Chapter 1

Introduction

1.1 The marine benthic food web

The marine environment is the largest ecosystem on earth, covering 71% of the earths surface. This vast ecosystem is predominantly supported by primary production of phytoplankton in the photic zone, where it sustains the pelagic food web (Fig. 1.1). A small part of the organic carbon produced in the photic zone is exported and ultimately reaches the sediment, where it fuels the benthic community.

The benthic community is biologically diverse and consists of prokaryotic bacteria, protozoan flagellates and ciliates, and different metazoans. A rough classification of this diversity is generally based on organism size (Table 1.1 and Fig. 1.2) and is practical from several perspectives. First, abundance data of benthic communities typically show distinct size classes (Schwinghamer, 1981). Second, weight-specific physiological processes scale with body size (Peters, 1983), which makes size a useful parameter in the process of constructing energy budgets. Third, the presence of sediment particles makes sieving a practical way of separating organisms from the sediment and sieving divides a sample in size classes by definition.

Table 1.1: Size-based classification of the benthic community with size range, typical abundance level in coastal and estuarine sediments and the main groups in each size class.

Size class	**Size range**	**Abundance**	**Main groups**
Bacteria	0.5 - 4 μm	10^9 ml^{-1}	aerobic and anaerobic respirers, chemo-autotrophs, fermenters
Microbenthos	$<$ 63 μm	10^3 ml^{-1}	flagellates, ciliates
Meiobenthos	63 - 1000 μm	10^6 m^{-2}	nematodes, foraminifera, copepods, turbellaria, ostracods
Macrobenthos	$>$ 1000 μm	10^3 m^{-2}	polychaetes, bivalves, crustaceans gastropods, echinoderms

Benthic communities in continental shelf or deep-sea sediments rely predominantly on vertical sinking of phytodetritus or horizontal bed-load transport of detritus (Graf, 1992). In contrast, estuaries and coastal systems are much shallower and therefore benthic fauna have direct access to their food sources. Benthic suspension feeders have direct access to

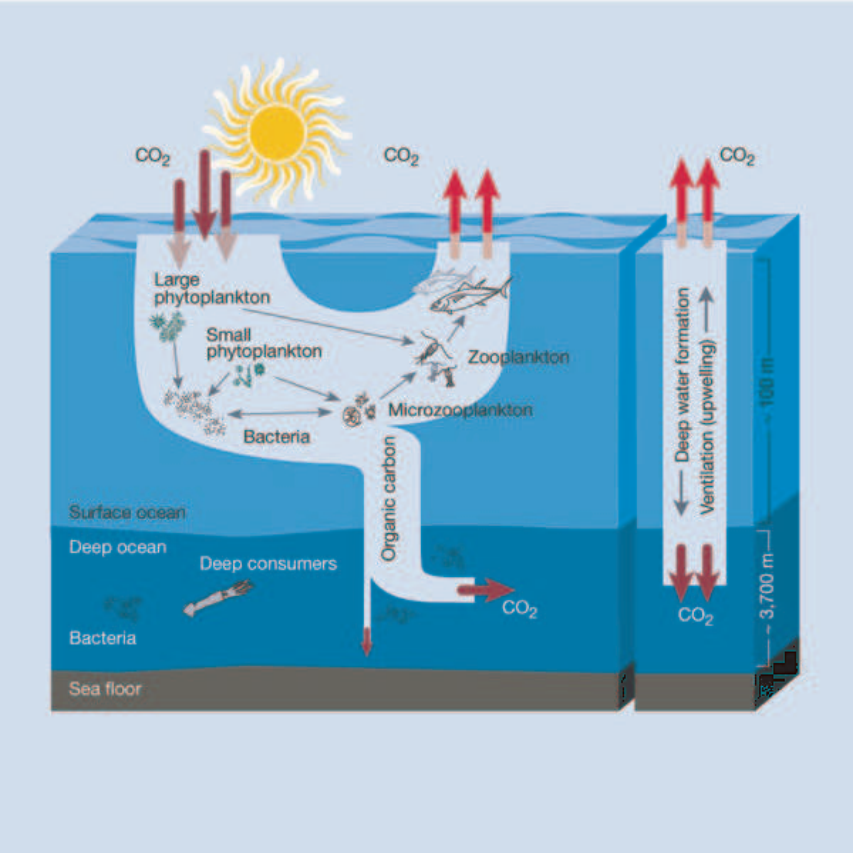

Figure 1.1: Major carbon flows in the marine ecosystem. Production of organic matter by phytoplankton supports the pelagic food web. Some organic matter escapes the surficial water layers and settles on the sediment, where it fuels the benthic food web. Remineralization of organic matter releases nutrients that are transported to the photic zone during upwelling events, where it in turn supports primary production (Chisholm, 2000).

phytoplankton and directly affect phytoplankton dynamics (Herman et al., 1999). Intertidal sediments are directly exposed to sunlight and are inhabited by microphytobenthos, unicellular eukaryotic algae and cyanobacteria, that have a high productivity (MacIntyre et al., 1996; Underwood and Kromkamp, 1999). Moreover, the close proximity of estuaries and coastal waters to other ecosystems results in additional carbon inputs. For example, salt marshes may be responsible for a large outwelling of organic carbon produced by salt marsh vegetation and river discharges bring in allochthonous organic matter derived from upstream terrestrial and freshwater ecosystems.

Our conceptual understanding of how these carbon inputs flow through the marine food web grew in complexity during the last decades. This discussion has focussed mainly on the pelagic food web, but is of equal relevance for the benthic food web. Initially, Steele (1974) developed marine food web theory on the herbivorous pathway in which phytoplankton is grazed by zooplankton which in turn is food for fish (Fig. 1.3). Another pathway that has received considerable attention is the detrital pathway. The

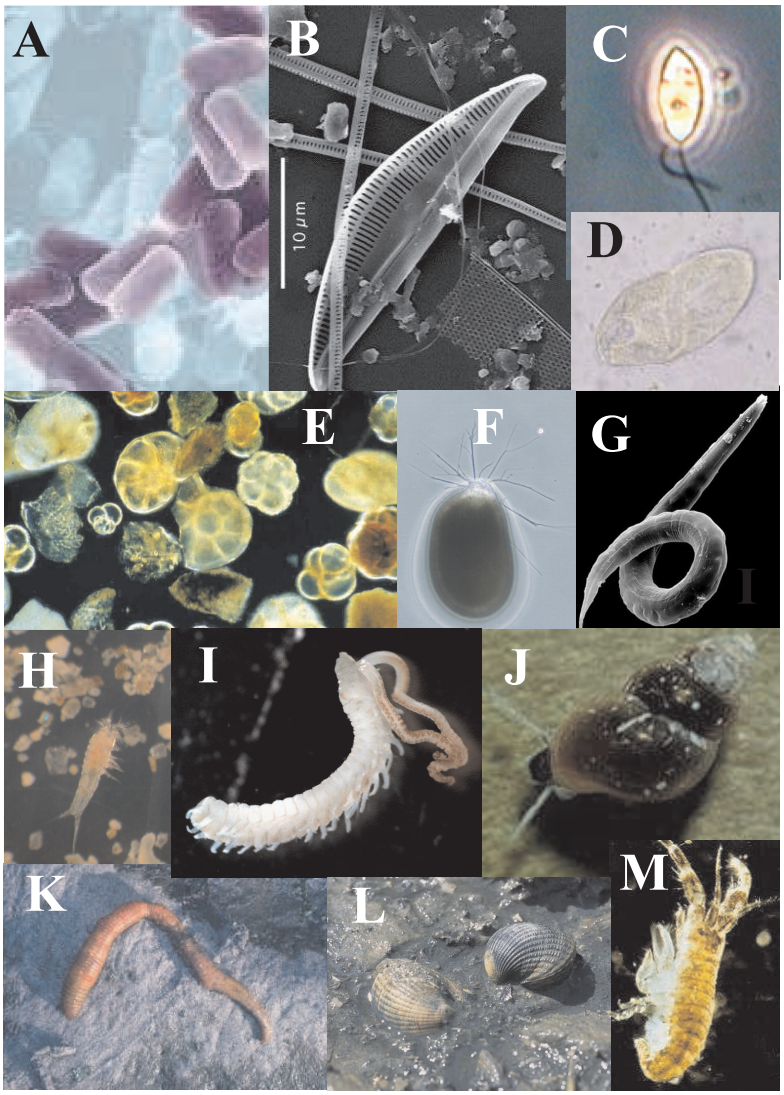

Figure 1.2: Compilation of different members of the benthic community A) bacteria, B) microphytobenthic diatoms, C) flagellates, D) ciliates, E) hard-shelled foraminifera, F) soft-bodied foraminifera, G) nematode, H) copepod, I) *Polydora cornuta* (polychaete), J) *Hydrobia ulvae* (gastropods), K) *Arenicola marine* (polychaete), L) *Cerastoderma edule* (bivalve), M) *Corophium volutator* (crustacean).

importance of detritus has been emphasized particularly for systems adjacent to salt marshes (Teal, 1962) (Fig. 1.3). Later discussion centered around the question whether assimilation of detrital carbon or attached bacteria is most important (Lopez and Levinton, 1987). The last major addition to marine food web theory has been carbon transfer through the microbial loop (Pomeroy, 1974; Azam et al., 1983) (Fig. 1.3). Dissolved organic

matter, produced by algae and bacteria, viral lysis and sloppy feeding zooplankton (Jumars et al., 1989), is assimilated by bacteria and through several transfer steps may eventually feed higher trophic levels. In this way, dissolved organic carbon that is otherwise not readily available for higher trophic levels may still contribute to the traditional food chain.

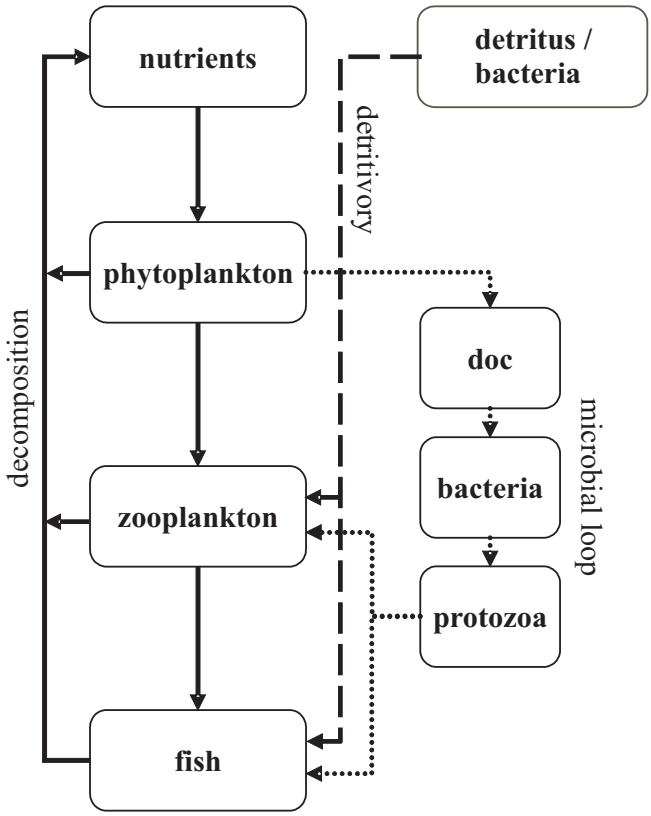

Figure 1.3: The complexity of marine food webs. In the traditional herbivorous food chain (line arrows), algal growth support zooplankton, which in turn support fish. In this view, bacteria are responsible for degradation of organic substances. The detrital pathway (long dashed arrows) is an additional carbon input to the traditional food chain by assimilation of detritus and associated bacteria. The microbial loop (dotted arrows) is assimilation of dissolved organic carbon by bacteria, subsequent grazing by protozoans and then transfer of carbon to the traditional food chain.

Our quantitative understanding of this complex 'spaghetti' of interactions in natural food webs is still very limited (Cohen et al., 1993; Polis and Strong, 1996; Herman et al., 1999; Azam and Worden, 2004; Moore et al., 2004), which is partly due to the large number of possible pathways and the difficulty to distinguish among them. Another important reason is that methodological and logistical limitations prevent from making all the necessary measurements at the same time at the same place in the field. The sedimentary environment is particularly notorious in this respect, because of high spatial and temporal variability, difficult accessibility and sampling, adsorption - desorption

reactions and simply the presence of sediment particles that hinders the process of isolating benthic fauna. Because of these difficulties, our knowledge of benthic food webs is still limited (Herman et al., 1999). Hence, it remains a challenging task to decipher the element flows among the benthic community members and use this knowledge to come to a mechanistic and eventually predictive understanding of the structure of benthic food webs.

One way to study the structure of food webs is by means of mathematical modeling, which is taken up in this thesis by means of linear inverse modeling (LIM) and dynamic modeling. Both approaches are based on mass balances and aim to quantify the relations among the food web compartments (Fig. 1.4). In a LIM the flow values have a 'fixed' value and are considered to be a long-time average. Data are added as a value that represents the value of a flow or a combination of the flows. In a dynamic model, flows are described by mechanistic relationships and are a function of time. Data are used to fit the model parameters of the dynamic model such that the data are optimally reproduced. In general, linear inverse models have fewer data requirements and do not require detailed knowledge of mechanistic relationships (Gaedke, 1995). In contrast, dynamic models may harbor mechanistic knowledge, but require large data sets to assess the validity of the model and its parameters (Gaedke, 1995). Irrespective of the differences, both approaches have proven to be valuable tools in the quantification of food webs (Vézina and Platt, 1988; Cole et al., 2002; Van den Meersche et al., 2004). One basic requirement for effective use of these modeling approaches is the availability of data, that serve both as input for the model and as validation of the model output. Typically however, data availability is rather limited and adding new data resources that bring in additional pieces of information is a valuable strategy to increase the reliability of a food web model. In this thesis, we take advantage of developments in stable isotope techniques to increase the amount of data in our food web models. The next paragraph will briefly introduce some stable isotope techniques in food web research.

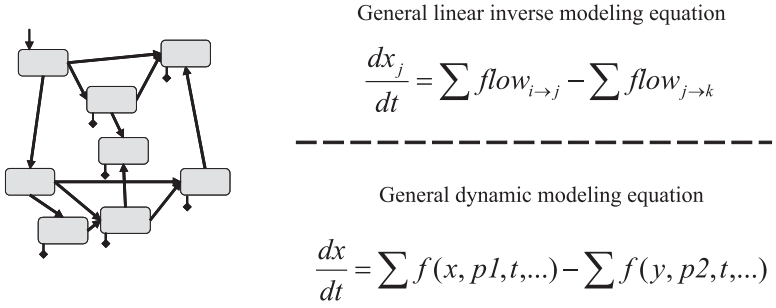

Figure 1.4: General equations for linear inverse modeling and dynamic modeling. On the left is a conceptual model of a food web. The connecting arrows define the incoming and outgoing flows for each compartment. In a linear inverse model these relationships are presumed to have a fixed value ($flow_{i\to j}$). In a dynamic model, the relationships are not fixed but expressed as a function of e.g. state variables (x), parameters ($p1$) and time (t). The resulting mass balance is a function of various different relationships with the other compartments in the food web.

1.2 Stable isotopes in food web research: Natural abundance and tracer level

1.2.1 Stable isotopes at natural abundance level

Isotopes are forms of one element, which differ in numbers of neutrons and therefore have a different atomic weight. Many isotopes are unstable and the nucleus disintegrates, releasing energy and radiation along the way, until a stable form is reached. Stable isotopes, as their name suggests, have a stable nucleus that does not disintegrate nor emits radiation. Elements that are mostly used in ecological research (C, N and S), consist for $>$ 95 % of the lightest isotope, i.e. the stable isotope with the least number of neutrons. The isotope composition of matter is usually denoted in the δ notation; the deviation in the ratio of heavy to light isotope from a reference expressed in parts per thousand. For example the carbon δ^{13}C (‰) of a sample is

$$\delta^{13}C = \frac{\frac{^{13}C}{^{12}C}sample - \frac{^{13}C}{^{12}C}reference}{\frac{^{13}C}{^{12}C}reference} \cdot 1000 \tag{1.1}$$

The reference material for carbon is carbonate Vienna Pee Dee Belemnite which has a isotope ratio R of $\frac{^{13}C}{^{12}C}$ of 0.0112372.

Biological processes may have different reaction rates for the heavy and the light isotope and reaction product tends to be depleted in heavy isotope with respect to its source. This process is called fractionation and resulting differences in isotope compositions may be used to trace the flow of elements through ecosystems (Peterson and Fry, 1987; Lajtha and Michener, 1994). For example, primary producers fractionate differently with respect to their inorganic carbon source and consequently have different δ^{13}C values (Fig. 1.5). Since there is limited fractionation associated with transfer up the food web, consumers have a δ^{13}C signature close to its resource according to the 'You are what you eat' principle (Post, 2002) (Fig. 1.5). Transfer of nitrogen up the food web is associated with $\sim$ 3 ‰ enrichment per trophic level and may therefore be used to estimate trophic levels (Minagawa and Wada, 1984; Vander Zanden and Rasmussen, 1999; Post, 2002)).

Several estuarine food web studies have taken benefit from stable isotopes to link producers and consumers. Peterson and Howarth (1987) clearly showed that macrobenthos in a salt marsh depended on phytoplankton and *Spartina* detritus, whereas sulfur-oxidizing bacteria and terrestrial organic matter were much less utilized. Herman et al. (2000) use the δ^{13}C difference between phytoplankton and microphytobenthos (see Fig. 1.5) to estimate dependence on local microphytobenthic production or pelagic production of an intertidal flat community. Rossi et al. (2004) used δ^{13}C signatures to demonstrate the existence of an ontogenic shift in the life-cycle of the bivalve *Macoma balthica*.

The main advantages of natural abundance stable isotope signatures are that they can be measured from comparatively small samples and isotope signatures integrate information on food sources that are assimilated in body tissues of field collected organisms over a longer period of time. However, in many cases there are more potential food sources than isotopes available to disentangle food web relationships (Phillips and Gregg, 2003). Also differences in δ signatures may be small and fractionation factors are not accurately known and may be variable (Gannes et al., 1997). An alternative way in which stable isotopes may be used to study food web dynamics is by means of a deliberate isotope tracer addition. This topic will be detailed in the next paragraph.

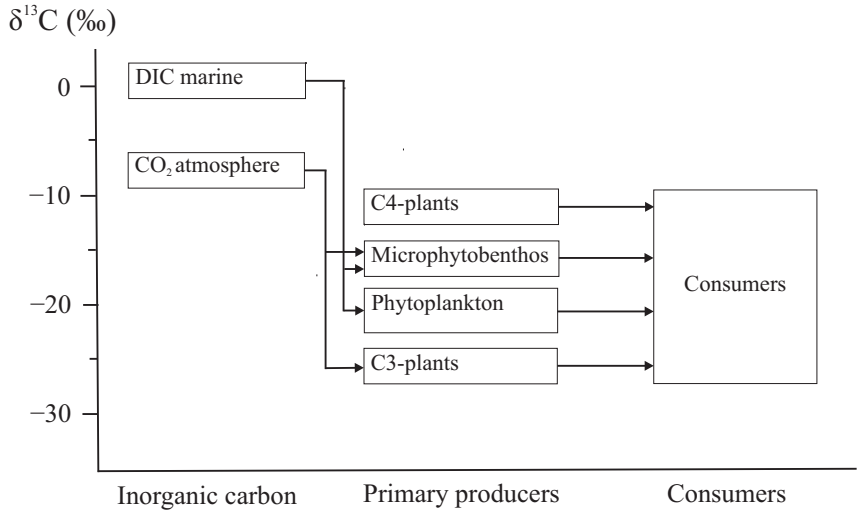

Figure 1.5: Relationship among δ^{13}C values of inorganic carbon sources, primary producers and consumers. The δ^{13}C value of the consumer depends on the importance of the different resources in its diet, e.g. a dominance of C4-plants will result in a δ^{13}C value close to -10 ‰. Adapted from Boschker and Middelburg (2002).

1.2.2 Stable isotopes at tracer level

One way to advance the use of stable isotopes in food web research is to deliberately modify the isotope composition of an (in)organic resource and monitor the transfer of this modification to other food web components. As compared to radioactive isotopes, stable isotopes have the advantage that they can be used to measure transfer rates *in situ* without legal restrictions or negative environmental effects. A change in the isotope composition compared to the background composition is denoted in the $\Delta\delta$ notation: $\Delta\delta = \delta_{modified} - \delta_{background}$. A modification of the isotope composition of an (in)organic resource will result in transfer of this modification to other food web components (see Fig. 1.6 for an example). The rate and magnitude of transfer depends on the importance of the resource for the consumer and turnover time of the consumer.

Stable isotope enrichment studies are basically done in two ways. One way is the introduction of inorganic nutrients or simple organic substrates in a food web. As such, inorganic nitrogen has been used as a tracer in rivers (Hamilton et al., 2001, 2004), estuaries (Holmes et al., 2000; Tobias et al., 2003) or sediments (Tobias et al., 2001) to quantify nitrogen transformation rates. ^{13}C-bicarbonate was used to quantify transfer up the food web of microphytobenthos in intertidal sediments (Middelburg et al., 2000) and phytoplankton in lakes (Cole et al., 2002; Pace et al., 2004). ^{13}C-acetate has been used in small streams to determine the importance of streamwater dissolved organic carbon and bacterial carbon for the invertebrate community (Hall et al., 2000). The other way is to culture algae in an isotopically enriched medium, harvesting the algae and feed them to retrieved sediment cores (Moodley et al., 2000) or *in situ* sediments (Blair et al., 1996; Levin et al., 1997; Moodley et al., 2002, 2005; Witte et al., 2003; Nomaki et al., 2005). In both cases, transfer of label to aquatic and benthic organisms is monitored in time and

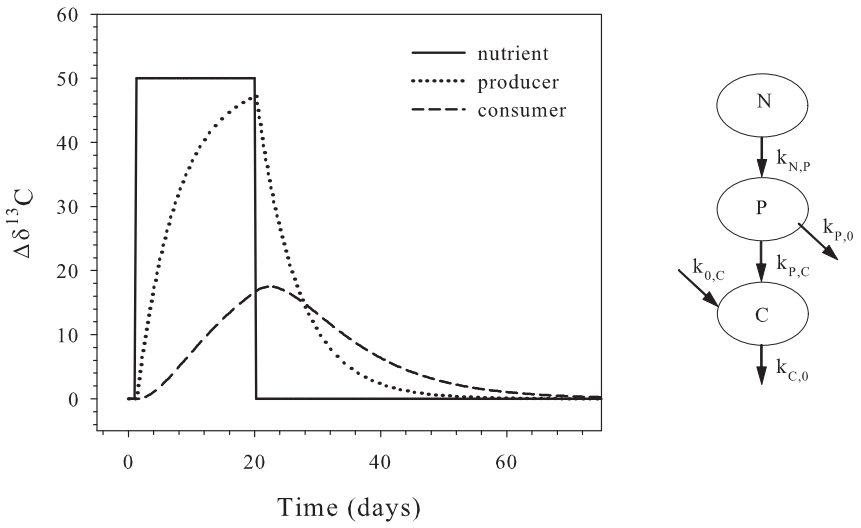

Figure 1.6: Transfer of label among a nutrient (N), producer (P) and consumer (C) compartment due to an pulse enrichment of the nutrient compartment (see Hamilton et al. (2004)): $\frac{d\,\Delta\delta P}{d\,t} = k_{N,P} \cdot \Delta\delta N - (k_{P,C} + k_{P,0}) \cdot \Delta\delta P$ and $\frac{d\,\Delta\delta C}{d\,t} = k_{P,C} \cdot \Delta\delta P - k_{C,0} \cdot \Delta\delta C$, with $k_{N,P} = k_{P,C} + k_{P,0} = 0.15$, $k_{P,C} = 0.05$ and $k_{C,0} = 0.10$, such that P obtains 100% of its nutrient requirements from N, but C obtains only 50% from P. An assumption of the model is a steady-state biomass of P and C.

used to quantify assimilation of the added substrate. These studies have revealed important insights in the structure of natural food webs through in a way that was previously not available.

1.3 Research questions and thesis lay-out

The material presented in this thesis is intended to expand our methodological capabilities to study the structure of marine benthic food webs and to apply these to answer some pressing ecological questions. The focus in this thesis is on the importance of the herbivorous, microbial and detrital pathways in an intertidal food web, with special attention for the microbial pathway. A large biological data set is required to accurately decipher the large number of carbon transfers in benthic food webs (Fig. 1.3). Therefore, we integrate a data set that consists of biomass data, process rates, natural abundance stable isotope signatures and isotope tracer data and quantify the carbon flows in the food web of the Molenplaat intertidal flat. Another important topic in benthic food web research has been the fate of bacterial carbon in marine sediments (Kemp, 1990). The main focus so far has been on transfer to grazers, but bacterivory has been found insufficient to account for measured bacterial production rates (Kemp, 1987; Epstein and Shiaris, 1992; Hondeveld et al., 1992; Hamels et al., 1998). Another possible fate is burial of bacterial carbon, e.g. Grutters et al. (2002) report the preservation of refractory cell wall components in marine sediments. However, data that allow to disentangle the possible fates of bacterial

carbon production simultaneously are currently lacking.

Specifically, the main research questions that motivated our work were:

1. How can biomass, process rates, natural abundance and tracer stable isotope data be integrated to quantify food web flows?
2. How are the carbon flows in a intertidal flat partitioned among the herbivorous, microbial and detrital pathways?
3. What is the fate of benthic bacterial carbon production?
4. What is the importance of bacterial carbon in diets of benthic fauna?

Chapter 2 gives a review of current approaches in linear inverse modeling. The aim of these approaches is similar: reconstructing element flows in food webs from incomplete data sets. However, the inverse problem is approached from different viewpoints. We present a framework that can be used to identify the origin of these differences. In addition, several methods are proposed that allow to analyze the properties of the food web reconstruction, a topic that has been overlooked in most previous LIM applications.

Chapter 3 builds on the previous chapter and provides a case-study on how knowledge on carbon processing and carbon stocks, stable isotopes at natural abundance and tracer level can be integrated by means of linear inverse modeling. In addition, it is explicitly shown how the uncertainty in the food web reconstruction decreases when new data are added to the LIM. The ecological implications of the reconstructed benthic food web are discussed.

One conclusion in chapter 3 is that the majority of bacterial carbon production recycles back to detritus and/or dissolved organic carbon and only a limited amount of bacterial carbon production is grazed. This conclusion provided the starting point for chapter 4, in which the results from an in situ ^{13}C isotope tracer experiment that aimed to quantify the importance of different fates of benthic bacterial carbon production are presented.

The key question to be answered in chapter 5 is: How much of their carbon requirements do meiobenthos and macrobenthos derive from bacterial carbon? Data from an in situ ^{13}C labeling experiment are interpreted with a simple isotope model to estimate the dependence on bacterial carbon. The ecological implications of the bacteria-benthos interaction are discussed.

Chapter 6 compares the dynamics of different bacterial biomarkers: polar-lipid-derived fatty acids (PLFAs), D-alanine as a marker peptidoglycan and total hydrolyzable amino acids (THAA). These biomarkers represent different parts of bacterial biomass and are expected to show different degrees of degradability. The results are discussed with respect to the use of biomarkers to quantify total bacterial label uptake, the importance of carbon burial as a sink of bacterial production and differences in degradation rates of bacterial compounds.