kasih banyak" for your altruism and your kindness.

Thank you so much NIOO-CEME colleagues for your feedback and help during my stay: Eric Boschker, Leon Moodley, Dick van Oevelen, Francesca Rossi, Bart Veuger, Nicole Dijkman (thank you very much for your help with the analysis of pigment data), Peter Herman, Tom Ysebaert. I thank the NIOO soccer team for all those nice games. Thank you Jeroen Jansen for all the good extracurricular eolian activities.

Thank you all de Kêête people for what you are, a nice, rich and powerful mixture of different characters, colours and tastes. Thank you Arnon, Ellen, Ina, Karel, Nienke, Testuro, Sayuri, Stef, Olivier, Caroline, Sara… and all the people who were there and brought fun and joy in the house. I really enjoyed my time breathing, eating, drinking and swimming in the melting pot. I especially thank Els Sandee for taking care of us and making de Kêête a comfortable place.

Le NIOO pendant 4 ans, cela aura surtout été une bonne ambiance, la complicité, l'amitié et le soutien d'une petite brochette de gens à qui je dois de gros remerciements. Merci à vous deux Sandra (merci pour la traduction) et Stéphane pour votre sympathie et votre générosité. Merci de m'avoir supporté dans le bureau pendant plus de 4 ans (cela n'a pas du être facile tous les jours), merci pour toutes les discussions intéressantes et aussi toutes les discussions futiles, pour les rigolades et les moments sérieux, bref pour tous ces moments passés ensemble. Merci aussi à Fred G. et à Francesc (merci aussi pour la traduction) pour leur soutien et leurs conseils et, surtout leur bonne humeur.

Merci maman et papa de m'avoir permis de suivre de longues études et pour votre précieux soutien moral et affectif. C'est en grande partie grâce à vous si je suis arrivé à réaliser mes projets jusqu'à présent. Merci à Augustine et Gus qui n'ont toujours pas vraiment compris pourquoi je fais de la recherche, ni ce que cela va m'apporter, mais qui m'ont bien soutenu quand même tout au long des années. Merci à Thibault, mon poteau de 30 ans, qui a tout suivi depuis le début et qui n'a jamais manqué de paroles et d'actions encourageantes ou réconfortantes. Merci aux autres potes qui ont suivi la progression et m'ont encouragé, comme Damien, David, Erwan, Fred C., Julien, Sophie, Rado. I warmly thank Paula, Aimo and Katie Vanhala for their support and their interest in my work.

Finally, I want to thank Lisa for being the best support one could imagine. Thank you for being so understanding, generous, patient with me and particularly with Us during these years of long distance. I am so grateful for everything you've done for me: understanding me and my work, helping me schedule when the stress was freezing me, correcting my papers and providing precious comments, cooking nice treats after painful hours of work, soothing, counselling, encouraging… waiting for me for such a long time.

Curriculum vitae

Victor Evrard was born on the 9th of April 1976 in Paris, France. He spent his early years in West Africa stimulating his curiosity for nature. He went to primary and secondary school in Grasse and Nice in the Southeast of France. His growing interest for life science and marine ecology was confirmed after he attended lectures by Prof. Meinesz during his undergraduate studies at the University of Nice Sophia Antipolis. He soon embarked on field research to investigate the impact of the invasive alga *Caulerpa taxifolia* during a summer internship at the University of Nice Sophia Antipolis. In 1998, he decided to pursue his undergraduate studies in Paris at Pierre & Marie Curie University and to specialize in oceanography and marine biology. During his undergraduate studies, he met Dr. F. Ibañez and discovered the importance of numbers, and decided to specialize in numerical ecology. After he graduated in 2000, he followed a one-year research training and wrote a thesis on the long term evolutions of benthic and planktonic populations. In 2001 he enrolled in a biomathematics MSc programme at Pierre & Marie Curie University and did a research training at the Institut National Agronomic Paris-Grignon. His MSc thesis, supervised by Prof. R. Arditi, dealt with the study of population dynamics. From November 2002 to April 2007 he worked as a PhD student at the Netherlands Institute of Ecology in Yerseke, the Netherlands. In September 2007 he will be joining Dr. M. Xenopoulos' group at Trent University in Canada as a postdoctoral fellow to study the biogeochemistry of freshwater ecosystems.