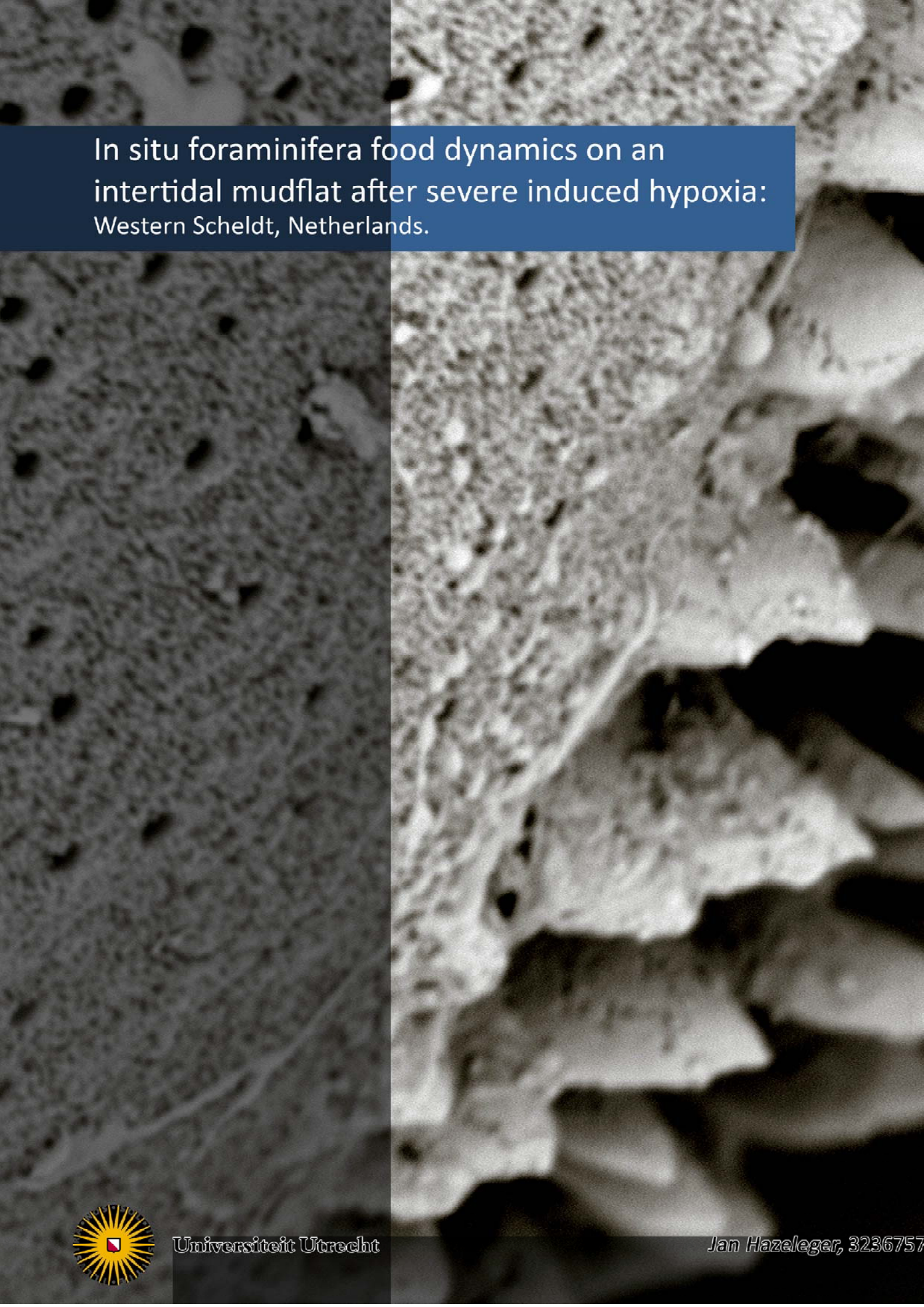
In situ foraminifera food dynamics on an intertidal mudflat after severe induced hypoxia: Western Scheldt, Netherlands.



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Summary

In order to predict natural and anthropogenic effects that are expected on benthic fauna on intertidal mudflats, a large in situ defaunation/refaunation experiment has been conducted at the NIOO (Yerseke, 2005). Species identities, diversity and a ^{13}C food pulse are used to identify the microbial carbon flow to higher trophic levels during habitat recovery.

For my Msc Thesis, I've used the foraminifera samples that were collected in this study and studied the foraminifera as an intermediate step between microbial carbon flow and the higher trophic levels. Foraminifera could be a major constituent of the carbon flow in times of recovery due to their prolonged anoxia resistance and the absence of other meio- and macro-fauna. Several methods have been used to determine the role of the foraminifera in the recovery of the benthic ecosystem on the intertidal mudflat.

First the species identities, numbers and size distributions per foraminifera species are used to identify changes in foraminifera population after two and five months of recolonisation in June and September. The size distribution of the individual foraminiferal species are found to be a surprisingly good indicator for changes in habitat conditions in which the foraminifera live. The size distribution can be used to identify physical and ecological habitat changes induced by seasonal differences over the year, but also habitat conditions that are changed by an severe induced stress are well identified.

The *Ammonia beccarii*, *Elphidium excavatum* and the *Haynesina germanica* are most abundant in this sediment. They survive the hypoxia and seem to be overall positively correlated to the recovery of a severe induced hypoxia in terms of size distributions and quantities. This is in contrast to the microbial fauna and the other meio- and macro-fauna that are negatively correlated to the severe hypoxia and are dependent on their subsequent dispersal from the surrounding sediment. Therefore, foraminifera behave out of context in terms of habitat recovery in contrast to the other fauna. Moreover, population behavior of individual foraminiferal species in terms of size distributions and numbers is different over the year in terms of habitat recovery to stable 'conditions'.

Secondly, the ^{13}C food pulse measurements at 96 hours after labeling the sediment with bicarbonate and glucose are used to determine role of foraminifera as a whole in the carbon cycle. Moreover they are used to track the assimilation of different carbon sources in foraminiferal species. Results indicate that the foraminifera overall switch to the carbon source they prefer when food is plenty available, but also that foraminifera only play a minor role in the carbon cycle (up to 0.3%). Moreover the results are used in combination with the ^{13}C label signature of the bacteria and microphytobenthos to identify seasonal differences in carbon sources that could have led to different size distributions of foraminifera over the year.

Also others reasons are discussed and evaluated and size distribution together with the quantities of the foraminifera are used to describe population behavior in times of recovery after a severe induced hypoxia.

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Introduction

The earth as we know it today has many coastal areas in which highly developed benthic ecosystems are present. Due to increasing natural and anthropogenic induced disturbances there is an increase of interest in the recovery of benthic ecosystems. Present knowledge is inadequate to predict effects of increasing structural and functional changes induced by these disturbances. Structural and even functional changes of the ecosystem follow upon the increasing magnitude and frequency of perturbations in local ecosystems. Moreover the return to their normal state appears to take considerably longer (Andersson, 2007). Estimation of the SEDAC (the Socioeconomic Data and Applications Center) human populations in the coastal areas (first 100 km off the coast) is around 40% (SEDAC, 2006), which is much higher than the inland population. These high population near the coast produce waste in these benthic ecosystem by harbor activities etc., but also for the protection of these coastal areas dredging of sediment in rivers and coastal areas is used to protect the land of flooding. Other perturbations include destruction by intensified storms, hypoxia due to increasing nutrient runoff and excessive flooding (Murray, 2006), that are expected by a continuous increase of natural and anthropogenic induced effects in these local habitats over the next years. For example, an increased nutrient runoff by intensified use of fertilizer in agriculture will lead to local hypoxia in rivers, deltas and oceans, in which fauna dies-off by the lack of oxygen (Colen *et al.*, 2008)(Rossi *et al.*, 2009)(Andersson, 2007). Restoration of the oxygen gradient in these local habitats will set ecological, but also biogeochemical processes in motion to recover the re-oxidized land (Andersson, 2007). The interaction between these two is not well understood and therefore needs to be studied in order to predict future developments of these habitats after severe perturbations.

This process is also of interest in geologic surveys that involve perturbations (anoxia) in benthic habitats throughout geologic history. Information on the succession of the opportunist and colonist species after perturbation can be used as a modern analog and will help us to understand more of the ecological information in these proxies.

Since the habitats of benthic fauna are highly versatile and many elements involve spatial and temporal distributions (Diz *et al.*, 2009), research on benthic ecosystems is very complex. Geochemical, physical and the ecological interactions between fauna are important in establishing new habitats (Ernst *et al.*, 2002). Since consistent effects are heavily studied in laboratories using experimental cosms (Ernst *et al.*, 2002), (Moodley, L. *et al.*, 1997), (Widbom *et al.*, 1995), eliminating many of the original parameters (Langezaal *et al.*, 2004), still minor is known about the functioning *in situ* in terms of structural and functional behavior of the ecosystem.

Effort has been made (Cardinale *et al.*, 2000), (Rossi *et al.*, 2008), (Colen *et al.*, 2008),(Montserrat *et al.*, 2008) and others doing i*n situ* experiments to predict inconsistent patterns in the recovery of benthic ecosystems in context dependent (complex and non-monotonic) areas. In other words, 'how would the real world be'?

Experimental overview and results NIOO study

In order to investigate the carbon cycle as a property of the ecosystem functioning and transport from primary producers to higher trophic levels, a study was conducted by the NIOO in 2005. They made use of a deliberately experimentally induced hypoxia on an intertidal mudflat in the Western Scheldt in the Netherlands. Patches of 4x4 meter were covered by a plastic seal for two months and were intended to make the sediment anoxic and completely stop the carbon transport. After restoration of the oxygen gradient, the dynamics of species diversity and species identities of the macro-fauna, the microbial fauna and the biogeochemical properties of the patches were closely examined (Rossi *et al.*, 2009). Addition of ^{13}C bicarbonate and ^{13}C glucose label has been included to investigate the restoration of the carbon cycle between the microbial-fauna and the macro-fauna. The results of the study conducted by the NIOO and the related biogeochemical and ecological processes are summarized below.

Habitat destruction by a severe induced hypoxia dies-off all the aerobic bacterial life, micro-phytoplankton, part of the meio-fauna and all of the macro-fauna (Rossi *et al.*, 2009), (Colen *et al.*, 2008), (Andersson, 2007). Also the biogeochemical properties of the habitat are heavily disturbed, including the nutrient and carbon flow (figure 1).

In an inconsistent, but orderly (not random) way biogeochemical and ecological processes follow up on each other in order to restore the benthic live after a severe hypoxia. At the start, just after the hypoxia, the oxygen gradient will raise by diffusion of oxygen into the sediment. Penetration will be limited to the upper part of the sediment, but is sufficient to let bacteria and the microphytobenthos (microbial-fauna), including the cyanobacteria and the diatoms colonize the intertidal mud flat. Oxygen measurements one day after reoxidation of sediments confirmed the diffusion of oxygen into the upper layers of the sediment (Colen *et al.*, 2008).

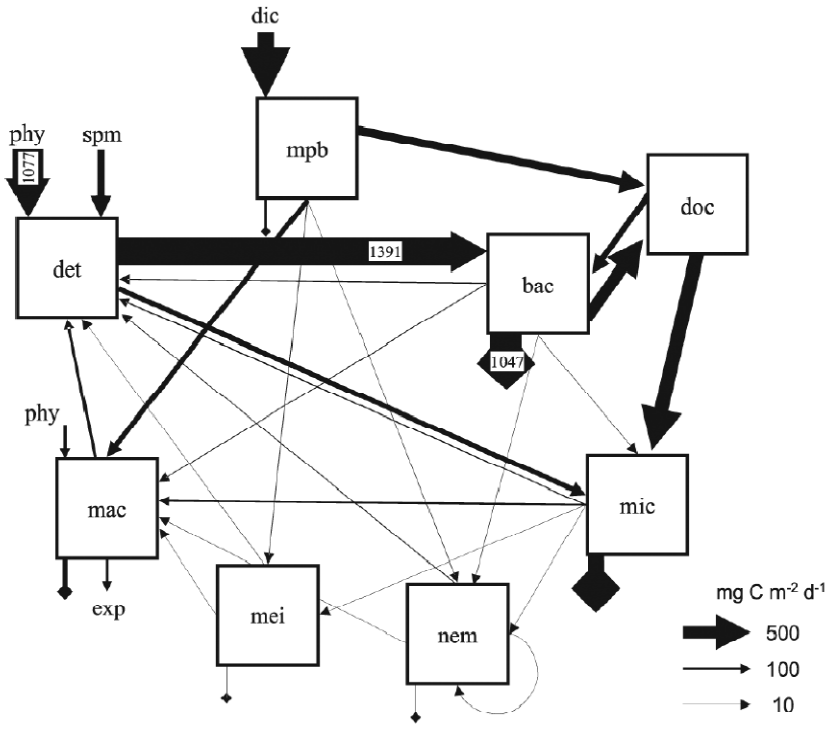

Figure 1: shows a systematic representation of an stable intertidal food web, with all major components, including the micro-, meio- and macro-fauna (van Oevelen et al. 2006). Gain and losses of carbon by the different fauna originate from the conversion of DIC by microphytobenthos into organic carbon, grazing of other fauna, respiration, mortality, import and export. Mpb = microphytobenthos, bac = bacteria, mic = microbenthos, nem = nematodes, mei = meiobenthos, mac = macrobenthos, doc = dissolved organic carbon, det = detritus, phy = *phytoplankton, spm = suspended particulate matter, dic = dissolved inorganic carbon and exp = export from the system.*

The microphytobenthos and bacteria can be resuspended into the water column as dissolved organic carbon (DOC), they can be mixed into deeper layers of the sediment

or can function as an important carbon source for heterotrophs (Middelburg *et al.*, 2000). In this study the latter is the most likely to function, due to the absence of bioturbation by macro-fauna and the presence of high quantities of labile carbon. In this way the present micro-phytobenthos and bacteria enables the micro-, meio- and macro-fauna to enter the disturbed intertidal flat in search for food.

The restoration of the carbon cycle is one of the fundamental functional properties in the restoration of an ecosystem and once established allows species on higher trophic levels to enter the system. The introduction of the micro-, meio- and macro-fauna will enhance bioturbation of the sediment (Herman *et al.*, 1999), (Meysman *et al.*, 2006). Bioturbation by these fauna increases the delivery of oxygen to the deeper sediments where it is used for the degradation of organic matter. Inorganic carbon can be converted by autotrophic fauna and subsequently can be consumed by the heterotrophic fauna. Within two months the microbial fauna and its carbon flow is completely restored (Rossi *et al.*, 2009), but the restoration of the higher trophic fauna, including its carbon flow and subsequent bioturbation takes at least to 5-6 months after reoxidation of the sediment (Rossi *et al.*, 2009).

Measurements of ammonia deeper in the sediment (2 to 8 cm deep) confirm that oxygen did not penetrated up to 8 cm depth after 6 months of refaunation. Ammonia (NH_4^-) that is present deeper in the sediment due to the breakdown of organic matter in depletion of free oxygen was not yet converted back into NO_3^-, during the time of this study. This indicates that the deeper bio-irrigation by the macrofauna, normally present in undisturbed sediments to keep the deeper sediments oxygenated, was not yet restored (Colen *et al.*, 2008). Also suspension feeding did not recover during the time of this study.

Foraminiferal information

In this study the focus is on hard shelled foraminifera, they form a part of the benthic intertidal meio-fauna. The meio-fauna is of minor constitute in the study conducted by Rossi, but could be an important link between ecological and biogeochemical interactions during recovery (Moodley *et al.*, 2000). Moreover, foraminifera are among the first protozoa to recolonize new benthic habitats (Alve, Elisabeth *et al.*, 2003) (Alve, Elisabeth, 1999) and are more resistant to prolonged anoxia (Moodley, L. *et al.*, 1997) than macro-fauna. As an addition to the study by Rossi, the results of this study will yield information about foraminifera identities, abundances and their role in the carbon cycle and will be an addition to the already obtained information on the recovery of microbial and macro-fauna species.

Foraminifera are unicellular eukaryotic heterotrophic organisms and many of them produce a shell (figure 2). This shell is constructed out of calcite, which has a very high preservation potential. Foraminifera are present in the oceans since the beginning of the Cambrian and due to their continuously changing morphological features, they can be used for biostratagraphic dating. Foraminifera usually grow until a length of around 400 to 500 µm, but cases have been reported of modern foraminifera that grow up to 19 cm (Hemleben, C., 1989).

Prolonged anoxia could be beneficial for some foraminifera species that are adapted to low oxygen levels and possibly are facultative anaerobic (Moodley, L. *et al.*, 1997), (Bernhard, Joan M. *et al.*, 1999). Nevertheless many foraminifera species will at least be affected by severe oxygen depletion (Murray, 2006). The prolonged anoxia will eventually lead to a strong reduction in generic diversity of the total fauna, because of a decrease in ecological and biochemical interaction between micro, meio- and macro-fauna, but could promote the foraminifera that are not limited by this.

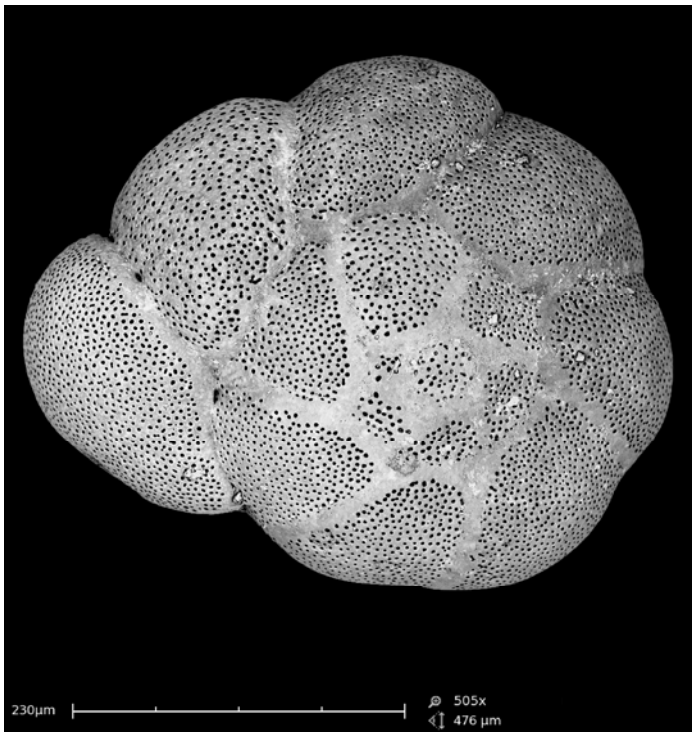

Figure 2: *Ammonia beccarii* imaged by a Scanning Electron Microscope (SEM). The scale bar in the lower left corner is 230µm.

Foraminifera species that are present or arrive in an area that is heavily disturbed; physical, chemical and ecological parameters of the surrounding sediment in combination with the limits of the species will eventually determine the success of it. Seasonal properties and related ecological interactions, for instance the arrival of food by phytodetritus (Morvan *et al.*, 2006) and temperature may influence this. Prolonged absence of types of food may promote other foraminifera genera, that have different food strategies and consequently different structural habitats. Another scenario is the switch to another carbon source by foraminifera in absence of its preferred carbon source and might imply that seasonal parameters are not that prominent at all. This is already stated by Moodley, (2000 and references herein) in which he stated that foraminifera in shallow environments in the presence of different sources of carbon may promote selective feeding.

Since benthic foraminifera are one of the first protozoa to recolonize the sediment, they may hypothetically play a major role in the recovery of the carbon cycle. According to calculations (Oevelen *et al.*, 2006) by Linear Inverse Modeling (LIM) benthic foraminifera and other meio-fauna in stable habitats play only a very small role in the carbon link (less than 1%). This 1% dominantly consist of microphytobenthos (93%) followed by microbenthos (7%) and minor to no carbon is transferred up to higher trophic levels (figure 1). Percentages could be considerably different during the early stage of recovery. Already present and new successful arriving foraminifera will reproduce and consume different amounts of carbon than in undisturbed stable ecosystems. Moreover food patterns of opportunistic meio-fauna species may have been different during refaunation (Murray, 2006), in terms of their strategy.

Incorporation of labeled ^{13}C by micro-phytobenthos and bacteria.

Tests conducted by (Montserrat *et al.*, 2008) between several Chlorophyll (chl) pigments show after two months of refaunation that almost the complete microphytobenthos population consist of diatoms (90 - 95%) and a minor component is filled by green algae and cyanobacteria. As a result of the defaunation a decline in diatoms was noticed to ± 60% of the microbial fauna, but this was already restored up to control values on sampling moments 2J, 2S and 5S. Moreover the microphytobenthos quantity (measured by chl A content) increased in the first months of recolonisation up to three times the control values (Montserrat *et al.*, 2008), till 3 months of recolonisation. The same results are found for quantities of microphytobenthos and bacteria measured by PLFA concentrations (Rossi *et al.*, 2009). Moreover bed stabilization of the sediment due to production of extracellular polymeric substances (EPS)(Smith *et al.*, 1998) by diatoms was clearly present after 2 months of recolonizing, resulting in slightly elevated sediments (figure 3). These highly hydrated carbohydrate-rich exopolymers are also ecologically significant since they can be utilized by bacteria, meio- and macro-fauna (Decho *et al.*, 1990) and can be used as a source of energy. This intense bloom of diatoms and subsequent excretion of EPS is probably related to an abundance in food, a decrease in grazing rates and a reduction in bioturbation.

Figure 3: Bed stabilization and elevation by EPS excretion of diatoms. High chl A content is measured up to 3 months three months of recolonisation (Montserrat *et al.*, 2008).

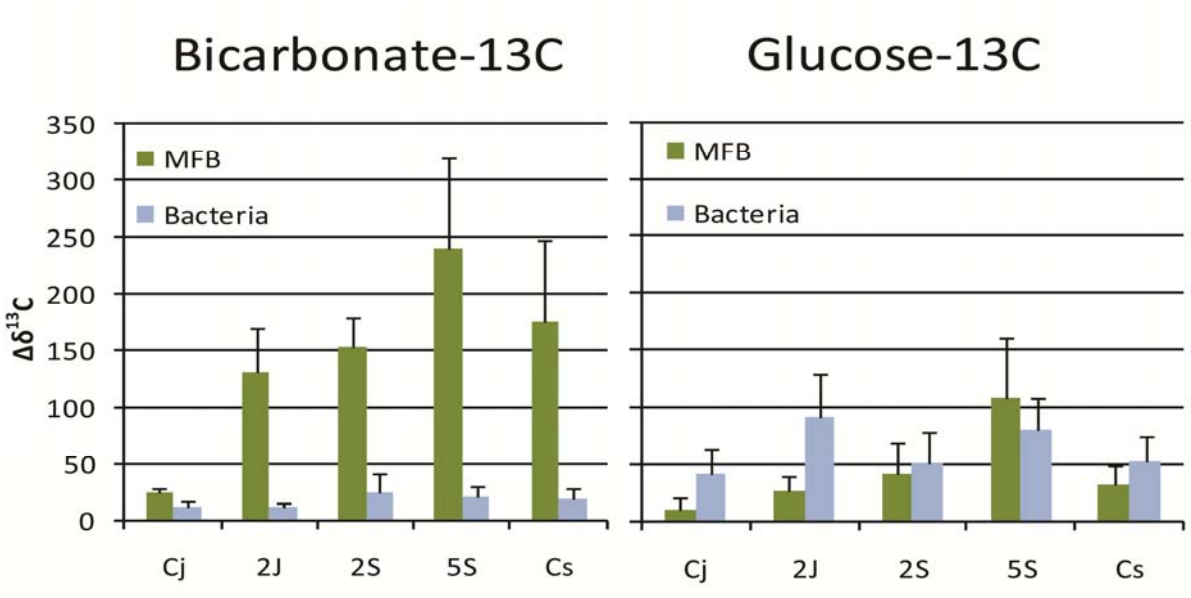

Figure 4: Incorporation of ^{13}C in MFB (microphytobenthos and bacteria (Rossi, Transport of carbon in re-colonizing webs. PowerPoint).

Labeling the fauna with ^{13}C bicarbonate was intended to track uptake and assimilation by autotrophic micro-organisms only. Subsequent enrichment of this label in higher trophic fauna would then imply grazing of the autotrophic micro-organisms, which include diatoms, green algae and cyanobacteria. The labeling with ^{13}C glucose was intended to label the bacteria only. However, uptake and assimilation of ^{13}C glucose by the microphytobenthos is was also observed, although at lower amounts than the ^{13}C bicarbonate label (figure 4). Still, considerably amounts of assimilation ^{13}C glucose is measured in the microphytobenthos. This amount is comparable to the assimilation of ^{13}C glucose by bacteria. Therefore the autotrophic pathway is labeled though a combination of assimilation of ^{13}C bicarbonate and ^{13}C glucose.

Assimilation of ^{13}C bicarbonate is also measured in the bacteria. However, assimilation of ^{13}C bicarbonate in bacteria is much lower than in microphytobenthos and therefore now a good distinction can be made between these two food sources and the food source of an individual foraminifera species. Summarizing, uptake by foraminifera on the labeled glucose only is due to grazing of bacteria. While a mixed signature of labeled glucose and bicarbonate results from grazing on microphytobenthos (and potentially also bacteria).

Hypothesis and research questions

The severe hypoxia events will occur on larger areas in natural settings in contrast to the small defaunated patches in this study. Moreover local extinction over a much larger area of sensitive meio- or macro-fauna in depletion of oxygen will 'exclude' this fauna to reenter the disturbed habitat or at least delay it for a much longer time span. Simply because dispersal strength of the fauna is insufficient to allow the fauna to reenter the ecosystem quickly. Dispersal of the fauna into the defaunated plots is relatively easy in contrast to hypoxia events over larger areas. Therefore we expect the foraminifera to recover differently in terms of structural properties over the year in contrast to a natural hypoxia over a larger surface, induced by a range of different physical and ecological factors that are seasonal related. However in terms of functional properties we expect the ecosystem, including its ecological and biogeochemical state, will return to its normal 'undisturbed' state over time. However the time that is needed for this complete restoration, including the deep bio-irrigation and suspension feeding is probably much longer and must be interpreted in terms of years.

Foraminiferal recolonisation after a severe oxygen depletion on an intertidal mudflat is examined in terms of species diversity, size distribution and abundances over the year. Two patches of 4x4 meter have experienced a severe oxygen depletion of two months till March and July, directly followed by recolonisation of benthic fauna, respectively till June and September 2005. Sampling of the benthic fauna has been done after addition of ^{13}C bicarbonate and ^{13}C glucose on several time intervals of incubation, including control samples without defaunation. Results are used to obtain information on ecological and biogeochemical sensitivity of individual foraminiferal species and functional and structural properties of the foraminiferal species in a recovering habitat.

General questions that I discus in this paper are:

- How do foraminifera behave in terms of dynamics of diversity, species identities and ecological relationships during recovery of an experimentally induced hypoxia over different times of the year.
- New foraminifera carbon isotope data is implemented in the already excessive knowledge derived from the defaunation/recolonisation experiments and is used to study the carbon transfer to higher trophic levels.
- Are there differences in the allocation of labeled carbon between the different foraminiferal species and what does this imply for their food preferences.
- Does the hypoxia event has an impact on the sizes and biomasses of different foraminiferal species and is there a seasonal influence on these differences.
- As a result, what would the functional end product of the ecosystem after recovery be (Rossi *et al.*, 2008) in different times of the year.

Methods

Location

The ^{13}C tracer experiments were done on an intertidal mudflat in the Scheldt estuary (figure 5)(Rossi *et al.*, 2009). The intertidal mudflat covers an area of around 1.0 km^2 and has a semidiurnal regime with a mean tidal range of 3.9 m. The sediment has an average silt content (% of particles <60µm) of 50%. On location (51°21'23"N, 3°42'49"E) in between a surface of around 50 x 50 m, four patches (4 x 4 m) were covered by a black plastic sheet for two months. The patches are separated 5 to 10 meters apart from each other. The two months that the plastic sheet covers the sediments are intended to impose a severe hypoxic environment.

Figure 5: The large photo in the middle shows the beginning of the Scheldt estuary in the Southwest of the Netherlands. The box in the lower left corner shows an enlargement of it. The white line in here represents a kilometer. The box in the right lower corner shows again an enlargement. The black and white dots in the white square show the 4 x 4 defaunated patches. Since the picture is taken in 2009 and visibility of the plots by Google Earth has decreased in contrast to 2005, plots are visible only very faintly.

Setup of the experiment.

Two patches of 4x4 meter are covered with a black water-proof polyethylene sheet of 0.1 mm thick (figure 6), from 30th of January until 30th of March 2005 and two others from 9th of May until 6th of July, 2005. As a control two patches per time span are chosen that did not experience any human induced disturbance (hypoxia). The four patches have been left alone for two months after defaunation, respectively to 6th of June and 5th of September. In this way recolonisation of the sediment could take place and fauna could reenter the sediments. After these two months the ^{13}C bicarbonate and glucose is added to the sediment, intended to label the fauna. The patch that has experienced two months of recolonisation till June (2J) is referred as 2J and the one that experienced 5 months of recolonisation in September 5S. The patch that has experienced two months of recolonisation till September is called 2S hereafter (figure 7). The control patches that are sampled in June and September, are named respectively (CJ) and (CS), due to adverse weather conditions only one control patch is sampled in June.

Figure 6: The plastic seal that covers 4x4 meter of sediment during ebb. Picture is taken directly after placing.

In April samples have been taken that have experienced only defaunation, no recolonisation. Addition of the ^{13}C bicarbonate and glucose label started on April the 6th.

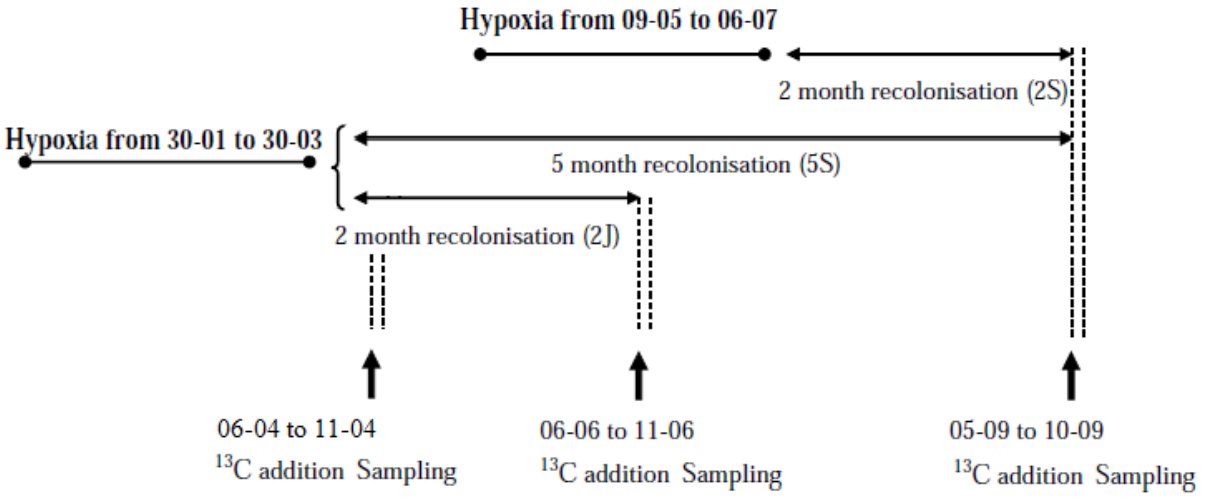

Figure 7: Setup of the experiment, including defaunation and the recolonisation periods of 2J, 2S and 5S. The samples of April are labeled and taken more a less on the end of the defaunation and the beginning of the recolonisation stage of 2J and 5S. Picture is modified after original one (Rossi et al. 2009).

For the ^{13}C-labelling, 250 mg ^{13}C-labelled bicarbonate (99% ^{13}C) or 114.75 mg ^{13}C glucose (99% ^{13}C) is used that were both individually dissolved in 250 ml of filtered artificial seawater. Per solution one patch of 50 x 50 cm (figure 8) was labeled. The labeled bicarbonate was sprayed over the sediment and intended to label the microphytobenthos. The ^{13}C-labbeled glucose was injected till 8 cm of sediment depth using injections homogenized over the surface with a grid of 2.5 x 2.5 cm holes and were intended to label heterotrophic bacteria (van Oevelen et al. 2006). The concentrations are chosen according previous studies (Middelburg *et al.*, 2000), (Oevelen *et al.*, 2006) in order to significantly label fauna, without significantly enrich the sediment with carbon.

The labelling started on low tide and was added once. Half of the patches is labeled with ^{13}C bicarbonate and half with ^{13}C labeled glucose. Sampling was done after 4, 24, 48, 96 and 144 hours after labelling (figure 9). Every sampling time, two randomly selected plots of 50 x 50 cm in each patch per labelling was chosen and sampled to a depth of 8 cm and an internal diameter of 5 cm (total sampled area per patch and labelling: 39,25 cm^2). All cores were stored at 4°C, until arrival at the laboratory (two hours after sampling). In this study only the samples that were taken 96 hours after incubation are used.

Figure 8: 50 x 50 cm tray (patch) in which the two labels in artificial sea water are added.

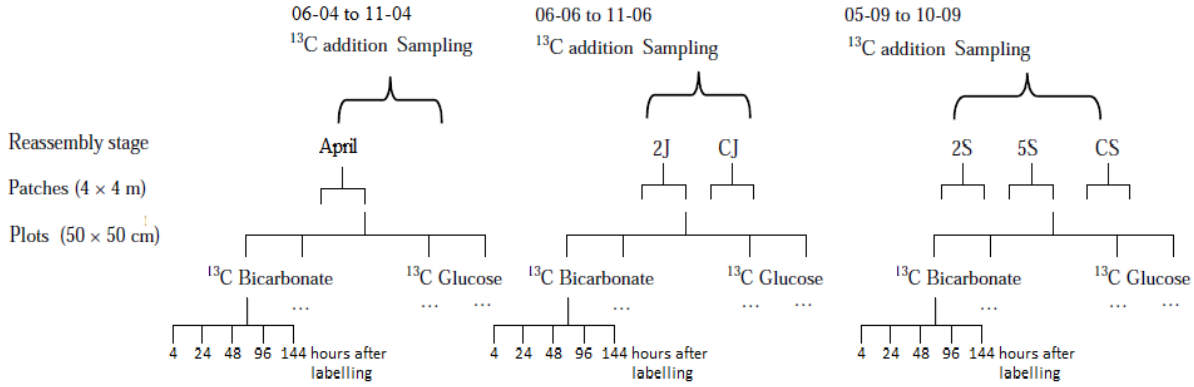

Figure 9: Schematic overview of the months of labelling, quantity of patches and plots and the corresponding labelling. Also the sampling moments after the labelling are depicted in the bottom of the overview. Picture modified after original one (Rossi et al. 2009).

The cores were sub-sampled in the laboratory, with a cut syringe that has an internal diameter of 2 cm. Some of the sub-cores were used in the experiment of Rossi, 2009, for the bacterial-isotope signature with the help of PLFA (phospholipid fatty-acids) biomarkers and identification and determination of the macro-fauna signature. The remaining cores that were intended for foraminiferal ^{13}C-label determination are sliced horizontally, making slices of resp. 0-1, 1-2, 2-4 and 4-8 cm. In this experiment only the samples of the upper cm were used. In Appendices 1 the treatments of the samples are listed. The sub-slides are stored in 4% buffered formaline with Rose Bengal staining. Formaline may alter isotopic composition, but since incorporation is calculated as a difference between sample and background value (both treated with formaline), this potential bias is eliminated.

Foraminiferal processing and ^{13}C measurements

In this experiment the 'living' foraminifera of the T4 (time after labeling: 96 hours) samples of 0-1 cm depth were picked. First the samples were sieved over a 63µm sieve with tap water to remove the formaline and the fraction smaller than 63µm. All other following proceedings were performed in demy water, unless otherwise stated. Afterwards the samples were spitted in one half and two quarters splits. First the foraminifera of half a split were picked and counted, when the amount is insufficient for a good labeling measurement, the other quarters were picked. Splitting of the samples is implemented to cut down picking time, since this is very time consuming. After picking sufficient forams that were alive at the time of sampling per sample (Appendices 2), they are stored at -20°C, until all samples are processed. Live foraminifera were easily determined since they are very well stained by the rose Bengal staining. Foraminifera that do not have completely filled chambers with cytoplasm until the last two were discarded. Also foraminifera that are not completely stained by Rose Bengal or show indications of bacterial grazing on the cytoplasm are discarded. Difficulties arise in the separation of young *H. germanica* and *E. excavatum*, since they are very similar to each other when small. See discussion for implications. Eventually after picking all the samples, one sample per time is melted on room temperature. The foraminifera of this sample were determined and divided into groups containing only forams of one species. Photo's of the forams were taken with a calibrated scale burned (figure 10) into it to retrieve the size and the number of the forams later on. This is done with a Hitachi Camera, type Hv-c20A connected to a loop (Leica MZ12(5)). The camera is connected to a computer where the photos were stored. Photos were analyzed by the software Analysis, version 3.00 (build 428).

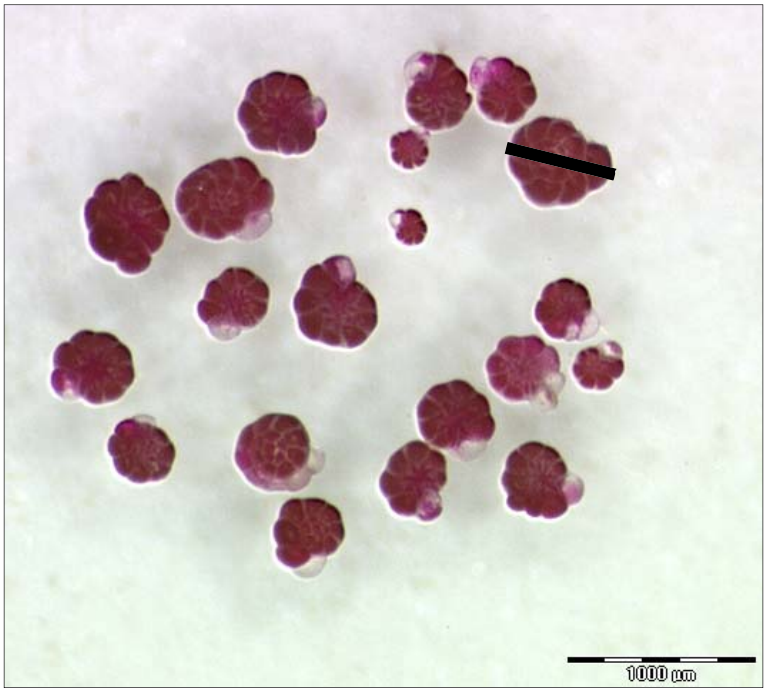

Figure 10: One of the pictures taken of specific foraminifera species (here: ventral view of the A. beccarii). In the upper right corner the black line indicate how the length of the individuals is determined. This represent the maximum length of the foraminifera.

After taking the photograph, 20 specimens per species were randomly selected and cleaned to remove any adhered particles and thereafter transferred to silver boots (silver weighting boats D5024, 12 x 4 x 4 mm). Foraminifera species that do not have 20 individuals were examined on their sizes. If sizes were sufficient the samples are still used to measure the ^{13}C label incorporation. After transporting the foraminifera into silver boots they are decalcified by adding 2.5% HCl (Moodley et al, 2000). At first 50µl is added to the silver boots and the foraminifera are visually checked by a microscope to see whether all the shells are decalcified. If necessarily another 50µl is added to remove any last $CaCO_3$. After decalcification, the samples were dried overnight in a stove on 50°C. The tray in which the samples were placed to dry is loosely packed in silver foil, with small openings to prevent the air stream of the stove to go directly over the samples. When the samples were dried, the silver boots were carefully closed, to form a small round package including the foraminiferal

tissue. Thereafter the samples were measured for $\delta^{13}C$ content at the NIOO with a Carlo Erba 1106 Elemental Analyser coupled online with a Finnigan Delta S isotope ratio mass spectrometer.

The sub-samples that have been photographed were analyzed on size and quantity per species. The length of the foraminifera shell is measured from its last chamber to the other side of the foraminifera in its ventral view. In this way, the maximum length of the shell is measured (fig. 10).

Biomass calculations

Moodley et all., 2000 and 2008, determined the length of the shell and the corresponding biomass of two *Ammonia beccarii.* On the hand of these measurements the power of distance of the size and corresponding biomass (figure 11) is calculated for the *A. beccarii*. However only one size/biomass proportion of *E. excavatum* and the *H. germanica* is known (Moodley at all., 2000 and 2008) no formula can be calculated. Nevertheless, they both seem to fit well in the power of distance formula that is determined for the *A. beccarii*.

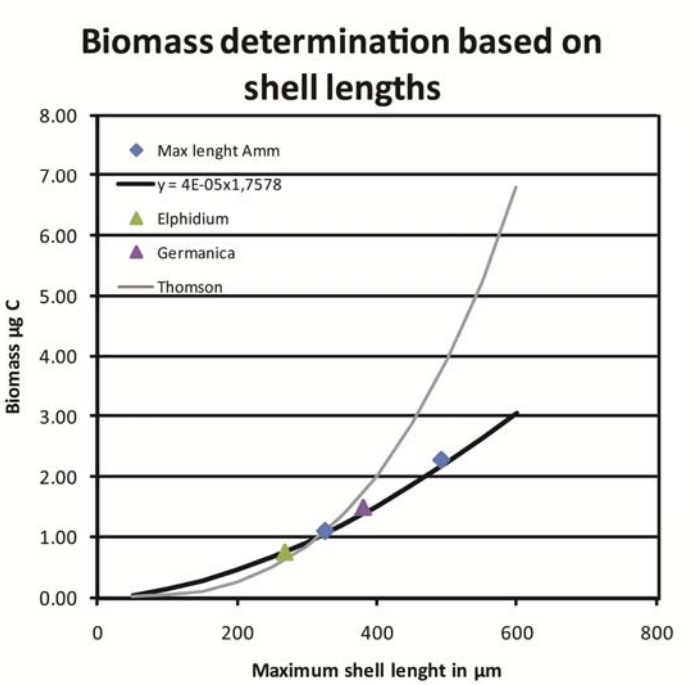

Figure 11: Power of distance (red line) calculated by two size/weight proportions of the *A. beccarii* (blue dots). The size/weight proportions of the *A. beccarii*, *E. excavatum* (green triangle) and the *H. germanica* (purple triangle) are from (Moodley *et al.*, 2000)

Using the formula of Thomson (Thomson *et al.*, 1993) in order to calculate the biomass based on the length of the foraminifera, more or less the same biomass is found for the *A. beccarii*. When the foraminifera are assumed to be spherical the following formula can be used: volume = (π/6) length × width^2 (Thomson *et al.*, 1993). Subsequently the biomass C is determined by the foraminiferal volume (µg C) = 0.06 x volume$_{(nanoliter)}$. In case of the *E. excavatum* and the *H. germanica*, which (in terms of volume and biomass) are more similar to each other, lower biomass over the total range of sizes are found in contrast to the biomass calculated with the formula that is constructed by the data from Moodley(2000 and 2008). Visual inspection (figure 26) of the latter two shows that they are indeed much more spherical than the *A. beccarii*. Moreover only one size/biomass proportion is known (Moodley at all., 2000 and 2008) and different sizes of specimens could behave completely different in terms of biomass in contrast to the power of distance that was calculated for the *A. beccarii*. Therefore for biomass calculations of the *H. germanica* and the *E. excavatum* the biomass formula of Thomson were used.

#

^{13}C label calculations

Carbon isotopes are expressed in delta notation relative to Vienna Pee Dee Bellemnite (PDB): $\delta^{13}C = [(^{13}C/^{12}C)_{sample} /(^{13}C/^{12}C)_{ref} - 1] * 1000$. The $\delta^{13}C_{background}$ values that were used for A. beccarii, E. excavatum and the H. germanica are respectively: -16,62‰, -14,94‰ and -12,93‰ (Moodley *et al.*, 2000). The $\Delta\delta^{13}C$ was calculated with the biomass together with the $\delta^{13}C$ proportion of the sample which produce the weight of the ^{13}C label in each sample in ug^{13}C/3,14 cm^3(Middelburg *et al.*, 2000). All measurements of the label incorporation in microphytobenthos and the macro-fauna that are accomplished by F. Rossi are in mg^{13}C/39,25cm^2, therefore the data is recalculated to this proportion for comparison.

In order to compare the quantity of ^{13}C label incorporation in foraminifera to the total quantity of the added label, some specific recalculations of the added labels must be made. This will account for the difference in label addition, since the ^{13}C bicarbonate is sprayed over the sediment, while the ^{13}C glucose label is injected into 8cm depth of sediment. The 250 mg ^{13}C bicarbonate and 114.75 mg ^{13}C glucose that are used to label the 50 x 50 cm^2 plots are therefore present in different quantities at the start of the labelling and proportional uptake calculations have to be corrected for this. In the case of the 250 mg ^{13}C bicarbonate that is added to label the 2500 cm^2 of sediment is corrected for the 39,25 cm^3 (1cm depth) of sediment in which the biomass of the foraminifera are determined (table 1). Since the bicarbonate is sprayed over the sediment and is assumed not to label the sediment below the first cm (assumption are discussed in the next paragraph), no correction is made for the sediment depth. While addition of 114,75 mg ^{13}C glucose also has to be corrected for the 8 cm of sediment depth in which it is injected (table 1). This leads to a relatively much lower concentration of ^{13}C glucose present in the upper cm of the sediment in contrast to that of bicarbonate. Proportions of incorporation of the total added label are produced by dividing the duplo mean specific mg ^{13}C/39,25 cm^3 uptake per label in the three foraminifera species in each plot by the calculated present ^{13}C label in the 39,25 cm^3 (upper cm of sediment) in which the foraminiferal ^{13}C content is measured and biomass is calculated.

Recalculation of the added bicarbonate in mg ^{13}C/39,25 cm^2	
surface plot cm2	2500
mg ^{13}C added	250
mg ^{13}C/cm2	0,1
mg ^{13}C/39,25 cm2	3,925
Recalculation of the added glucose in mg 13C/39,25 cm2	
surface plot cm2	2500
mg ^{13}C added	125
mg ^{13}C/cm2	0,05
mg ^{13}C/39,25 cm2	1,9625
mg ^{13}C/39,25 cm3 (= divided by 8 cm)	0,2453125

Table 1: Recalculation of the added label to mg^{13}C/39,25cm^3 to compare it with the data of F. Rossi that she conducted in her label measurements.

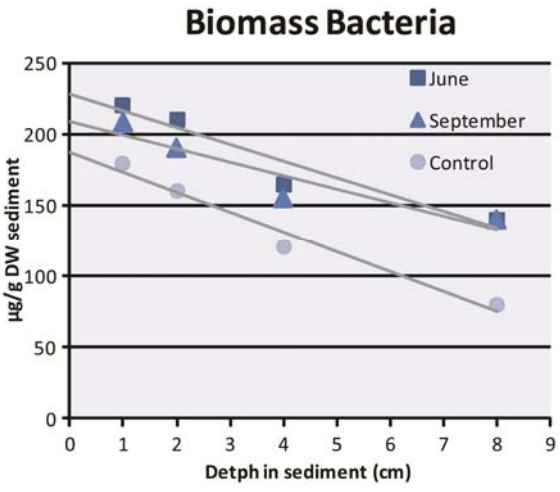

Figure 12: Bacterial biomass distribution over 8 cm of sediment in the samples of June (J), September (S) and the Control plots(C).

For the proportion of ^{13}C label in the foraminifera to the total microbial fauna signature another correction is needed. The bacterial uptake of ^{13}C glucose label over the total 8 cm (Rossi *et al.*, 2009) of sediment is

different, since biomass of the bacteria is lower deeper in the sediment. The biomass for bacteria decreases linearly in the 8 cm of sediment (39,25 cm^2) and eventually 2/3 of biomass is left over in the deeper parts (figure 12). Assuming that (assumption are discussed in the next paragraph) the biomass and uptake of label occur in the same proportions, then the weight of the label in the upper 39,25 cm^3 is 17% of the label that is present over 8 cm depth. No correction is needed for the uptake of ^{13}C glucose by microphytobenthos, since they are assumed to live only in the upper cm. Correction for the ^{13}C bicarbonate label into the microbial fauna is also not necessarily since this is sprayed over the sediment and is assumed to label only the upper cm of sediment.

Proportions of microbial (microphytobenthos + bacterial) ^{13}C label in the foraminifera are calculated by dividing the ^{13}C mg C/39,25cm^3 of label incorporation in the foraminifera by the weights of the incorporated label in microbial fauna. The exact food patterns of the three prominent foraminifera could not be determined and therefore the ^{13}C label of microphytobenthos and bacteria per label are added to have a total microbial quantity of ^{13}C incorporation.

Assumptions and corrections for the recalculated data

For label uptake calculations of the ^{13}C bicarbonate and glucose in mg/39,25 cm^3 (upper cm of sediment), assumptions have been made (see methods: Biomass and ^{13}C label calculations). This is necessarily in order to correct for the difference that arises in experimental design, between the one conducted by (Rossi *et al.*, 2009) and this study. Due to time constraints the foraminifera specimen in this experiment are counted and measured for their specific label uptake only in the upper cm of the sediment. The uptake ratio's and biomass of the micro- and macro-fauna in the study of F. Rossi are measured over a total of 8 cm of sediment depth. Therefore assumptions are needed to calculate the proportional incorporation of the total present available ^{13}C label and to compare label uptake of the foraminifera with that of micro- and macro-fauna, while keeping in mind that the bicarbonate is sprayed over the sediment intended to label the microphytobenthos and that glucose is injected till 8 cm into the sediment for labelling the bacteria.

Bicarbonate

First the bicarbonate will probably not penetrate (diffuse) deep into the sediment in the 96 hours after addition of the label. Bioturbation would increase penetration of the label deeper into sediment depths, but bioturbation is not completely restored and thus of minor influence. Since diatoms, which are the major autotrophs that assimilate the ^{13}C bicarbonate, are restricted to the upper millimeters of the sediment, presumably only the species that live in this upper layer of sediment can graze the labeled autotrophs. Overnight migration of the diatoms deeper into the sediment in the deficiency of light occurs, but this migration is not sufficient to transport the label much deeper into the sediment. Therefore the method of adding the label and the behavior of the diatoms, the ^{13}C bicarbonate label uptake will be restricted to the upper cm of sediment. This assumption makes the values that are measured by F. Rossi in mg ^{13}C/39,25 cm^2 (8cm depth) bicarbonate uptake by MFB comparable to the values mg ^{13}C/39,25 cm^3 (upper cm of sediment) that are measured in our study. If the penetration of the label is deeper into the sediment it will introduce an underestimation of the proportional uptake by foraminifera of the total added ^{13}C label. Moreover, since bicarbonate is sprayed over the sediment, resuspension of the label during flood is likely and can introduce another underestimation of the calculated proportional uptake.

Subsequently the foraminifera in the upper cm of sediment are assumed to have equal access to the labeled microphytobenthos. Therefore the label measured in the foraminifera will be a

representative mean of the total uptake of bicarbonate. This also includes a laterally equally distributed quantity of microphytobenthos over the measured 39,25 cm^3. In the recolonisation plots this assumption is most likely correct, since a stable equally distributed mat of microphytobenthos was present in the upper part of the sediment in times of sampling (Montserrat *et al.*, 2008). This stable equally distributed mat was absent in the control plots and therefore patchiness in quantity of microphytobenthos could have been present.

Glucose

In case of additions of ^{13}C glucose injected into 8 cm of sediment assumptions will be different. Labelling with ^{13}C glucose was intended to label the bacteria, but is also taken up by diatoms. This labelling of diatoms by ^{13}C glucose will then probably also be restricted to the upper cm of sediment in which the diatoms reside in order to receive sunlight. In terms of uptake and assimilation of ^{13}C glucose in bacteria, this will happen in the total 8 cm of sediment in which the label is injected. Presuming that the migration of bacteria in 96 hours is negligible and that foraminifera sampled in the upper cm are restricted to eat the bacteria only from the upper cm, the injected label must be divided by 8 in order to compare it with the biomass and measured label uptake in this study.

Microbial ^{13}C incorporation

More assumptions and corrections were necessarily to calculate the percentage ^{13}C label in foraminifera over the total ^{13}C label present in microphytobenthos and bacteria. The assimilation of ^{13}C bicarbonate and glucose into foraminifera is assumed to be only obtained by grazing microphytobenthos and bacteria. Correction is only needed for the weight of ^{13}C glucose uptake in bacteria for an assimilation over the upper cm of sediment (39,25 cm^3). Since bicarbonate is sprayed over the sediment and uptake is assumed to be restricted to the upper cm and glucose can only be assimilated in the upper cm by microphytobenthos in order to receive sunlight no correction is made for these values.

Since the uptake of ^{13}C bicarbonate and glucose is controlled by the proportion of specific PFLA (phospholipid ester-linked fatty acids), assimilation of the ^{13}C in there must be assumed to be equal over all plots. However proportional assimilation could for example differ between control and recolonisation plot, since environmental parameters are different. Incorporation of ^{13}C in these specific PFLA could be higher in the recolonisation plots in times of stress, while other PFLA are build by microphytobenthos and bacteria in 'stable' times. After reassessment of the method, this error seems to be very low (Middelburg, personal comm.). Although there is no data available that can validate this, introduction of a error in the proportions of the microbial ^{13}C label incorporation might be possible.

More deviation of the proportion of incorporation of microbial fauna in foraminifera can occur, due to patchiness of quantities and activity of individuals. This is discussed in "Difficulties in ^{13}C label measurements of the foraminifera" in the discussion section.

SEM pictures

For identification of the three most prominently present foraminifera species scanning electron microscope (SEM, Phenom) pictures were taken. The pictures are summarized in figure 26.

Statistical analysis

The total standing stock of the three prominent foraminifera were used in descriptive statistics and reproduced in figure 13 with 1 S.E. For the measurements of the length of the foraminiferal shells, the foraminifera were subdivided into size classes of 25 µm, starting at 75 µm, the next one being 100 µm up to the size class of 600 µm. The smallest size class was 63-75 µm, since the sediment is sieved on a 63 µm sieve. The number of foraminifera in the different size classes are recalculated to percentages of the total sample, unless stated otherwise, to reduce bias due to patchiness in the quantity of foraminifera per sample. For comparison and identification of the differences in the size distributions in the early recolonisation stages (2J and 2S, with CJ and CS as controls) and the recolonisation stages till September (2S and 5S, with CS as control) univariate analysis of variance (ANOVA) is used. Levene's test for equality of variance is used to choose between the Tukey HSD and Game-Howell test for post-hoc comparison.

The isotope signature of the individual foraminifera species per sample are mostly measured in duplo. However in some of the samples the numbers of *A. beccarii* and *E. excavatum* is too few to have a good isotopic measurement, in these cases there is only one measurement instead of the duplo measurement. No statistics have been performed on the duplo isotopic measurements in which large variation can occur (see "Difficulties in ^{13}C label measurements of the foraminifera"). The duplo isotopic means are used for the calculations of the percentage of total quantitative ^{13}C uptake to the total added label and the percentage quantitative ^{13}C uptake of foraminifera in contrast to the microbial signature. However no statistics can be applied to the isotopic measurements and the assimilation of the label is highly variable, the ^{13}C isotope values and calculations that make use of these values can be used in a descriptive way. This has to be kept in mind while interpreting the labelling data.

Results

Foraminifera quantity and distritrubution of sizes

In all patches the following foraminifera species are most prominent; *Ammonia beccarii*, *Elphidium excavatum* and the *Haynesina germanica*. In April also the *Brizalina variabilis* and the *Elphidium willansoni* are present, but in low numbers and only in April, so that no statistical calculations can be applied on them. In figure 13 and appendices 2, the amounts of the most abundant forams are calculated for ind./3.14cm^3 (mean of 4 samples), for April, 2J, 2S, 5S, CJ and CS.

The quantity of the *A. beccarii* is higher in the defaunated plots than in the control plots, but not significant for the 2S plot (significances based on twice the S.E., descriptive statistics)(fig. 13).

The is opposite is observed for the quantities of *E. excavatum*, except the defaunated plot in April, which are twice as high as all the other plots. The quantity of *E. excavatum* in the plots that have been sampled in June are higher in all plots than the numbers found in the samples of September, but there is no significant differences in quantity between 2J and CJ (fig 13).

The quantities of *H. germanica* in April are more than twice as high as the other measurements. CJ is higher than 2J, in contrast to the samples taken in September, in which the quantity is highest in 2S, lower in 5S and eventually lowest in CS (fig 13).

Haynesina germanica is also the most abundant foraminifera species found in the samples. The *H. germanica* is present in numbers at least 3 to 6 times as high, compared to the *A. beccarii* and the *E. Excavatum*. In April this is almost 10 times as high.

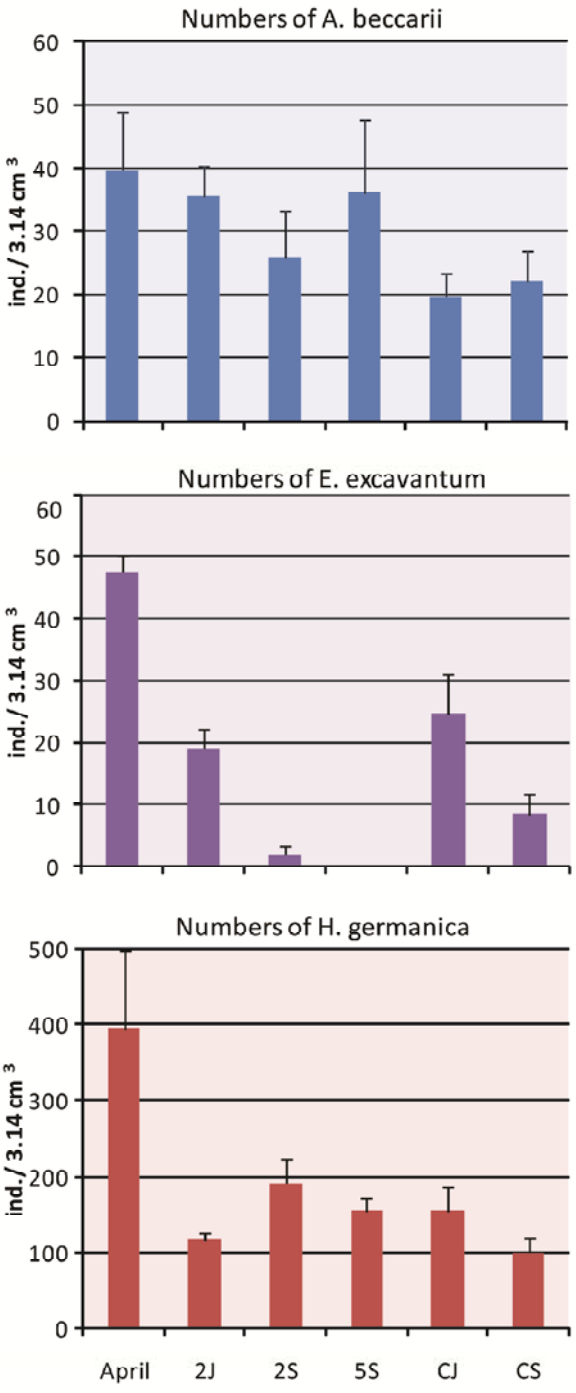

Figure 13: The numbers of A. Beccarii, E. Excavatum and H. germanica that are sampled in April, June and September (mean of 4 samples). April = 2 months of defaunation (def.)) and no refaunation (ref.), 2J = 2 months of def. and 2 months of ref., 2S = 2 months of def. and 2 months of ref., 5S = 2 months of def. and 5 months of ref. CJ = no def., CS = no def. Error bars are 1 SE. Note the different Y-axis scale.

Size distributions: 2J, 2S, CJ and CS

In the histograms of fig. 14 the percentage of forams per length class are displayed. Histograms show the three prominent species in the CJ,CS, 2J and 2S samples in percentages, divided in boxes of 25µm. Samples that have had the same treatment, but had different label addition were summed, assuming that there is no difference in length between the foraminifera after the 96 hours of label incubation with bicarbonate and glucose.

The size distribution of *A. beccarii* in the control plots of June and September are similar (sig.=0.077, CJ = CS). The controls are not similar to the defaunated plots and also the defaunated plots are different from each other (sig.=0.016, CJ ≠ 2J), (sig.=0.012, CS ≠ 2S),(sig.=0.02, 2J ≠ 2S). The mean sizes of *A. beccarii* of sample CJ, 2J, CS and 2S are 274µm, 330µm, 232µm and 273µm respectively and they have no equal variances. The median of the individual sizes of the *A. beccarii* in CJ, 2J, CS and 2S are respectively 263 µm, 334 µm, 228 µm and 265 µm. Clearly, *A. beccarii* is bigger in the recolonizing plots.

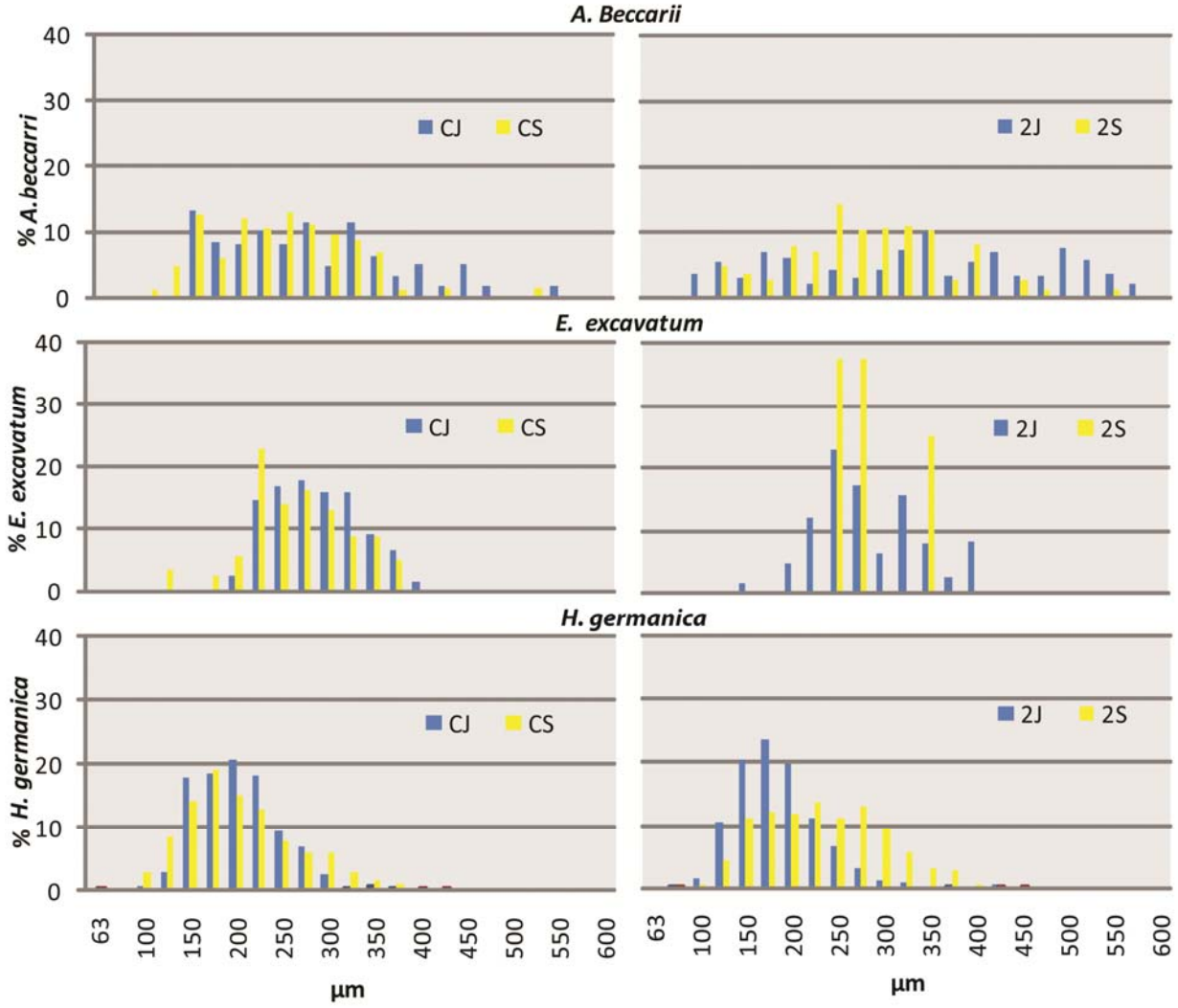

Figure 14: Size distribution of the *A. beccarii, E. excavatum* and the *H. germanica* in 2J and CJ in percentages of the total quantity present. 2J = 2 months of def. and 2 months of ref., CJ = no def. Box size is 25 µm.

E. excavatum shows overall similarity in the size distribution between the all samples (F=0.811, sig.=0.489, 2J = 2S = CJ = CS). Mean sizes of *E. excavatum* of sample CJ, 2J, CS and 2S are 271µm, 275µm, 258µm and 267µm, respectively. Note that the quantity of the *E. excavatum* in 2S are very low (fig. 13) and percentages are based on 7 individuals.

The control size distributions of *H. germanica* are similar (sig.=0.843, CJ = CS). The size distribution of the *H. germanica in* the control plots differs significantly to that in the defaunated plots (sig.=0.000, CJ ≠ 2J), (sig.=0.000, CS ≠ 2S), (sig.=0.000, 2J ≠ 2S). The mean sizes of *H. germanica* of sample CJ, 2J, CS and 2S are; 189µm, 174µm, 192µm and 222µm respectively and samples have no equal variances. The median of the sizes are 186 µm, 168 µm, 177 µm and 217 µm, respectively.

Size distribution in September: 2S, 5S and CS

In the histograms (figure 15) the size distribution of *A. beccarii* and *H. germanica* of 2S, 5S and CS are also compared

The *A. beccarii* has a significant difference in size distribution between CS and 2S (sig.=0.002 CS ≠ 2S), but this is not the case between CS and 5S (sig.=0.171 CS = 5S) and 2S and 5S (sig.=0.133 2S = 5S). The mean sizes 2S, 5S and CS are 273µm, 251µm and 231µm, respectively and they have equal variances. The median sizes are respectively, 266µm, 253µm and 229µm.

E. Excavatum was not found in the 5S samples and found in insufficient numbers to test them on significance in size distribution in September.

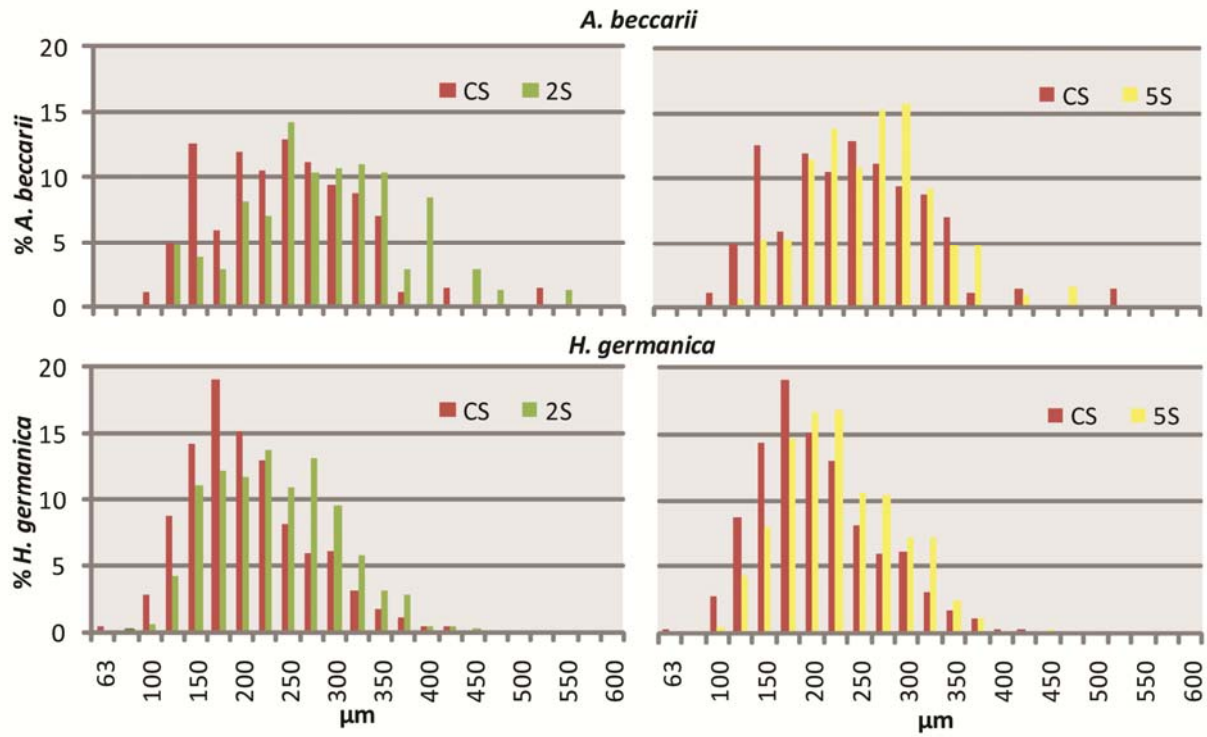

Figure 15: Size distribution of the *A. beccarii* and the *H. germanica* in 2S, 5S and CS in percentages of the total quantity present. 2S = 2 months of def. and 2 months of ref., 5S = 2 months of def. and 5 months of ref. CS = no def. Box size is 25 µm.

In case of the *H. Germanica,* 2S and 5S are different in size distribution in contrast to the control samples (both sig.=0.000 CS ≠ 2S = C5), while 2S and 5S are the same(sig.=0.165 2S = 5S). The mean sample sizes of 2S, 5S and CS are 222µm, 215µm and 192µm, respectively and do not have equal variances between the groups. The median sizes are respectively, 217µm, 208µm and 177µm.

April and 2J

In April the patches did experience 2 months of defaunation and are labeled and sampled directly after the defaunation. The same patches were samples after two months of refaunation, in June. No control patches of April were sampled and therefore the patterns in the size distribution of April are compared to 2J, to show the effect what happens in the two months of recolonisation in terms of size distributions of the three dominant foraminifera species. In figure 16, the histograms are not in percentages, but in quantities of the species in 3,14cm^3, so the large difference in individuals between April and June becomes clearly visible. The sizes distribution of *E. excavatum* and *H. germanica* are the same in April and June. *A. beccarii* has a higher number of larger individuals in June.

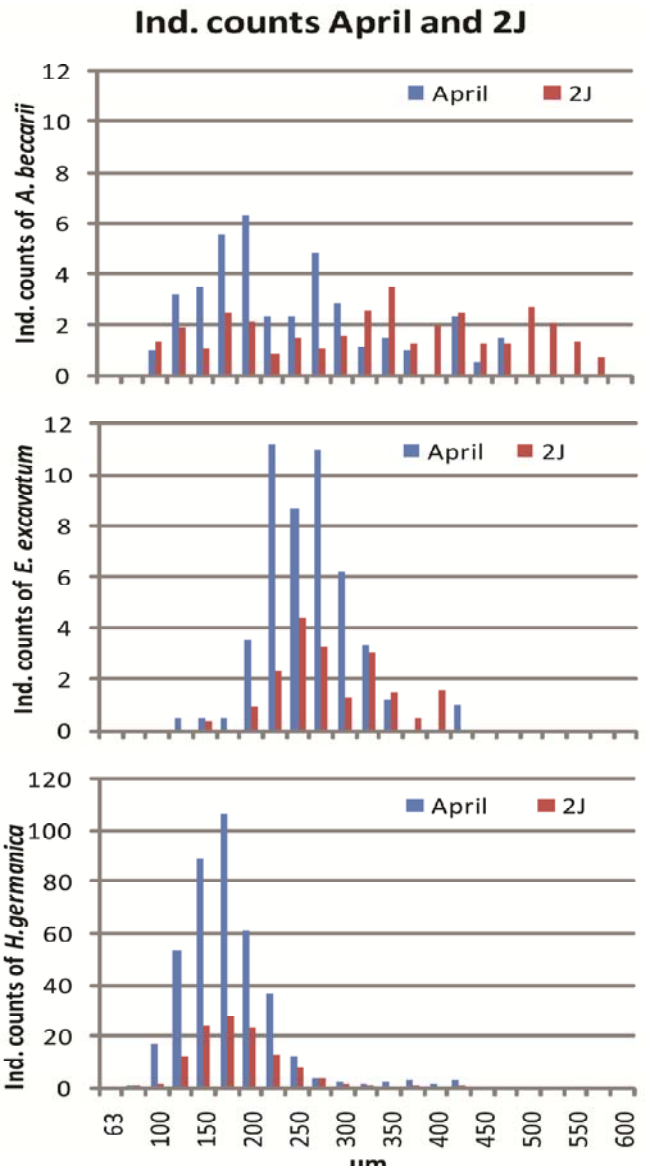

Figure 16: Size distribution of the *A. beccarii, E. excavatum* and the *H. germanica* in April and 2J in total quantity present. April = 2 months of def. and no ref., 2J = 2 months of def. and 2 months of ref. Box size is 25 µm.

Incorporation of ^{13}C bicarbonate and glucose:

Early recolonizing stages (CJ and 2J).

In figure 17 the $\Delta\delta^{13}C$ bicarbonate and glucose measurements for the three prominent foraminifera are shown: *A. beccarii*, *E. excavatum* and *H. germanica.* For some samples, no duplicate sample was present or foraminiferal specimens were too low in abundances to measure it in duplicate.

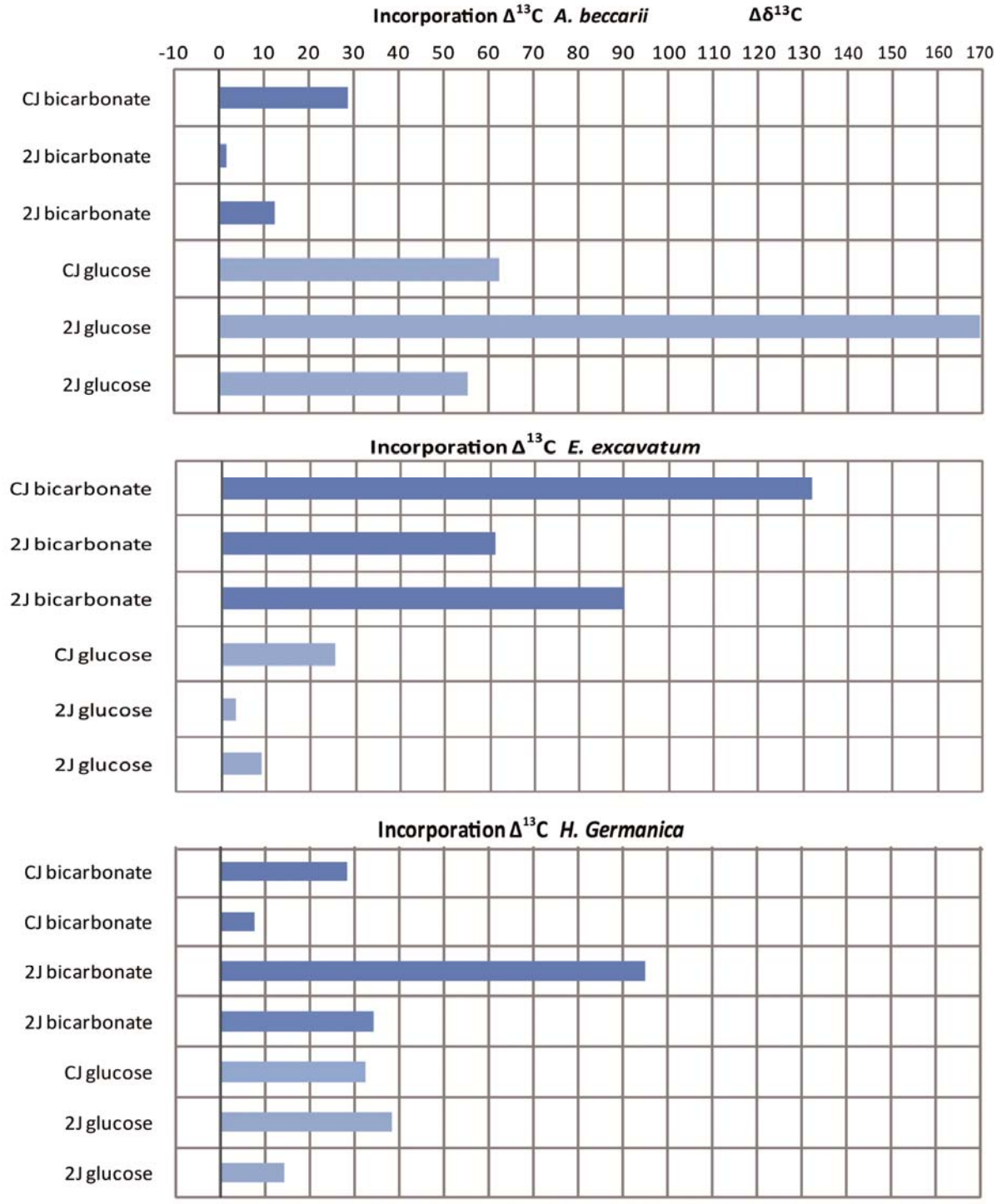

Figure 17: $\Delta\delta^{13}C$ uptake in the *A. beccarii, E. excavatum* and the *H. germanica* of the ^{13}C bicarbonate and glucose label in the 2J and CJ plots. 2J = 2 months of def. and 2 months of ref., CJ = no def. $\Delta\delta^{13}C$ uptake = $\delta^{13}C_{measured} - \delta^{13}C_{bacbground}$.

The $\Delta\delta^{13}C$ bicarbonate label in *A. beccarii* in June is lower in sediments that have experienced defaunation, while one label incorporation measurement of ^{13}C glucose is very high in the defaunated plots. Mean incorporation of ^{13}C glucose is however much higher for the *A. beccarii* in the defaunated plots in contrast to the control ones. For *E. excavatum* all incorporation measurements of the ^{13}C label in the defaunated plots are lower than the controls. Mean incorporation of $\Delta\delta^{13}C$ bicarbonate in the defaunated plots in the *H. germanica* are higher than the control plots, while the mean ^{13}C glucose uptake in the recolonisation plots is lower than the control plots.

Early recolonizing stages (CS and 2S).

The $\Delta\delta^{13}C$ of *A. beccarii* and *H. germanica* in September are graphed in figure 18. The *E. excavatum* is absent in the samples of 2S and 5S and therefore only the *A. beccarii* and *H. germanica* are depicted. The incorporation of ^{13}C bicarbonate in *A. beccarii* is lower in the defaunated plots than in the control plots, while mean uptake of ^{13}C glucose in the defaunated plots correspond to the control measurements. In terms of the *H. germanica*, $\Delta\delta^{13}C$ bicarbonate is higher in the defaunated plots, while incorporation of ^{13}C glucose is difficult to interpreted. The duplo measurements are highly variable in the defaunated plots. In terms of mean uptake of the label it is slightly higher in the defaunated plots.

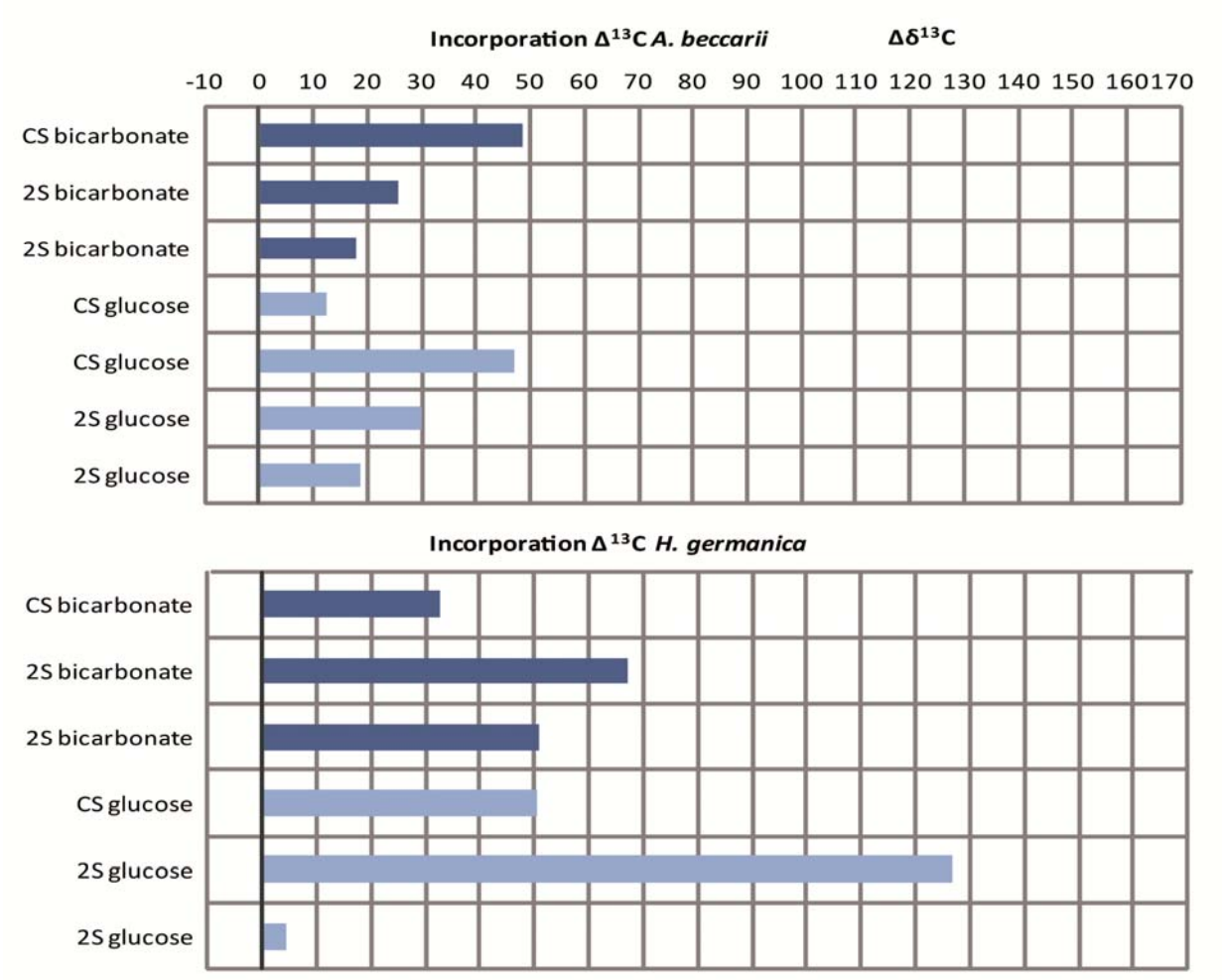

Figure 18: $\Delta\delta^{13}C$ uptake in the *A. beccarii* and the *H. germanica* of the ^{13}C bicarbonate and glucose label in the 2S and CS plots. 2S = 2 months of def. and 2 months of ref., CS = no def. $\Delta\delta^{13}C$ uptake = $\delta^{13}C_{measured} - \delta^{13}C_{bacbground}$.

Recolonization stages till September

As already noted *E. excavatum* is almost absent in the samples taken in the defaunated plots of September and therefore there are no incorporation measurements available.

In terms of incorporation of ^{13}C bicarbonate by the *A. beccarii* (figure 19), the recolonizing plots are lower than in the control plot. In which subsequently the 5S samples are lower than 2S. Keep in mind that there is only one control sample measured.

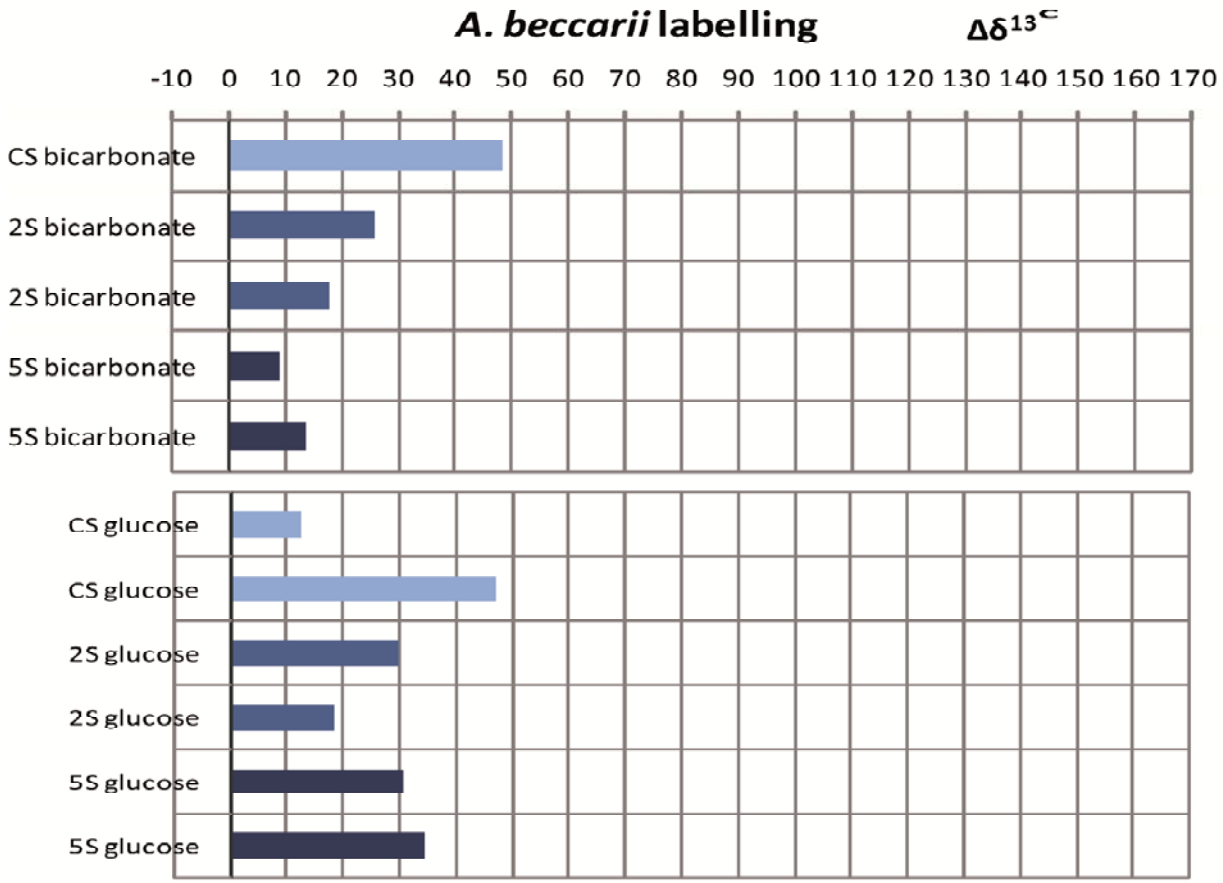

Figure 19: $\Delta\delta^{13}C$ uptake in the *A. beccarii* of the ^{13}C bicarbonate and glucose label in the 2S, 5S and CS plots. 2S = 2 months of def. and 2 months of rec., 5S = 2 months of def. and 5 months of ref., CS = no def. $\Delta\delta^{13}C$ uptake = $\delta^{13}C_{measured}$ - $\delta^{13}C_{bacbground}$.

The $\Delta\delta^{13}C$ glucose assimilation does have a high variable duplo control and therefore it is hard to conclude whether $\Delta\delta^{13}C$ glucose is higher or lower in the recolonisation plots than the control $\Delta\delta^{13}C$ measurements. If we mean the control samples, more or less the same uptake values are found for the incorporation in the recolonisation plot and control, with only a slightly smaller assimilation of ^{13}C glucose in the 2S plot.

The uptake and assimilation of ^{13}C bicarbonate by the *H. germanica* in the recolonisation plots is higher than control plot (figure 20), although one of the duplo $\Delta\delta^{13}C$ measurements of 5S is very low. This exception is hard to explain (see discussion: Implications in measurements of labeled ^{13}C in foraminifera) in terms of label uptake in contrast to the other 5S measurement.

H. germanica does have a higher ^{13}C glucose assimilation in contrast to the control when the duplicate samples of 2S and 5S are averaged. The $\Delta\delta^{13}C$ glucose assimilation in 2S and 5S are similar to the 5S assimilation of bicarbonate, which is highly variable and has one high and one low $\Delta\delta^{13}C$ incorporation.

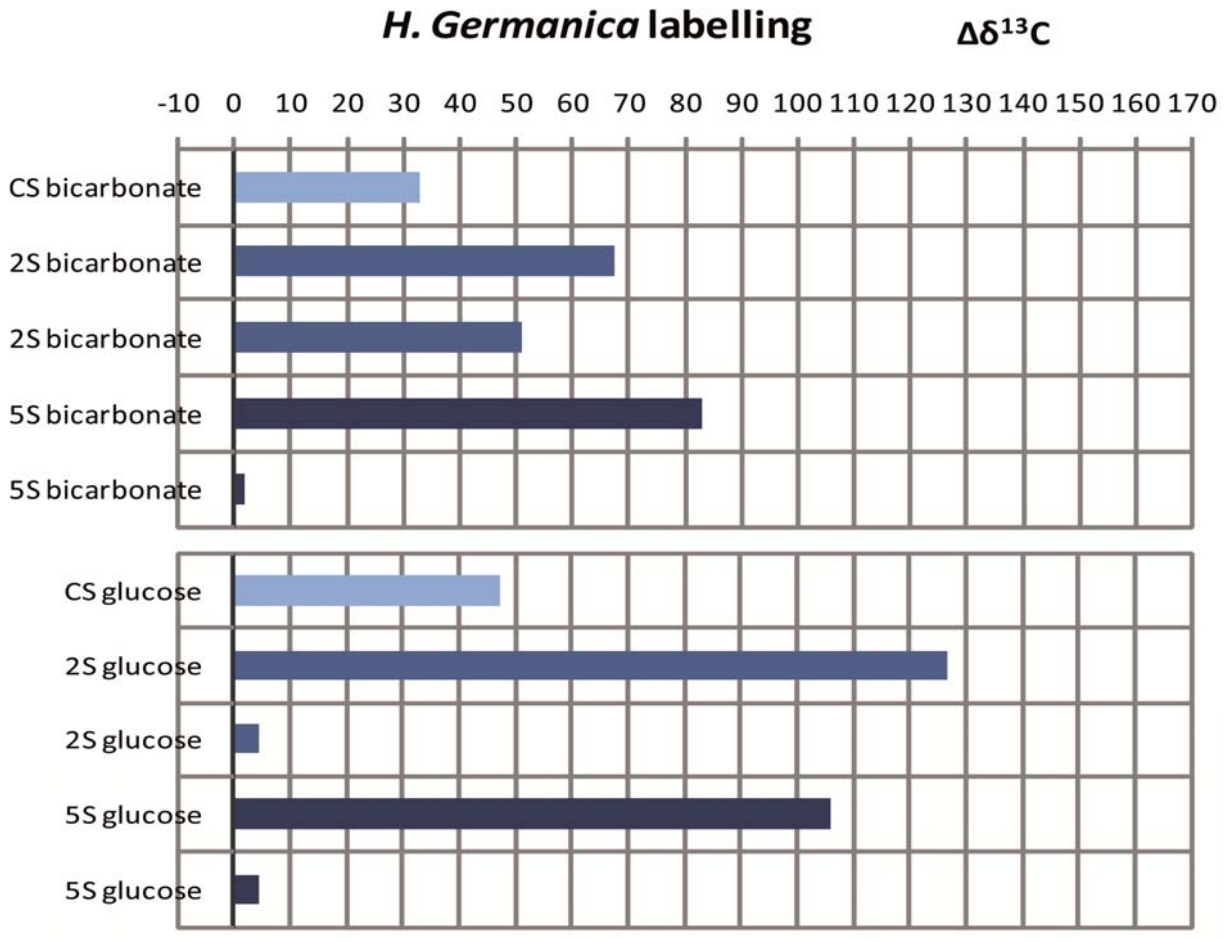

Figure 20: $\Delta\delta^{13}C$ uptake in the *H. germanica* of the ^{13}C bicarbonate and glucose label in the 2S, 5S and CS plots. 2S = 2 months of def. and 2 months of rec., 5S = 2 months of def. and 5 months of ref., CS = no def. $\Delta\delta^{13}C$ uptake = $\delta^{13}C_{measured}$ - $\delta^{13}C_{bacbground}$.

April and 2J

Uptake of the ^{13}C bicarbonate label in the *A. beccarii* is relatively high in the plots sampled in April directly after defaunation, while ^{13}C glucose assimilation is high after two months of refaunation in June (figure 21).The assimilation by the *H. germanica* is in both labels overall relatively high, however large fluctuations occur and therefore difficult to interpret (figure 23). The assimilation of ^{13}C bicarbonate by the *E. excavatum* is relative high in April and 2J, while ^{13}C glucose assimilation only occurs in April (figure 22)

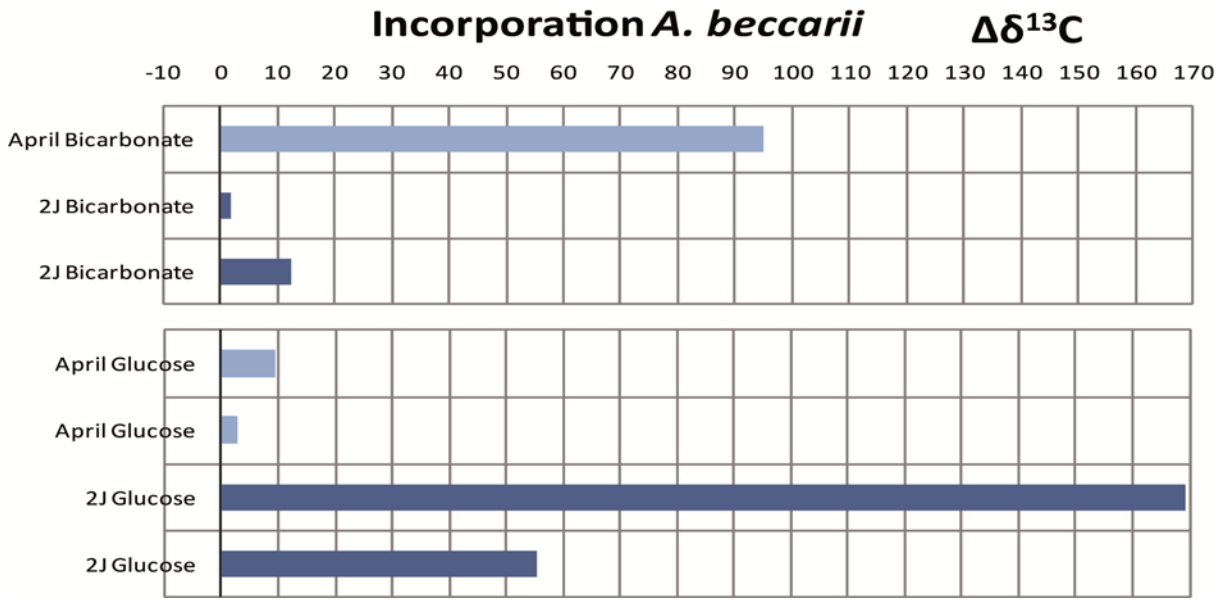

Figure 21: $\Delta\delta^{13}C$ uptake in the *A. beccarii* of the ^{13}C bicarbonate and glucose label in the April and 2J plots. April = 2 months of def. and no rec. , 2J = 2 months of def. and 2 months of ref. $\Delta\delta^{13}C$ uptake = $\delta^{13}C_{measured} - \delta^{13}C_{bacbground}$.

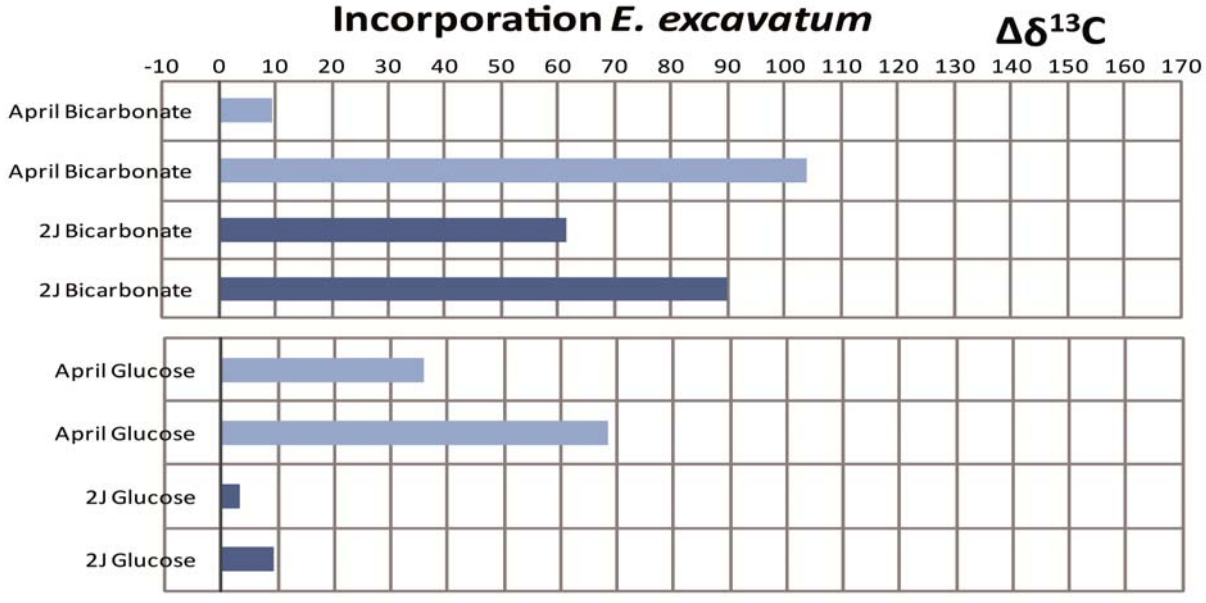

Figure 22: $\Delta\delta^{13}C$ uptake in the *E. excavatum* of the ^{13}C bicarbonate and glucose label in the April and 2J plots. April = 2 months of def. and no rec. , 2J = 2 months of def. and 2 months of ref. $\Delta\delta^{13}C$ uptake = $\delta^{13}C_{measured} - \delta^{13}C_{bacbground}$.

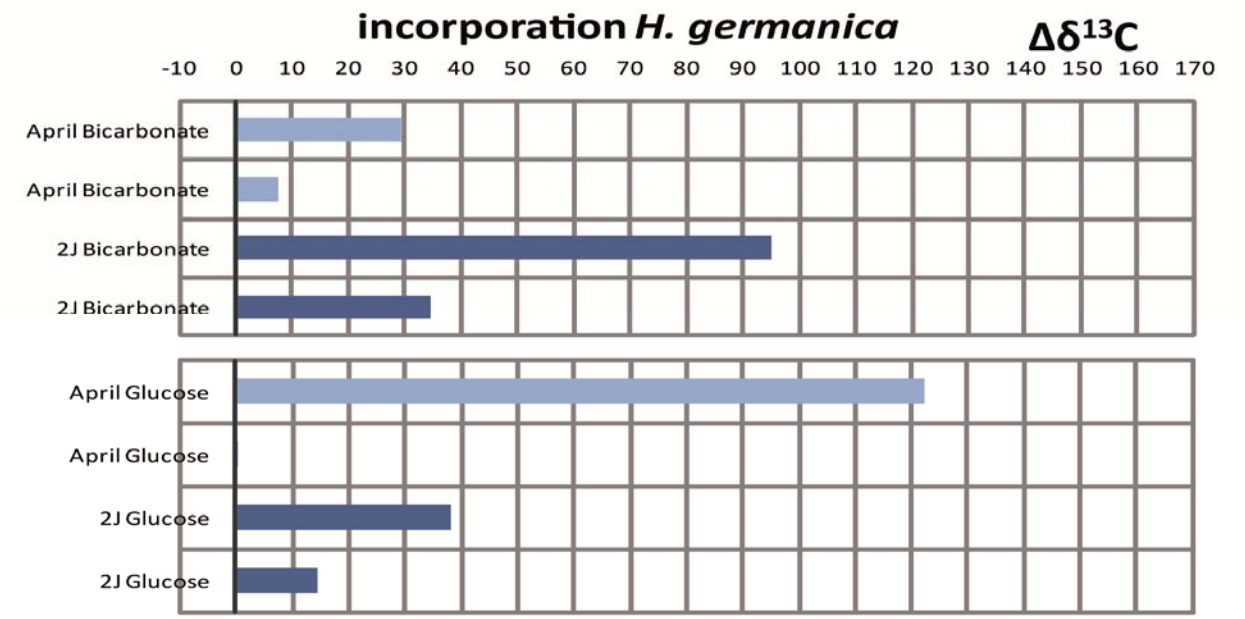

Figure 23: $\Delta\delta^{13}C$ uptake in the *H. germanica* of the ^{13}C bicarbonate and glucose label in the April and 2J plots. April = 2 months of def. and no rec. , 2J = 2 months of def. and 2 months of ref. $\Delta\delta^{13}C$ uptake = $\delta^{13}C_{measured} - \delta^{13}C_{bacbground}$.

Proportional label uptake in the foraminiferal species.

Most pronounced in the total proportional label uptake of bicarbonate and glucose is the around ten times higher uptake of ^{13}C glucose in all the test cases (fig. 24). Furthermore the relatively high incorporation of ^{13}C bicarbonate by the *H. germanica* and low incorporation in the *A. beccarii* in the recolonisation plots in contrast to the controls are interesting. The uptake of ^{13}C glucose in the recolonisation plots is relatively high for the *A. beccarii* in June, while it is more similar to the control plots in September. The *H. germanica* shows a lower uptake of ^{13}C glucose in June and a higher one in September relative to the control plots. Measurements of the ^{13}C label incorporation of bicarbonate and glucose in the *E. excavatum* are restricted to June, but are highest in the control plots.

Since the proportional uptake of labeled bicarbonate could be heavily underestimated (see: discussion for the assumptions and corrections of the recalculated data), quantities have to be interpreted as proportions in contrast to the glucose labelling.

Overall, the % of incorporation of the available ^{13}C label in foraminifera is comparable to the $\delta^{13}C$ measurements. Which is expected, since the $\Delta\delta^{13}C$ is the ratio between the measured $\delta^{13}C$ and the added $\delta^{13}C$. Thus the graphs show similar proportions only now in percentages to the total available label.

One should keep in mind that this uptake and incorporation of label is highly depended on the $\Delta\delta^{13}C$ incorporation of the microphytobenthos and the bacteria that are grazed by the foraminifera. For example, lower uptake of label in the control plots by the micro-organisms in contrast to the defaunated plots will lower the total $\delta^{13}C$ of the incorporation in the foraminiferal species, although grazing rates between control and recolonizing plots are similar and visa versa. Therefore, comparison with biomass and $\Delta\delta^{13}C$ of the micro-fauna is needed to circumvent this.

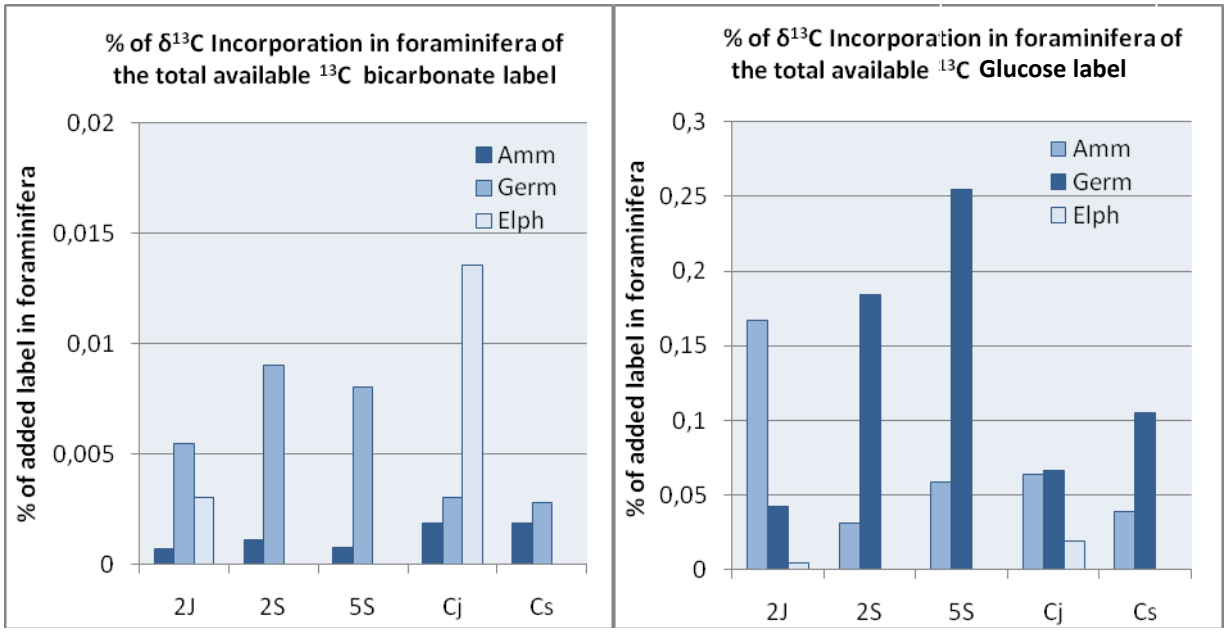

Figure 24: Percentage of the quantity of $\delta^{13}C$ that is incorporated in foraminifera to the total available ^{13}C bicarbonate and glucose that is added to the sediment. The added label is recalculated to 39.25 cm^2 of sediment and only the upper cm. The quantity $\delta^{13}C$ that is incorporated in the foraminifera is calculated by the formulas of Middelburg et al., 2000. 2J = 2 months of def. and 2 months of ref., 2S = 2 months of def. and 2 months of ref., 5S = 2 months of def. and 5 months of ref. CJ = no def., CS = no def. Note the different y-as scale.

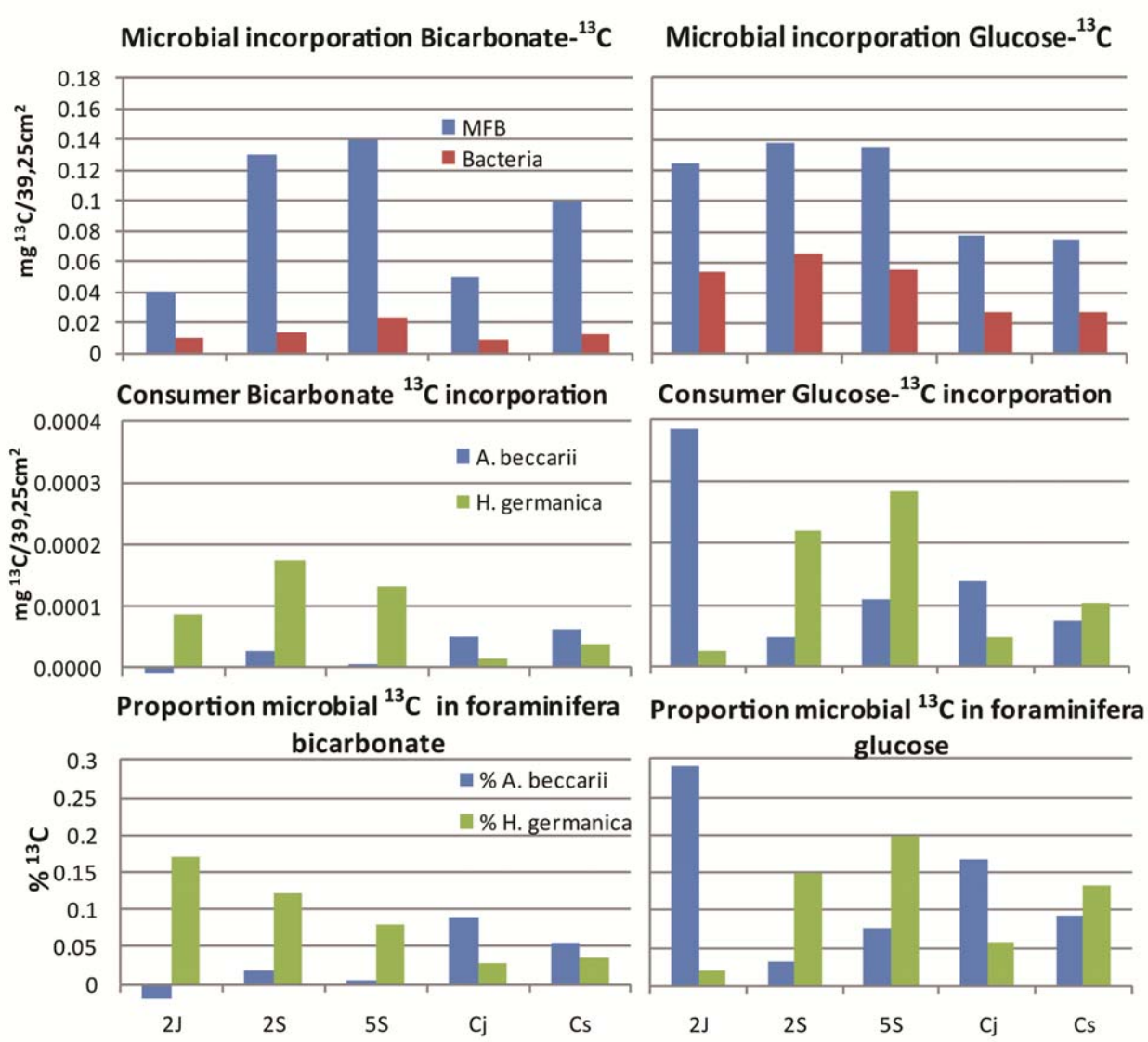

Figure 25: Upper two graphs show the quantity ^{13}C assimilation after 96 hour of incubation with ^{13}C bicarbonate and glucose in microphytobenthos and bacteria. The middle two graphs show the recalculated quantity ^{13}C assimilation after 96 hour of incubation with ^{13}C bicarbonate and glucose in the *A. beccarii* and *H. germanica*. The lower two graphs show the percentage of ^{13}C of the microphytobenthos and bacteria in the foraminifera. See text for calculations. 2J = 2 months of def. and 2 months of ref., 2S = 2 months of def. and 2 months of ref., 5S = 2 months of def. and 5 months of ref. CJ = no def., CS = no def.

The results of the calculations of the proportion of microbial ^{13}C in foraminifera are graphed in figure 25. The weight of foraminifera (consumer) ^{13}C label incorporation is divided by the corresponding quantity ^{13}C label uptake of the microbial fauna (food). The trend that is found between proportion to the total available ^{13}C label in foraminifera and proportions of incorporation of microbial label is similar, however small deviations are present. These deviations occur due to correction of the foraminiferal incorporations by the ^{13}C bicarbonate and glucose incorporation of the microbial fauna per plot.

SEM pictures of the three prominent foraminifera.

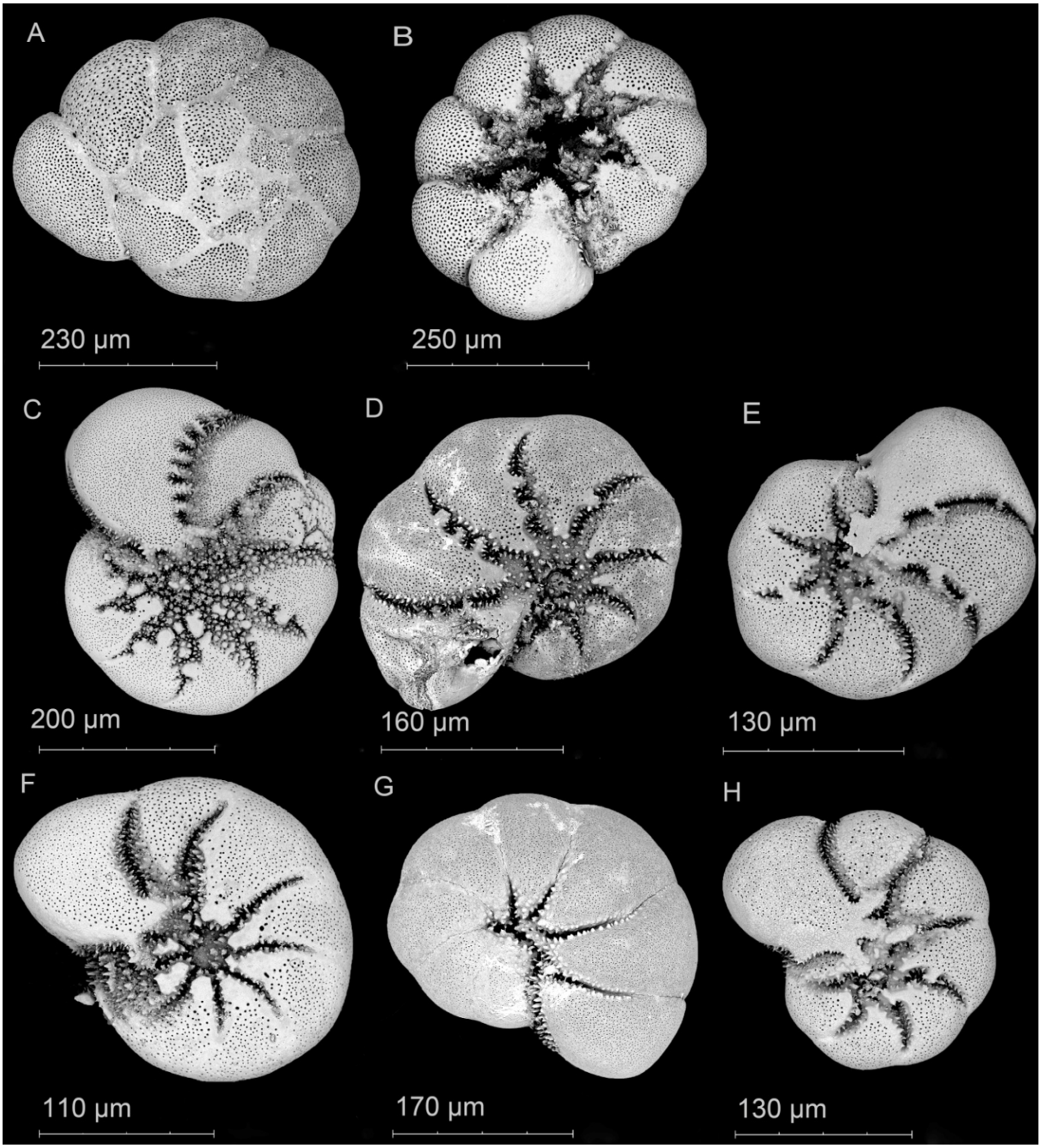

Figure 26: SEM pictures of the three prominent foraminifera. (A,B) Respectively, dorsal and ventral side of the *Ammonia beccarii.* (C,D,E) Ventral side of the *Elphidium Excavatum* (F,H) Ventral and (G) dorsal side of the *Haynesina germanica*.

Discussion

Foraminifera species and behavior during recolonisation

The three most prominent foraminiferal species are identified as the *Ammonia beccarii* (Alve *et al.*, 1994; Goldstein *et al.*, 1993), *Elphidium excavatum* and the *Haynesina germanica* (Austin, 2005)(fig. 26). See the foraminifera reference list for other pseudonyms and extra information. The foraminiferal species that is identified as *Ammonia beccarii* is presumably not correct and it probably a closely related one of the *A. beccarii (Moodley et al., 2000)*. However since related research in the Western Scheldt has identified it as the *A. beccarii* the same name is used to minimize confusion.

Besides the three prominent foraminifera species also the *Brizalina variabilis* and the *Elphidium willansoni* are found in the assemblage in April (Appendices 2). These two species are not found in June and September with a stained cytoplasm. Since April was sampled directly after two months of defaunation, when the plot is highly anoxic, organic degradation of organic material slowed down or completely stopped. Also the bacterial community is heavily disturbed (Rossi et al, 2009) and only anaerobic bacteria could survive this hypoxia. This slower degradation and grazing of organic material could be the reason that the found *Brizalina variabilis* and the *Elphidium willansoni* in April look alive, since the cytoplasm is still capable of taking up the Rose Bengal staining (Bernhard, J.M., 1988). In this case also the three prominent foraminifera in April could also be heavily overestimated (Berkeley *et al.*, 2008; Gooday *et al.*, 1990). However, the presence of *Brizalina variabilis* and the *Elphidium willansoni* in April could also be the result of normal seasonal reproduction behavior during this time of the year, as a response to a spring bloom for example. Since the *Elphidium excavatum* and the *Haynesina germanica* show much higher numbers in April, this reproduction behavior on the availability of food e.g. could also apply for them and will then a be seasonal signature rather than be caused by the defaunation. Since this cannot be excluded no conclusions can be made on the high numbers of these latter two foraminiferal species in April.

No living *Brizalina variabilis* and *Elphidium willansoni* are found In June and September, while empty shells of the two are present. Degradation or grazing by bacteria in the 2 or 5 months during refaunation might have been the reason for this. Hence also no living *Brizalina variabilis* and *Elphidium willansoni* are found in CJ and CS and the absence could also be explained by other ecological and/or seasonal fluctuations, including a very high patchiness of the species. Nevertheless the absence of living ones and presence of corresponding empty shells show that organic breakdown of the cytoplasm by organic degradation and/or bacterial scavenging in the two months of recolonisation occurs. Moreover during the picking of the samples of June and September, three distinct groups are present that could be easily visual separated. One group consists of empty shells, one group that is highly degraded and/or grazed by bacteria and one group in which the shells are completely filled with cytoplasm and intensely stained with the Rose Bengal. The shells that are empty are interpreted as foraminifera that died before the experiment. The degraded and/or grazed ones are interpreted as individuals that did not survive the hypoxia, while the fully filled and stained foraminifera are interpreted as living ones that have survived the anoxia. Therefore due to the distinct contrast between these three groups, overestimation in the samples of June and September in quantity of the foraminifera that are stained by Rose Bengal when dead is presumed to be of minor influence.

The quantities of *E. excavatum* en *H. germanica* in April are almost twice as high as the samples taken in June and September (figure 13). This could be an overestimation by the false positive Rose Bengal staining but also an increased reproduction in order to survive the anoxia. Although this is not the case for *A. beccarii*, this species could have kept the same quantities by going into a sort of dormancy and using another strategy to overcome the stress conditions. Conclusions cannot be made, since we do not have any control for the defaunated samples in April. The samples were taken originally as a starting point of no macro-faunal carbon flow(Rossi et al, 2009), so seasonally influences, ecological and overestimation by false positive staining cannot be tested in this setup.

Relatively the same number of the *A. Beccarii* in April, June and September and the controls (figure 13) indicate that many individuals did survive the hypoxia. Besides the large decline between April and June in individuals of the *E. excavatum* and *H germanica*, the overall defaunated samples have more individuals than the corresponding controls. If most of the specimens were killed by the hypoxia, release of gametes, zygotes, embryonic agamonts, gamonts and dispersal by adaptation to a meroplanktonic juvenile life stage is the most plausible mechanisms for introducing new individuals. It is not likely that the same or an even higher quantity of foraminifera is found in the defaunated sediments as a result of these introduction mechanisms of new individuals. Also the individual foraminiferal sizes measured in the defaunated plots are overall larger than the controls plots (see next paragraph), for both *A. beccarii* and *H. germanica,* but not in every sample moment.

The lower numbers of the *E. excavatum* in the defaunated plots (2J, 2S, and 5S) in contrast to the numbers in the control plots (CJ and CS) indicate that the hypoxia has a negative effect on its total abundances (figure 13). In CJ the numbers of *E. excavatum* are slightly higher than in 2J, but quantities in 2S and 5S show a large decline in contrast to CS. The low numbers in 2S and 5S could be related to seasonal or ecological limits of the species in the time before sampling(Gerlach *et al.*, 1985), instead of being killed by the hypoxia. Moreover (Nooijer, 2007) already stated that *E. excavatum* is more influenced by environmental variability than *H. germanica* and *A. beccarii* are. However the misidentification of *E. excavatum* and *H. germanica* could also have caused this large fluctuation in *E. excavatum* quantities between June and September. When the specimens are small, almost no differences is visible between these two. In a larger stage (over around 160 µm) the *E. Excavatum* start to show identifiable characteristics in terms of sutural bridges over the suture lines (figure 26). Since this is not visible and counted for in the samples of September that were identified first it could have created deviations in quantities in the smaller sized *E. excavatum*. The *H. germanica* is less influenced by this misidentification, since it does have much larger quantities in contrast to the *E. excavatum*. Furthermore, more care was taken during the identification of the smaller ones and deviations are presumably smaller.

Early recolonisation stages: 2J, 2S

The recolonisation stages 2J and 2S, which were sampled in June and September will help us to understand habitat build up over the year, while comparison to the controls taken in the same moths help to distinguish seasonal differences. Are structural difference in the habitat present and on what scale does it influences structural and functional habitat build up?

Size distributions

The shell sizes of the three prominent foraminifera (figure 14) in 2J and 2S show some remarkable differences. First, the mean sizes of *A. beccarii* in June and September are 40 – 50 µm larger in the defaunated plots than in the control plots. In case of the *H. germanica* this is only true for the samples taken in September (+30µm), while the *H. germanica* is smaller in 2J (-10µm). In terms of opportunistic/generalist strategy, larger sizes of individual species in general occur when habitat conditions are favorable and there is no specific need for reproduction in order to survive. Thus habitat conditions in June seems to promote the *A. beccarii* over the *H. germanica*, while September suites both. The *H. germanica* shows a smaller size distribution in 2J in contrast to CJ, which could be related to a start in frequently reproduction in order to survive the harsh conditions (hypoxia). However numbers of *H. germanica* are lower in 2J than CJ which is not expected. Since the sediment is sieved by a 63µm sieve many smaller sized *H. germanica* could have been lost during processing. Therefore conditions before sampling of 2J are not favorable for the *H. germanica,* but no conclusions can be made on its reproduction behavior and strategy of survival.

Secondly, the control samples CJ and CS are the same for *A. beccarii* as well as for *H. Germanica*, indicating that seasonal differences between June and September are not present in terms of size distributions.

Since size distribution between CJ and CS is similar, differences in size distribution in their corresponding defaunated plots 2J, 2S and 5S are related to the induced hypoxia. If the control size distribution (CJ and CS, figure 27 blue shaded graph) is seen as a stable condition in which new small foraminifera are introduced by reproduction and larger foraminifera die-off by natural occurring

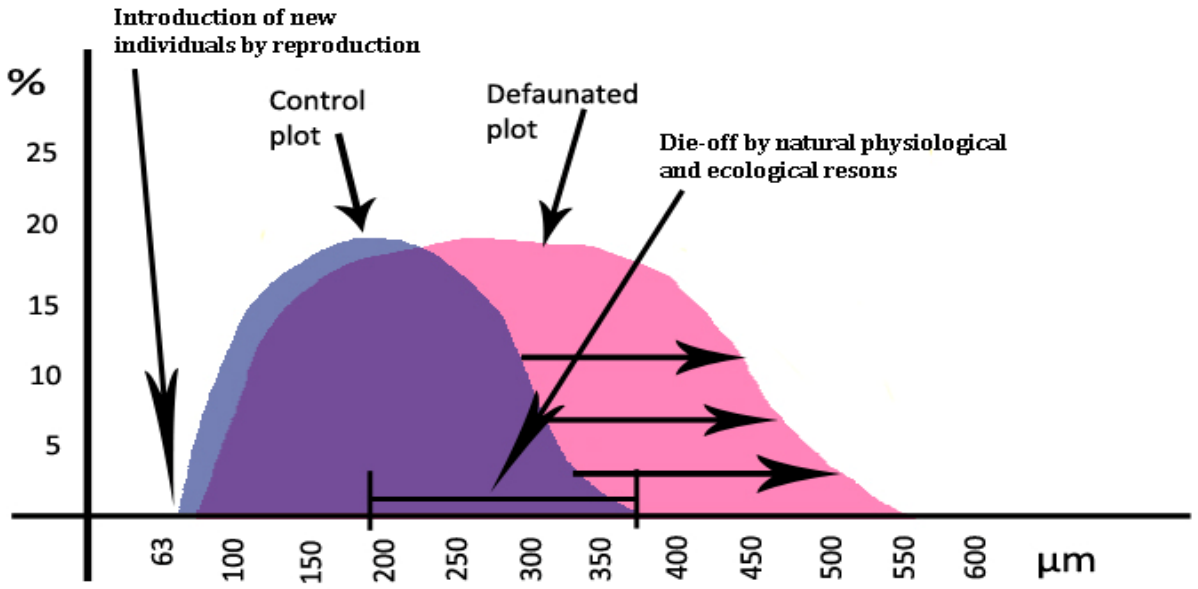

Figure 27: Schematic overview of an increase in a size distributions of the Foraminifera in percentages when normally present ecological and physical constrainments are limited.

physiological or ecological effects. Ecological and physical conditions during defaunation and recolonisation seem to reduce the die off and therefore mean size and the maximum size of the species can increase (figure 27, pink shaded graph). The increase in larger foraminifera and subsequent new smaller ones are in agreement with the higher number of individuals in both defaunation settings in contrast to the control. This decrease in die-off of the larger ones could be related to reduced grazing by macro-fauna and/or increased habitat size due to defaunation as well as an increase in food availability.

The numbers and sizes of *E. excavatum* are not that prominent as the other two and this species is almost absent in 2S in contrast to the 2J and the controls. Significant differences in mean sizes are absent. Since *E. excavatum* is present in September in such low numbers, presumably habitat conditions and/or present food is not suitable to sustain the species. Also the presence of the abundant and larger *A. beccarii* and *H. germanica* could have a suppressing impact on *E. excavatum* by competition.

Recolonization over time: 2S and 5S

Size distributions

The mean size of *A. beccarii* differs between CS and 2S. Moreover 5S seems to be an intermediate state between CS and 2S (figure 15), in terms of mean sizes and size distribution. The mean sizes of CS, 5S and 2S are 231µm, 251µm and 273µm respectively. Defaunation followed by a short period of recolonisation of the sediment seems to result in larger specimens. In contrast, the control plot shows the 'stable' situation and has the smallest individuals.

Since all macro-fauna has been killed by the defaunation and total abundances of macrofauna increases slowly again after reoxidation of the sediment, presumably grazing rates of the macro-fauna on organism of lower trophic levels is higher in 5S than in 2S. The macro-fauna as part of the carbon cycle is restored after 2-3 months after defaunation, but this is not the case for their total abundances (Rossi *et al.*, 2009). This die-off and slow recovery of macro-fauna biomass will subsequently also increase in habitat size and food abundances for the species that have survived the hypoxia, especially since bacteria and microphytobenthos are already present in quantities comparable to the control situation after two months of recolonizing. Limitation of food for the foraminifera is therefore unlikely in 2S. In the plot that has had more time for recolonisation (5S) this food is also eaten by the increasing macro-faunal abundances, which will subsequently also decrease the habitat size. In addition, grazing pressure on the foraminifera may increase with increasing macro-fauna numbers. Therefore the *A. beccarii* seems to use the defaunation as a profitable condition in which it could 'break-trough' the ecological boundaries that normally keep the sizes of the *A. beccarii* in a specific size range (figure 15).

The size of *H. germanica* does not significantly differ between 2S and 5S, but both are 25 -32µm larger than the control situation, (figure 15). The number of individuals of the *H. germanica* are highest in 2S (192±30 ind./3.14cm^3), intermidiate in 5S (155±16 ind./3.14cm^3) and lowest in CS (101±19 ind./3.14cm^3). This could be an artifact due to patchiness of the sediment, although the counts are measured in fourfold and a significantly large difference in quantities occurs. Therefore in terms of quantities it is presumably an effect of defaunation and subsequent recolonisation. Hypothetically the *H. germanica* could have reached a maximum in size and numbers in 2S, which could be species related or is restricted to recolonizing conditions of the plot. Nevertheless after this maximum in size and numbers in 2S, harsher conditions such as increased predation will

subsequently decrease the size distribution and numbers to the levels of 5S and CS. The 5S samples would then be a sort of intermediate step between 2S and CS in which the numbers of *H. germanica* decline by ecological boundaries, like macro-fauna predation and/or limitation of food. This intermediate character of 5S was also noticed in the sizes of the other prominent foraminifera *A. beccarii*. Boundaries that are present in the control situation, like habitat size, predation and the limitation of food do not longer restrict the *H. Germanica* to increase in size and in numbers after defaunation.

Care must be taken in defining the moment of most favorable conditions in which the *H. germanica* could increase in size and quantity. This moment could have been or exclusively restricted to the 2 months of defaunation. Nevertheless, the quantity of *H. germanica* in April is extremely high just after 2 months of defaunation, which might as well have been a seasonal effect. If the latter is true than the *H. germanica* does not endure favorable conditions on the moment 2S was sampled, but probably already declines in numbers, due to recolonisation of the grazers and a corresponding decrease in habitat size and food abundances. Depletion of oxygen would than directly or indirectly be a favorable condition in which *H. germanica* can flourish. This does not seem to be applicable for the *A. beccarii*, since the quantity in April do not significantly differ with the other sample moments in June and September (results, page 21).

Assimilation of ^{13}C bicarbonate and Glucose

Difficulties in ^{13}C label measurements of the foraminifera.

In the current experiment the sediment was made anoxic and the effects of hypoxia following up each other in a sequence of physical and ecological processes. For instance, re-oxygenation of the sediments will be followed by the recolonisation of the microphytobenthos and bacteria. Quantitatively the ecological and physical parameters are not completely restored after two months, while uptake in these plots is measured relative to a control that has a full potential of using the labeled carbon. Therefore high temporal resolution of the initial carbon concentration, microphytobenthos and bacteria is needed. Uptake of labeled ^{13}C bicarbonate and glucose by the autotrophs and bacteria is dependent on the concentrations of food just before labeling and the amount of added label. High concentrations of labile organic matter left over from the defaunation and die off of the original fauna, will have a diluting effect on the label assimilation in particular in the April, 2J and 2S data. As a result all defaunated labeled plots will have overall lower values of incorporated ^{13}C. However, also the opposite is likely, with new recolonizing sediments having high numbers of microphytobenthos and bacteria, much of the labile matter left over from defaunation could have been consumed. Then consumption of the added label will be equal or even more than the 'stable' plots. Also, direct uptake of ^{13}C glucose by the foraminifera cannot be ruled out, since this may be assimilated in the soft tissue of the foraminifera and is than measured together with the indirect ^{13}C glucose that comes via grazing. The presence of this direct grazing pathway of ^{13}C glucose by foraminifera is not tested yet, but is a process that is likely to happen. Therefore, it must be kept in mind while interpreting the data.

Another problem arises when look at differences in foraminifera measurements of ^{13}C labelling for the duplicate measurements which experienced the same treatment. For example the ^{13}C glucose measurements of *H. germanica* in 2S and 5S (figure 20): In both cases one measurement shows a high glucose uptake and the second one shows no label uptake at all. Since individual measurements are based on an average of 20 individuals per species and individual specimens could have odd incorporation of label, no conclusions can be made on the proportion of label in each individual foraminifera. Subsequently, incorporation of label in larger specimens will dilute in the already large amount of ^{12}C and normally present ^{13}C of the specimens in contrast to the smaller individuals. Measured ^{13}C label is an average of size and individual activity of the specimens in one sample. Since specimen are randomly selected from the bulk, this is expected to be on average a good representation of incorporation. Still, there are probably large differences in incorporation per foraminifera.

June versus September

Assimilation of ^{13}C bicarbonate and glucose in the individual foraminifera species shows large fluctuations in uptake in and between sample moments and therefore are difficult to interpret. Nevertheless, *A. beccarii* (figure 17 and 18, 2J and 2S) clearly shows a lower assimilation of labeled bicarbonate relative to the control. This is opposite in *H. germanica*, which has a higher assimilation of labeled bicarbonate in the defaunated plots during the 96 hours of labeling. Since bicarbonate is almost completely restricted to processing by autotrophic organisms, especially diatoms, we may conclude that that *H. germanica* prefers to graze on diatoms, or in minor quantities, the EPS excreted by them.

Grazing on microphytobenthos and subsequent assimilation of the ^{13}C bicarbonate label by the *A. beccarii* and *E. excavatum* is lower in the recolonisation plots than in the controls, while *H. germanica* has a high assimilation rate of the bicarbonate label in 2J and 2S. Competition for food between *A. beccarii*, *E. excavatum*, *H. germanica* and other fauna for eating the microphytobenthos is not likely, since there is a large amount of food present. Moreover the decline in numbers of *H. germanica* in 2J in contrast to CJ (fig. 13) is in contradiction and does not indicate that there was any competition for microphytobenthos that were labeled with bicarbonate. However, this could also be an artifact due to patchiness of the sediment.

Glucose is used by both bacteria and diatoms for respiration and assimilation,(Rossi *et al.*, 2009). Higher direct or indirect assimilation of the ^{13}C glucose by *A. beccarii* is observed in 2J than in 2S and 5S. Since the consumption of diatoms by *A. beccarii* is low in the bicarbonate label experiment and it is not likely that it has a preference for diatoms that specifically eat glucose, the higher assimilation of ^{13}C glucose is presumably due to the consumption of ^{13}C labeled bacteria.

Assimilation of the ^{13}C glucose label by *H. germanica* is high in 2S in contrast to the control and comparable to CJ in 2J (figure 17 and 18). Diatoms consume glucose as well as bicarbonate, although lower amounts. Presumably part of the uptake and assimilation of ^{13}C glucose by forams is related to grazing of diatoms as already stated above. Therefore high assimilation of labeled glucose in *H. germanica* in the 2J and 2S plots is probably related on grazing of microphytobenthos and minor amounts of bacteria. Keep in mind that this conclusion is based on descriptive interpretations of the ^{13}C label measurements, instead of being significantly proven.

The foraminifera seem to have a higher preference for one particular food source during reassembly of the habitat while this is not the case when the habitat is stable like in the control plots. In the reassembling plots ^{13}C bicarbonate assimilation by *A. beccarii* seems to be equal to, or lower than the *H. germanica* in contrast to control plots (figure 24). Therefore, the high assimilation rates of ^{13}C glucose in 2J by the *A. beccarii* in contrast to the very low assimilation of ^{13}C bicarbonate in all the reassembling plots seems to support the conclusion that the *A. beccarii* has a preference for grazing on bacteria (Austin, 2005; Langezaal *et al.*, 2005). Since the abundances of bacteria and diatoms is higher in the defaunated plots than in the control plot (Rossi *et al.*, 2009), it is unlikely to be an effect of limited food and subsequent competition for it. Therefore it is more likely that the foraminifera switch to a more specific uptake of one particular food source in times of reassembly. It may be concluded that specific grazing and assimilation of one particular food source by these two foraminiferal species is related to the high availability of the preferred food source during reassembly of the plots.

CJ, 2S and 5S

A. beccarii consumes quantitatively less microphytobenthos in the recolonisation plots (2S and 5S) than in the control plots (CS, figure 19). The opposite is observed for *H. germanica* which has a relatively high assimilation of bicarbonate label in the recolonization plots compared to the control (figure 20).

The incorporation of the ^{13}C glucose label by *A. beccarii* is slightly higher in 2S and 5S compared to CS. The ^{13}C glucose labeled organisms (and potentially the label itself) seems to be a relatively large source of the carbon used by *A. beccarii* for buildup of its soft tissue (figure 19), since bicarbonate assimilation in the defaunated plots of 2S and 5S are half the value of that in control. Assimilation of ^{13}C glucose by *H. germanica* is increased in the defaunated plots in compared to the control,

although this is variable between plots that endured the same conditions (figure 20). This label pattern corresponds to 2J and CJ in which the bicarbonate label assimilation was only high for *H. germanica* and assimilation of ^{13}C glucose was high for *A. beccarii* and *H. germanica* compared to the control. This result in CS, 2S and 5S correspond to our conclusion in June versus September. In which we concluded that in times of reassembly of the habitat and plenty availability of food, *A. beccarii* has a preference for bacteria and the *H. germanica* for microphytobenthos.

April and 2J

Assimilation of bicarbonate by the three prominent foraminiferal species (figure 21, 22, 23) in the samples of April implies the uptake of autotrophic carbon 9 days after removing the plastic seal, which is also found by Montserrat *et al.*, 2008. The ^{13}C glucose assimilation in April could also indicate the presence of bacteria, but presumably it is assimilated by the diatoms, since bacterial recovery takes longer (Montserrat *et al.*, 2008). Furthermore, the assimilation of ^{13}C bicarbonate by *A. beccarii* in April and ^{13}C glucose in 2J indicates that during recolonisation food uptake changes as a result of changes in the presence of and/or their preference for food. The absence of ^{13}C glucose assimilation by *A. beccarii* in April could be related to grazing on other autotrophs than diatoms, such as algae and cyanobacteria. However this cannot be concluded from our data alone. Overall the *H. germanica* and *E. excavatum* show a higher mean assimilation of ^{13}C bicarbonate and glucose in April compared to June, which indicates that they mainly graze on diatoms during the start of the recovery.

Proportion of total added label in the microbial fauna and foraminifera

The total uptake of labeled carbon by foraminifera is low compared to the total labeled carbon added, the role that foraminifera play in the total carbon cycle therefore is relatively small. Percentages up to 0.3% are found in the foraminifera in contrast to 40% of the macrofauna (Rossi *et al.*, 2009). Figures 24 and 25 show the proportion of total ^{13}C label that is incorporated in foraminifera and the assimilated label by microphytobenthos and the bacteria. The proportions of total label that is assimilated in foraminifera shows a ten times higher value for the glucose than the bicarbonate (fig. 24). This order of magnitude difference is due to the different biomass amounts of microphytobenthos and bacteria and can be corrected for (figure 25). Therefore initial label concentrations are chosen in good proportions and equally labels the fauna. After corrections for the ^{13}C label assimilation by microphytobenthos and bacteria small changes are present. Most pronounced is the 2J bicarbonate labelling of *H. germanica* which is much higher than 2S and 5S, since biomass of the microphytobenthos and the bacteria is lower June than in September (figure 25). Nevertheless, after correction of the uptake by the microphytobenthos and bacteria similar patterns remain in the assimilation of the labels compared to the uncorrected uptake patterns.

Conclusion

Size distributions

The ecological behavior of the foraminifera after an induced hypoxia differs from the benthic micro- and macro-fauna in the intertidal mudflat. In general, The foraminifera in this study survived the hypoxia and during or in the first months after the hypoxia they continue to reproduce and increase in size. In contrast, micro- and macro-fauna died off during the hypoxia and slowly increase in quantity over time by dispersal from the surrounding sediment. Therefore, the foraminifera are not context dependent in terms of recolonisation and the process to return to 'normal' conditions will differ. The foraminifera grew bigger than in 'normal' stable conditions when it is probably constrained by ecological interactions with the other fauna (in part grazing) and physical properties and the sediment. Whether the foraminifera in this relatively small defaunated area will completely return to their normal constrained size is not tested, since no samples were taken after 5 months of recolonisation (5S). However, the size distribution of *A. beccarii* and the *H. germanica* already ''return to normal'' in 5S in contrast to 2S. Furthermore the *H. germanica* also decreases in quantity. Therefore it is likely that due to recolonisation by other fauna and restoration of the physical conditions of the plot the foraminifera will return to their 'normal/control' size distribution.

A. beccarii and *H. germanica* show a corresponding increase in size in the defaunated plots of September, while in June only the *A. beccarii* significantly increased in size. Seasonal influences and related ecological and physical properties, like the presence of distinct food sources and water temperature could have constrained the *H. germanica* in June. Since the food preference of the *A. beccarii* is broad it feeds on both microphytobenthos and bacteria (Langezaal *et al.*, 2005), while the *H. germanica* prefers the microphytobenthos (Alve, E., et al. 1994 and this study). The much higher quantity of diatom biomass in September could have caused (part of) this differences. Nevertheless seasonal differences influence ecological and physical constraints of the size of the *H. germanica* during recolonisation.

The moment at which favorable conditions promote an increase in size of *A. beccarii* and *H. Germanica* could not be determined. This could be directly or indirectly related to the anoxic conditions or during habitat recovery after the plastic seal was removed. However, it seems that the *A. beccarii* kept a state of dormancy and continued to grow after the plastic seal was removed, since quantity does not highly fluctuate between April, June and September and the size distribution of *A. beccarii* in April corresponds to that in CJ. Contrastingly, *E. excavatum* and *H. germanica* have high quantities in April and already decline during sampling of 2J, 2S and 5S. This could reflect an opportunistic lifestyle in which these two started to frequently reproduce in order to survive. However, overestimation of total standing stock in samples of April could be present, due to 'false' staining of Rose Bengal and seasonal influences.

^{13}C food pulse

If there is ample food in the sediment, *A. beccarii* and *H. germanica* seem to selectively graze on their preferred food source. The *A. beccarii* overall consumes more ^{13}C glucose, which is related to grazing on bacteria, while *H. germanica* assimilates more ^{13}C bicarbonate and glucose than its control and this is related to grazing on diatoms. This behavior is caused by the higher availability of diatoms and bacteria after two months of recolonisation in contrast to the control plots. In the control plots diatoms and bacteria are limited, probably due to intenser top-bottom grazing rates by other fauna.

Model for population behavior of foraminifera in times of recovery

The increase in size distribution and numbers in the recovering plots in contrast to the control plots indicates that the foraminifera are positively correlated to the negative effects that the hypoxia has on the quantity of other meio- and macro-fauna. The quantity of food present in terms of diatoms and bacteria shortly after the reoxidation of the sediment is not limited, due to a decrease in top-bottom grazing of the other meio- and macro-fauna. This top-bottom grazing by macro-fauna is negatively related to the anoxia and is controlled by their dispersal rate after re-oxidation. Since the dispersal rate of the macro-fauna from the surrounding sediment is much lower than the recolonisation by the diatoms and bacteria, there is ample food, for foraminifera in the first months after defaunation. Moreover, if grazing of the foraminifera by the larger macro-fauna and habitat space would also limit the foraminifera in 'normal' stable conditions to grow larger, this would of course also be of influence in the months directly after defaunation. However the role of each of these factors constraining the size and numbers of foraminifera could not be determined quantitatively.

When recolonisation time increase and other meio- and macro-fauna are recovering, top-bottom grazing by macro-fauna on microbial fauna will increase. Therefore, the profitable conditions for foraminifera existing shortly after defaunation will disappear over time by the bottom-up control of food availability (microphytobenthos and bacteria). Therefore, constraining conditions in sizes and abundances of the foraminifera will eventually return to normal.

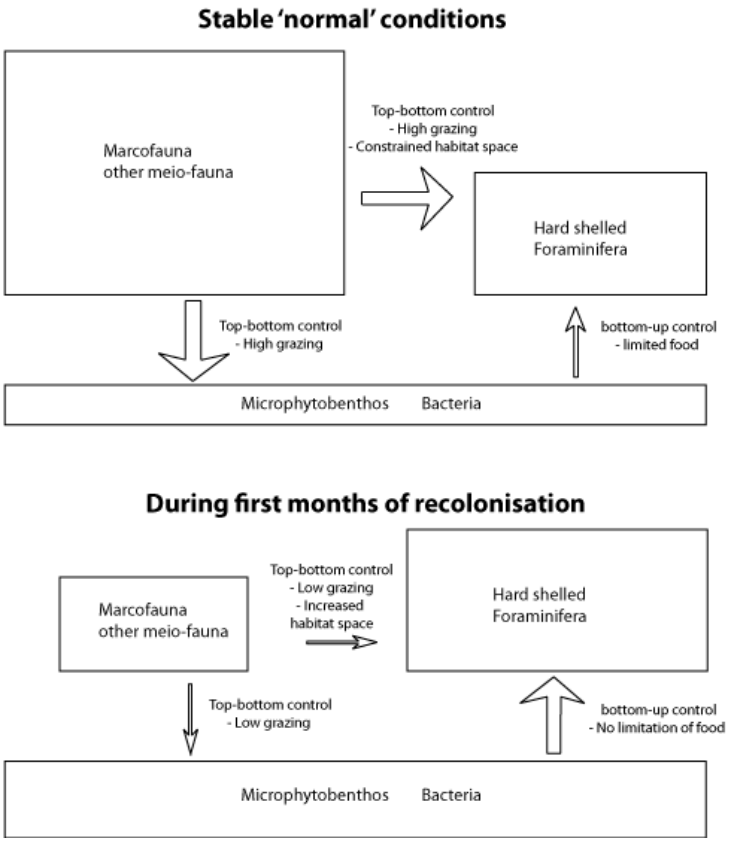

Figure 28: Schematic overview of the constraining parameters that control the size distribution of the foraminifera during the first months after defaunation and in stable 'normal' conditions. Size of the arrows represents the relative quantity of the parameter. Quantitative role of each parameter could not be determined.

There is a seasonal difference in the size distribution and labelling of *H. germanica* between June and September. The *H. germanica* is probably less influenced by ecological and physical constrainment during September. Nevertheless, this difference in recolonisation is probably temporal, due to the slow recovery of the larger macro-fauna. This will increase the constrainment on size distribution and decrease the grazing rate of *H. germanica* over the months/years. Presumably, this will eventually lead to the same functional end product as is present in the control situation. Since the surface of the perturbation is small and patches are surrounded by stable habitat.

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Ammonia beccarii (Linné) = *Nautilus beccarii* Linné, 1858

Bolivina Variabilis Williamson 1858

Elphidium excavatum (Terquem) = *Polystomela ecxavata* Terquem, 1875

Elphidium williamsoni Haynes, 1973

Haynesina germanica (Ehrenberg) = *Nonion germanicum* Ehrenberg, 1840

Appendices

Appendix 1: List of the samples in which the specific foraminifera species have been analyzed for their quantity, size and ^{13}C uptake of bicarbonate and glucose. In the second column B = bicarbonate, G = Glucose, D = Defaunated and C = Control. The first number of the two that follow represents the plot and the second is the patch in which the samples were taken.

	April	Def.	Labeled with:	Time of sampling	Sed. depth
1	B11	no	Bicarbonate	96 hours	0-1
2	B12	no	Bicarbonate	96 hours	0-1
3	G12	no	Glucose	96 hours	0-1
4	G22	no	Glucose	96 hours	0-1
	June				
5	B12	yes	Bicarbonate	96 hours	0-1
6	B22	yes	Bicarbonate	96 hours	0-1
7	G12	yes	Glucose	96 hours	0-1
8	G22	yes	Glucose	96 hours	0-1
9	C21 G	no	Glucose	96 hours	0-1
10	C22 G	no	Glucose	96 hours	0-1
11	C21 B	no	Bicarbonate	96 hours	0-1
12	C22 B	no	Bicarbonate	96 hours	0-1
	September				
13	DM B12	yes	Bicarbonate	96 hours	0-1
14	DM B22	yes	Bicarbonate	96 hours	0-1
15	DM G21	yes	Glucose	96 hours	0-1
16	DM G22	yes	Glucose	96 hours	0-1
17	DJ B12	yes	Bicarbonate	96 hours	0-1
18	DJ B22	yes	Bicarbonate	96 hours	0-1
19	DJ G12	yes	Glucose	96 hours	0-1
20	DJ G22	yes	Glucose	96 hours	0-1
21	CB 12	no	Bicarbonate	96 hours	0-1
22	CB 22	no	Bicarbonate	96 hours	0-1
23	CG 12	no	Glucose	96 hours	0-1
24	CG 11	no	Glucose	96 hours	0-1

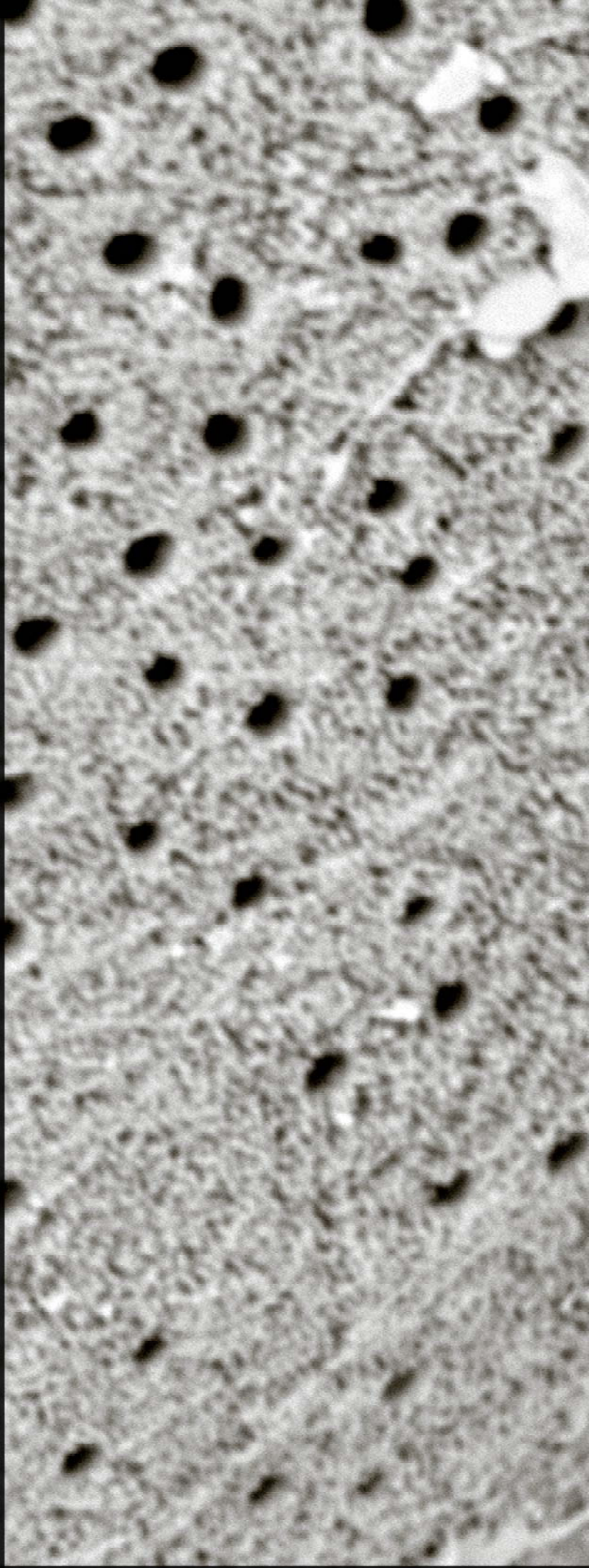
Appendix 2: Number of foraminifera individuals per species in the sample. First column show sample name (see appendix 1 for treatment). In the columns that follow the numbers of individual foraminifera per 39,25 cm^2 are given for the upper cm of sediment. For *Ammonia beccarii, Elphidium excavatum, Haynesina germanica, Elphidium willansoni* and Bolivina variabilis, respectively.

April	*A. beccarii*	*E. excavatum*	*H. germanica*	*E. willansoni*	*B. variabilis*
B12	45	48	321	1	
B11	14	52	192		1
G12	44	50	382	1	
G22	56	40	682		
Sum	159	190	1577		
June					
DB12	50	21	123		
DB22	31	16	133		
DG12	33	27	126		
DG22	28	12	84		
Sum	142	76	466		
C21 G	28	28	80		
C22 G	19	17	176		
C21 B	21	41	236		
C22 B	11	12	130		
Sum	79	99	622		
September					
DM B12	30	0	109		
DM B22	29	0	151		
DM G21	16	0	177		
DM G22	69	0	183		
Sum	144	0	620		
DJ B12	14	0	116		
DJ B22	21	0	207		
DJ G12	20	2	181		
DJ G22	48	6	264		
Sum	103	8	768		
CB 12	22	6	62		
CB 22	13	5	109		
CG 12	19	10	83		
CG 11	15	1	79		
CG21	41	20	172		
Sum	110	58	505		

In order to predict the anthropogenic effects expected for benthic fauna on intertidal mudflats, a large in situ defaunation/refaunation experiment has been conducted at the NIOO (Yerseke, 2005). Species identities, diversity and 13C labeling data were used to identify the microbial carbon flow to higher trophic levels (macrofauna) during the habitat recovery.

For my Msc Thesis, I've used the foraminifera samples that were collected in this study and studied the Foraminifera as an intermediate step between microbial carbon flow up to higher trophic levels. Foraminifera could be a major constituent of the carbon flow in times of recovery due to their prolonged anoxia resistance and the absence of other meio- and macrofauna.

Combining my data of the foraminifera with the extensive knowledge of the habitat recovery. Using species identities, quantities, sizes and the 13C food pulse experiment, the role of foraminifera species is determined during recovery of the carbon cycle. In combination with the biogeochemical properties of the sediment and the microphytoplankton and bacteria dynamics, the ecological interactions of foraminifera after times of stress is unraveled.



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