Summary

The rich benthic community of estuarine and coastal sediments thrives in an environment where organic carbon inputs of different origins are diluted with inedible sediment particles. Our knowledge on how the different carbon inputs are partitioned within the benthic community is limited, because of the intractability of the benthic environment, in particular due to difficulties with accessibility and sampling, and high heterogeneity. In this thesis, we combine stable isotope techniques with quantitative modeling approaches to gain additional insight in the structure of marine benthic food webs.

Chapter 2 is a review of applications of linear inverse modeling (LIM); a data assimilation technique that is used to reconstruct food web flows from incomplete data sets. The review discusses three aspects.

First, we make explicit account of the different elements that form a LIM: 1) five types of ecological information, 2) three linear equations and 3) two optimization norms. Differences among LIM applications can be explained by differences in the way these elements are combined, which can be translated to 1) a different appraisal of the quality of different information types and 2) differences in the chosen optimization norm.

Second, common practice in ecological modeling is to solve the LIM and discuss the results in terms of the food web flows. Typically, no or only limited attention is paid to the properties of the LIM solution. Five LIMs of food webs that differ in number of food web compartments, flows and available data were investigated with especial emphasis on the properties of their solutions. Strikingly, it appeared that the uncertainty surrounding the recovered flows was high. However, the uncertainty clearly differed among the LIMs and were directly related to the ratio of data to number of food web flows, the lower this ratio the higher the uncertainty. Moreover, it appeared that the linear optimization criteria did not always lead to one unique solution, but several alternative solutions may exist. Since the quadratic optimization criterium should always lead to a unique optimal solution, we recommend the use of this criterium in future studies. The gained insights in LIM problems showed that the analysis of the LIM solution is valuable and should be routinely included in LIM food web studies.

Finally, the large uncertainty in the reconstructed food webs pinpoints to the need for additional data resources that may reduce this uncertainty. We demonstrate that stable isotope signatures were very successful in reducing the uncertainty of the food web flows of a benthic food web (Chapter 3). Other data types, such as ecological stoichiometry and quantitative fatty acid signature data, are expected to be similarly effective and we suggest that LIM offers a ideal platform to integrate traditional data types (e.g. biomass data) with these modern data types to reduce the uncertainty in food web reconstructions.

Chapter 3 is a case-study of LIM to recover the carbon flows in an intertidal sediment. Conventional LIM methodology accommodates data on biomass and total carbon processing (e.g. primary production, community respiration). In this chapter we

extended the existing methodology such that natural abundance and tracer stable isotope data can also be added. Dedicated uncertainty analysis clearly demonstrated that these data additions decreased the uncertainty in the recovered food web: the uncertainty range for 60% of the flows decreased with $>$ 50%. Moreover, the conventional methodology uses a minimization criterium to solve the inverse model, this criterium has been questioned because it lacks a biological justification and introduces a bias in the reconstruction. In the extended methodology, the model is solved by assimilating tracer isotope data and therefore makes the arbitrary minimization criterium redundant.

Carbon flows in the intertidal food web were dominated by bacteria, both in terms of secondary production and respiration. Bacteria acquired carbon predominantly (85 %) from semi-labile detritus and the remainder from dissolved organic carbon. Only a limited fraction (9 %) of the bacterial carbon production was grazed by the benthos, the remainder was recycled back to detritus and dissolved organic carbon. Microbenthos was the second contributor to total secondary production. Its dominant carbon sources were detritus or dissolved organic carbon, the results from the inverse model did however not allow to quantify the importance of the sources individually. Surprisingly, bacteria contributed only a few percent to the total carbon requirements of microbenthos. Larger benthos, consisting of nematodes, meiobenthos and macrobenthos, fed selectively and relied primarily on microphytobenthos and phytoplankton. Surprisingly, detritivory was negligible for these larger benthic fauna. These observations suggest that the microbial community (i.e. bacteria and possibly microbenthos) is supported by semi-labile detritus with limited transfer to higher trophic levels, whereas local primary production by microphytobenthos and phytoplankton supported the meiobenthic and macrobenthic communities. This separation suggested that the detrital-microbial and algal-grazer pathways function rather autonomously.

Chapter 4 builds on the conclusion from chapter 3 that only a limited fraction of bacterial carbon production is grazed by the benthic community, but instead recycles back to detritus or dissolved organic carbon. This chapter reports on a dedicated in situ isotope labeling study that was conducted to quantify the fate of bacterial carbon in sediments on a time scale ranging from days to months. ^{13}C-glucose was injected in the surface 10 cm of an intertidal sediment and traced into specific bacterial polar-lipid-derived fatty acid (PLFA) biomarkers, particulate organic carbon (POC), dissolved inorganic carbon (DIC), meiobenthos and macrobenthos. A dynamic model describing the C and ^{13}C transfers in the sediment was successfully fitted to the C and ^{13}C observations of the different compartments. The model output was subsequently used to recover total bacterial carbon production and the importance of its potential fates: grazing, resuspension or recycling to dissolved organic carbon.

The model contained parameters describing bacterial growth, bacterial grazing, resuspension and growth efficiency. The values of these parameters were used in a published mechanistic relation that predicts the ratio $\frac{bacterial\ carbon}{particulate\ organiccarbon}$. The ratio prediction based on the model parameter was 0.005, and compared reasonably, although at the lower end, with the observed ratios (0.005 - 0.24). This correspondence suggests that mechanistic modeling may be used to model bacterial carbon dynamics, rather than the statistical regression models that are more commonly employed.

Total bacterial production was 67 mmol C m^{-2} d^{-1}. The primary fate of bacterial production was mortality, accounting for 65 % of the bacterial production, thus forming a recycling carbon in the bacteria - dissolved organic carbon loop. The major loss from this loop is bacterial respiration. Grazing of bacteria by meiobenthos (3 %) and macrobenthos (24 %) accounted for 27 % of the bacterial production. Due to the high meiobenthic and

macrobenthic biomass at the study site, we surmise that grazing pressure is high when compared to other systems. Therefore grazing on bacteria by benthos is not expected to be a major controlling factor of bacterial biomass in marine sediments.

The results from this experiment were also used in chapters 5 and 6.

Chapter 5 aims to answer the question: How much of their carbon requirements do intertidal meiobenthos and macrobenthos derive from bacterial carbon? To answer this question, the bacterial community in an intertidal sediment was labeled with ^{13}C, following the injection of ^{13}C-glucose. The appearance of label in bacteria (based on label incorporation in bacterial-specific phospholipid-derived fatty acids) and subsequent transfer to meiobenthos (group level) and macrobenthos (species level) was followed over a period of 36 days. The label dynamics of the benthos were either fitted with a simple isotope model or evaluated against enrichment of bacteria, to derive the importance of bacteria in the carbon budget of the meiobenthic and macrobenthic community. Bacteria constituted a maximum of 20 % and generally $<$ 10 % of total carbon demands of the different members of the meiobenthic and macrobenthic community. Therefore the trophic significance of bacterial carbon for intertidal meiobenthos and macrobenthos is limited.

The amount of labile carbon usually decreases with sediment depth. We therefore hypothesized that deeper dwelling benthos relies more on labile bacterial carbon than surface dwelling benthos that have direct access to other labile carbon sources. Contrary to this hypothesis, meiobenthos and macrobenthos living deeper in the sediment did not show a comparatively higher dependence on bacterial carbon. Possibly, benthic fauna cannot process sediment particles, with attached bacterial community, quickly enough to exploit the present bacterial carbon to a greater extent. If one assumes indiscriminate feeding on a homogeneous mix of sediment and bacteria, the expected removal of bacterial carbon at our study site is 0.36 % d^{-1}. The resulting carbon flows is well below the maintenance requirements of benthic fauna, and therefore we expect that bacterivory is limited by the processing rates of sediment particles. However, our observations do show that 6$\times$ more bacterial carbon is removed than expected from indiscriminate feeding, which clearly demonstrates selective feeding by benthic fauna.

Bacteria assimilate and respire both fresh and semi-labile organic carbon. The low dependence on bacterial carbon suggests that benthic fauna competes with bacteria for labile organic carbon, as labile carbon assimilated by bacteria is effectively lost for the faunal food web. Alternatively however, ingestion of bacteria might provide a means through which otherwise indigestible semi-labile detrital organic matter might enter the metazoan food web. Local circumstances, in particular the availability of labile organic carbon, will determine whether competition or subsidy dominates. The weak interaction between bacteria and benthic fauna implies that this interaction should not be viewed as a predatory, but as a competitive interaction for labile organic matter in the benthic food web.

In chapter 4 the most important fate of benthic bacterial carbon production was found to be mortality (65 %). Bacteria are made up of different compounds that different degrees of degradability, which may results in a build up of refractory compounds in the sediment. In **chapter 6** we evaluate the results from a ^{13}C-glucose labeling experiment and compared ^{13}C of different bacterial biomarkers. Polar-lipid-derived fatty acids (PLFAs) rapidly degrade in sediments and were therefore used as a marker for living bacteria. D-alanine (D-Ala) is a compound unique to peptidoglycan, a constituent of bacterial cell walls, which is known for its refractory nature. Therefore, D-Alanine is used as a marker for both living bacteria and bacterial remnants. Finally, since label uptake was dominated by bacteria, the label dynamics of individual amino acids (AAs) were assumed to be derived from bacteria

and were compared with amino acids that have been used as markers to quantify organic matter quality.

Dynamics in $\Delta\delta^{13}$C were very similar for PLFAs, D-Alanine and AA in the first weeks after labeling, indicating simultaneous production of the different biomarkers, which is expected when new bacterial cells are produced. Levels of $\Delta\delta^{13}$C were however lower for D-Ala and THAAs as compared to PLFAs, presumably due to the presence of an inactive background pool for D-ala and THAA that dilutes the $\Delta\delta^{13}$C signal of the living bacteria.

Surprisingly however, the $\Delta\delta^{13}$C dynamics were similar during the 4.5 months after labeling. If burial would have been an important sink of bacterial carbon, $\Delta\delta^{13}$C values of D-Ala and some AAs are expected to be relatively higher when compared to PLFAs. The correspondence in $\Delta\delta^{13}$C values between D-Ala and PLFAs therefore implies that burial of comparatively recalcitrant bacterially derived compounds is not a major sink of bacterial carbon. This is further indicated by the low contribution (< 1 %) of D-Ala carbon to total sediment organic carbon.

Absolute incorporation rates of ^{13}C for bacteria, inferred from conversion from the different biomarkers, are in good agreement and therefore provide an important validation for the use of biomarkers as a tool to quantify bacterial tracer incorporation.

Notable differences in the dynamics of $\Delta\delta^{13}$C and ^{13}C among the HAAs were only found 4.5 months after labeling. These differences confirmed the refractory nature of peptidoglycan, as more ^{13}C-D-Ala persisted when compared to the ^{13}C-L-Ala. Moreover the HAAs glycine (Gly), serine (Ser) and proline (Pro) behaved relatively refractory. Interestingly, these AAs have previously been linked to progressive states of organic matter degradation.