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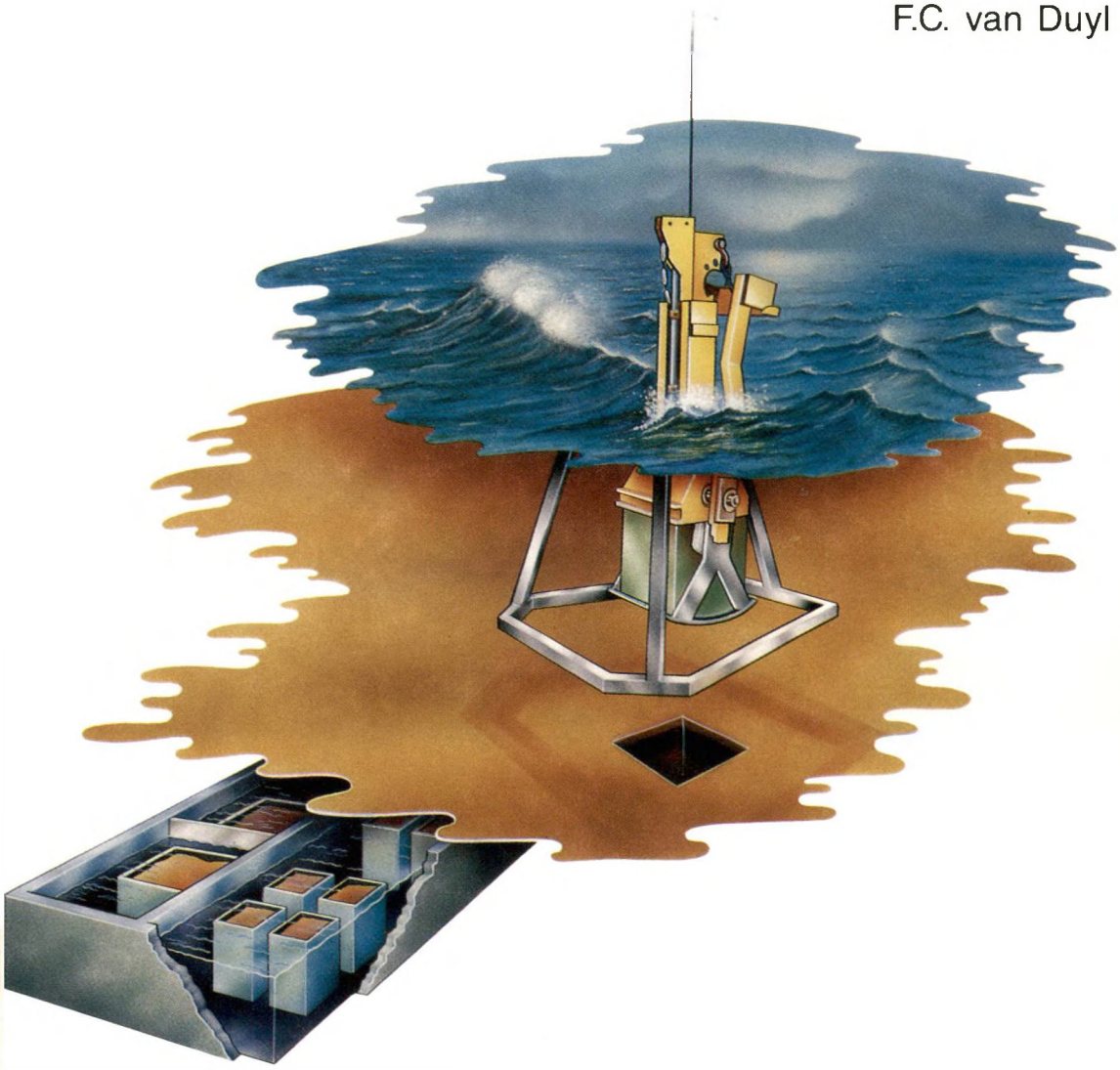

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1. INTRODUCTION

Chronical eutrophication is commonly indicated as a severe threat for the coastal areas of the North Sea. The need for detailed insight into the effects of eutrophication has been recognized, but notwithstanding major developments such as the enhancement of the nutrient load of the river discharges, the concurrent increase in primary productivity in the coastal zone and the spectacular doubling of the macrobenthic biomass of the Wadden Sea during the last decades, it is only possible to speculate on the kind of effects which chronical eutrophication exerts on ecosystem level and particularly how coastal ecosystems will develop in the future.

Ecosystems comprise extremely complex fabrics of living organisms and ambient factors, with numerous intra- and interspecific relationships between organisms. The main problem is that data obtained in the laboratory or on single species may and cannot be extrapolated to field situations or to the level of complete ecosystems, whereas field data are insufficient to obtain cause-effect relationships. Therefore, attempts have been made to enclose parts of natural systems for research, to transfer natural ecosystem sections to the laboratory or to compose experimental ecosystems - commonly referred to as mesocosms - from the basic components, water sediment and a variety of organisms, and to maintain them at *in situ* conditions.

Due to eutrophication the algal biomass in the coastal zones of the North Sea increased, enlarging the amount of organic matter sinking to the bottom. This enhanced the activity of the benthos, resulting in an increased oxygen demand of the sediment. The major adverse effect of this is possible oxygen deficiency, causing mass mortality of benthic organisms. Anoxic conditions occur locally, have so far been restricted and are unpredictable with respect to location and time and the time span over which such conditions prevail. This implies that oxygen deficiency is difficult to localize in the field. Studies on the circumstances and processes which lead to anoxic conditions have to be conducted under reasonably controlled conditions which can be provided in mesocosm systems. On the other hand changing inputs of organic matter into the sediment may lead to changing benthic biomasses and productivity. But which organisms will benefit most from the enrichment and how will the fauna composition react on changes in eutrophication level?

The Netherlands Institute for Sea Research (NIOZ) was among the first to recognize the large potential of mesocosms for marine research. In 1975 two large indoor mesocosms mimicking intertidal mudflat ecosystems were constructed. The usefulness of the concept was demonstrated by the apparent system reality expressed in the various structural and functional aspects of mudflat ecosystems. The expertise obtained was used in the so-called MOTIFs (MOdel TIdal Flats) in which TNO, RIN, NIOZ and DGW successfully investigated the effects of oil contamination and harbour dredgings on the Wadden Sea ecosystem. Meanwhile, NIOZ transformed the existing intertidal mesocosms into subtidal soft-bottom mesocosms with the aim to study the effects of eutrophication on North Sea sediments.

In 1989, the applicability of the North Sea mesocosms for eutrophication experiments was investigated in a cooperative pilot study carried out by the NIOZ departments 'Benthic Systems' and 'Applied Scientific Research' (BEWON). Benthic processes in starved mesocosms and in mesocosms enriched with organic matter were followed for 1 year and compared with those in the field. Comparisons rendered sufficient insight to assess:

1. the potential of mesocosms to maintain soft North Sea bottoms in a 'natural' condition over time
2. the nature of the deviation from this condition over time
3. whether the organic matter input condition can be manipulated in such a way that responses in benthic processes may be generated which also seem to occur in the field.

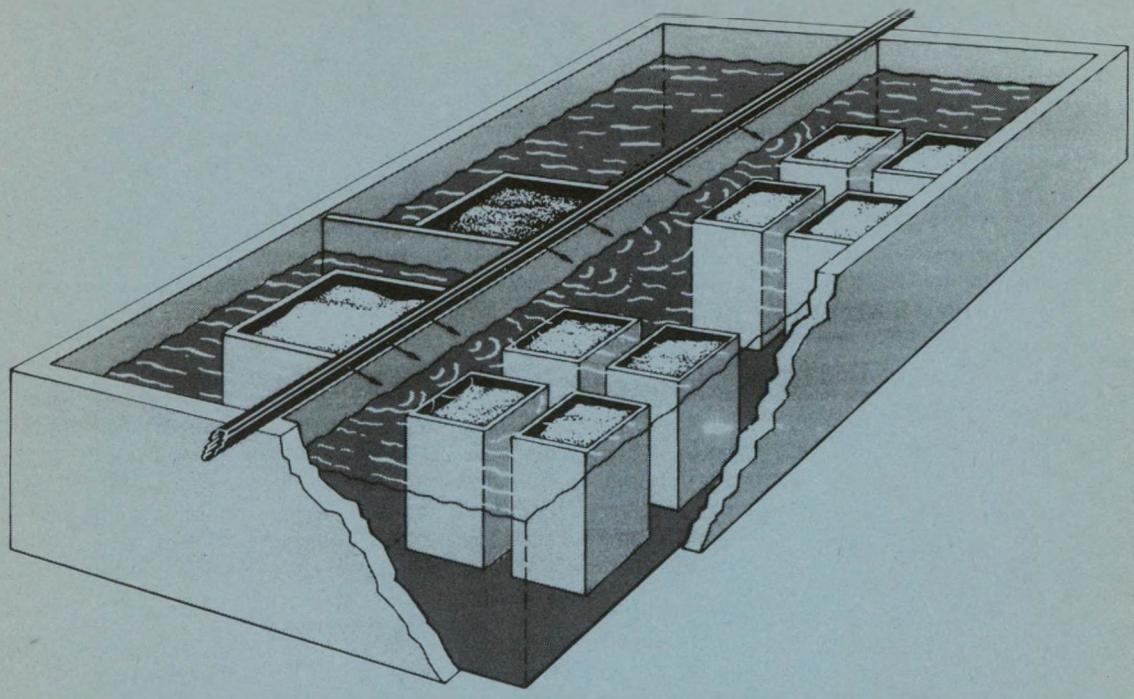
2. NORTH SEA MESOCOSMS

2.1. DESIGN

Essentially the NIOZ North Sea mesocosm facilities consist of 2 indoor concrete basins, 10.0 x 2.5 x 1.0 m each (Fig. 1), lined with fibre glass and installed within thermo-insulated rooms (Fig. 1). The basins are filled with sea water of selected quality, thermostatically controlled by means of graphite heat exchangers and kept in the dark. For the watersupply to the mesocosms there are two subterranean 60 m^3 reservoirs in which sea water collected by ship in the open sea or by pipeline from the Marsdiep can be stored. Before storage the sea water is filtered over sand filters (grain size 1-1.4 mm) removing the suspended matter. Membrane-driven pumps provide a flow-through of water from the storage to the mesocosms at a rate of ca 2 $m^3.h^{-1}$. Inflow in the systems occurs by a perforated pipe at one side of the basin at water level height; outflow by a skimming gutter at the opposite side. Then it flows back to the storage tank. Intact sediment cores are hoisted into the basins through the removable ceilings of the thermo-insulated rooms and accommodated in the basins.

2.2. EXPERIMENTAL SET-UP

In January 1989 2 bottom areas of 3 m^2 each were installed in one basin, one originating from sandy



Mesocosm set-up showing the two seawater filled basins housing submerged sediment sections in containers. Each water basin has its own watercirculation system. The inflow system in the right basin is shown. Basins are separated from each other by thermo-insultated walls (not shown).

sediments from the southern North Sea (Broad Fourteens), and one from muddy sediment from the Frisian Front. The uninterrupted sandy and muddy sediment sections were composed from 12 single 0.25 m^2 box cores carefully collected in the respective areas. In April separate sections of 0.25 m^2, both sandy and muddy sediment, were placed in the second basin housed in equally sized P.V.C. containers(Fig. 1). This group of mesocosms was supplied with about 23 g $C.m^{-2}$ in May-June, dosed as unthawed *Phaeocystis* cells, whereas the large bottom areas remained starved. It was decided to use a *Phaeocystis*-dominated algal suspension as the organic matter supply because this can be collected in considerable amounts and is a natural component in the coastal marine system. During the *Phaeocystis* bloom in April-May the material was collected with 50 μm plankton nets in the Marsdiep. The *Phaeocystis* colonies were scraped from the nets, deep frozen, subsequently thawed and divided into equal portions, which were administered to the bottoms over a period of 2 weeks to diminish the chances of oxygen depletion in the bottom water. During the experiment temperature in the mesocosms was tuned weekly according to existing temperature curves of the respective field stations, Broad Fourteens and Frisian Front, which were measured in previous years. For 1 year major chemical and biological system variables were followed in the sediment and the sediment-water interphase to study the development in starved and organic matter-supplied mesocosms. Emphasis in the study was on the benthic system and the response of the benthic system to organic matter supply. In conjunction with the mesocosm measurements, field observations were made in the North Sea every 1–2 months.

3. RESULTS

Results from mesocosm and field studies showed large similarities in development during the first few months of the experiment. In spring due to the supply of organic food, either derived from the algal spring bloom in the field situation or from artificial dosage of dead *Phaeocystis* material in the mesocosms, significant responses in community respiration, bacterial and protozoan production could be observed. Distinct effects in the starved systems were absent, despite the rise in temperature in these mesocosms. Notwithstanding observed differences between the sandy and muddy bottoms and between natural and experimental systems, the dominant role of organic matter deposition in the control of bottom ecosystems in the North Sea seemed evident. This implies that the systems are in potential suitable for eutrophication studies of North Sea bottoms just by

subjecting the mesocosm bottoms in the present set-up to increasing deposition of organic matter and assess the effects. Net deposition of organic matter at both the Frisian Front and the Broad Fourteens took place in spring (after the phytoplankton spring bloom) and in summer 1989. Particularly the sedimentation in early summer enhanced the benthic system. The magnitude of the enhancement was reasonably reflected by the organic matter supply to mesocosms with respect to benthic oxygen consumption, bacterial production and protozoan abundance. Compilation of the results of the 1989 mesocosm experiment clearly showed that biological variables in North Sea bottoms can be approached in mesocosms. The benthos can be manipulated with organic matter in a realistic way because the benthic ecosystem appears food limited. Organic matter supplies in the form of thawed *Phaeocystis* induce responses of the benthos comparable to those found in the field. Variations in inorganic nutrient fluxes appeared to be much more complicated to mimic in mesocosms. This is to be attributed to the smaller time scale on which chemical processes take place, to the higher sensitivity of such processes with respect to oxygen penetration, to high dependency on variations in apparent diffusion and to the supply conditions in the mesocosms. In comparison, most benthic organisms appear to cope with varying inorganic N, P and O_2 concentrations and fluxes without changing their productivity. The potential of mesocosms for chemical work lies not in mimicking the field situation but in analysing specific processes in a well-defined system such as the degradability of the *Phaeocystis*. The material appeared to consist for approximately 45% of refractory material and for 55% of material with decay times of approximately 1 month. Within 2 months after feeding, bacterial production and benthic oxygen consumption returned to values found in the starved mesocosms. Eutrophication with *Phaeocystis* suppressed nitrification and by this total denitrification.

Mesocosms prove to be a powerful tool to test the effects of different loads and degradability of organic matter on the oxygen consumption and biological response of different groups of benthic organisms. In future eutrophication research this needs to be further exploited.

4. CONCLUSIONS

1. In mesocosms North Sea bottom conditions can be simulated and manipulated, which make these systems suitable to assess the effects of eutrophication.

2. North Sea bottoms can be maintained in mesocosms for 1 year without losing their specific biological characteristics.

3. The present mesocosm set up is adequate to mimic activity and growth of zoobenthos.

4. To mimic *in situ* nutrient dynamics in mesocosms a better defined mesocosm set-up is required. The present set-up is adequate to study sediment-water exchange processes *per se*.

5. Input of degradable organic matter in mesocosms is required to generate and sustain activities in biological variables comparable to activities in the field.

6. 32–53% of the organic matter supply to mesocosms (23 $gC.m^{-2}$ equivalent to the estimated amount settling annually on sandy North Sea bottoms) was not recovered. The remainder (11–16 $gC.m^{-2}$) was insufficient to cause oxygen deficiency in sandy and silty North Sea bottoms and to keep the benthos active at field levels.

7. Supply of dead *Phaeocystis* material in mesocosms induces a direct response of the microzoobenthos.

8. Benthic response to the input of *Phaeocystis* extinguishes within 2 months and values of enhanced variables (*e.g.* benthic respiration, bacterial production) drop to initial levels.

9. The addition of *Phaeocystis* to the mesocosms suppressed benthic nitrification and denitrification.

10. In the 'fed' mesocosms pore water concentrations of ammonium and nitrate increased during the course of the experiments due to diminishing apparent diffusion (decreasing bioirrigation).

11. Burrow construction by *Callianassa* in silty sediments increases the sediment water interphase by at least 50%, enhancing sediment-water exchange.

12. Heterotrophic bacteria appear to be food limited in North Sea sediments.

13. Deposition of organic material at both the Frisian Front and the Broad Fourteens took place in spring (*i.e.* a few weeks after the phytoplankton spring bloom) and in summer 1989.

14. The organic matter supply to the bottom in June appears the major event generating the seasonal enhancement in biological variables *in situ*. At the Frisian Front more organic material settles on the bottom than at the Broad Fourteens.

SHORTCOMINGS MESOCOSM SET-UP

1. The variability in macrofaunal composition and biomass between replicates of intact bottom cores of 0.25 m^2 collected *in situ* is too large to assess (subtle) changes in mortality and growth.

2. The meiofauna standing stock declined in the present mesocosm set-up and could not be manipulated by dead *Phaeocystis* organic matter supply.

3. The benthic mesocosm set-up is inadequate to allow frequent sediment sampling without disturbing the bottom. In carbon and oxygen budget studies, short time-scale sampling is crucial to account for the rapid and intense initial response of the microzoobenthos and the oxygen consumption to an organic matter supply.

5. FUTURE RESEARCH

In future research the emphasis will be placed on quantification of the oxygen consumption and the carbon and nitrogen cycling in response to different loads of organic matter. The role of different groups of benthic organisms need to be assessed in order to be able to predict these processes.

Oxygen consumption
During biological degradation of organic matter, oxygen is consumed as long as it is available. The surface layer of North Sea sediments is usually oxygenated, because the flux of O_2 into the bottom exceeds the consumption at the sediment surface. By increasing the organic matter supply, the oxygen demand of the benthos will increase due to enhanced activity. As soon as the O_2 demand of the sediment exceeds the flux rate into the sediment, oxygen concentrations will drop and in due time the bottom becomes anoxic. When anoxic conditions prevail and the bottom water is depleted of oxygen as well, benthic organisms will suffocate and die. Particulary, macrofauna and meiofauna are sensitive to low oxygen concentrations. All bioturbation activities will stop as well as the related active aeration of the sediment through burrows.

The central theme of the boxcosm research will be the carrying capacity of North Sea bottoms with respect to loading of organic matter in the form of dead *Phaeocystis*. How much *Phaeocystis* material can be processed by the benthos without causing suffocation phenomena of the larger benthic organisms? And is the total amount of algal matter being processed related to the dosage, pulsed or continuous? The major consumers of O_2 and organic matter are bacteria. They will respond immediately to a *Phaeocystis* supply and dictate the oxygen consumption, which can result in anoxic conditions. The influence of macrofauna and related bioturbation on the bacterial production and nanoflagellate activity needs to be assessed.

Carbon and nitrogen cycling
Mineralization of organic matter and regeneration of carbon and nitrogen are essential functions of the benthos. Degradation of *Phaeocystis* material and nutrient cycling in the presence and absence of macrofauna will be studied. Frequent sediment sampling after *Phaeocystis* addition must enhance our insight into the labile fraction of this algal matter and its availability to organisms in deeper sediment layers. Besides biological processes, chemical processes (such as sorption) determine the recycling potential of the sediment and play a decisive role in exchange of nutrients between sediment and water. Sediments can act as suppliers or sinks of nutrients depending upon the environmental conditions. To get a better understanding of the time lag between uptake and mineralization, nitrification and denitrification, sediment-water fluxes need to be measured under different loads of *Phaeocystis* material.

Experimental set-up
For the experimental set-up the variability between bottom cores need to be reduced as much as possible. Therefore, manipulated North Sea sediments (oxygenated and sieved before use) will be put into 30 cm diameter boxcosms pre-incubated at fixed temperatures in running sea water. These sediments need to be faunated with different species of macrofauna in natural densities and with fresh surface sediment slurries to introduce a natural meiomicrofauna into the sediments. By excluding the macrofauna in half of the experimental containers we intend to analyse the role of macrofauna in oxygen and nutrient cycling and assess the role of the microfauna. The effects of eutrophication by pulsed or continuous organic matter supplies (*Phaeocystis* spec.) will be studied by monitoring changes in oxygen conditions, benthic respiration, bacterial and protozoan standing stock and production, growth of macrofauna, meiofauna, and the influence of bioturbation in the sediments in the boxcosms. The carbon and nitrogen cycling will be monitored by measuring flux rates and pore water concentrations. The particulate organic matter will also be measured. The response to a single organic matter pulse is compared with a situation in which organic matter is continuously supplied.

This project will be carried out in 1990 in cooperation between the NIOZ departments 'Benthic Systems' (NIOZ) and 'Applied Scientific Research' (BEWON), DIHO Yerseke and TNO Den Helder.

APPENDIX I

Nitrogen cycling in two sediments of the southern North Sea (Frisian Front and the Broad Fourteens): Field data, mesocosm results and mathematical modelling

(W. van Raaphorst, H.T. Kloosterhuis, E.M. Berghuis, A.J.M. Gieles, J.F.P. Malschaert & G.J. van Noort)

I. NITROGEN CYCLING IN TWO SEDIMENTS OF THE SOUTHERN NORTH SEA (FRISIAN FRONT AND THE BROAD FOURTEENS): FIELD DATA, MESOCOSM RESULTS AND MATHEMATICAL MODELLING*

Wim van Raaphorst, Henderikus T. Kloosterhuis, Eilke M. Berghuis, Adriana J.M. Gieles, Johannes F.P. Malschaert & Govert J. van Noort

* Publication no. 27 of the project Applied Scientific Research Netherlands Institute for Sea Research (BEWON)

ABSTRACT

Benthic nitrogen cycling was examined in a sandy station (Broad Fourteens, B14) and a silty station (Frisian Front, FF) in the southern North Sea. Both stations are shallow (B14: 29 m, FF: 39 m) and had a vertically well mixed watercolumn throughout the period of investigation (January 1989 - April 1990). Organic C and N contents were low, at B14 ca. 0.6 mg C and 0.12 mg N, and at FF ca. 5 mg C and 0.5 mg N per g of dry sediment. At FF a distinct maximum in org. C, N contents was observed at 3-5 cm depth, probably due to sediment reworking by Amphiura filiformis. Annually averaged benthic O_2 consumption and release rates of NH_4^+ and NO_3^-+NO_2^- (NO_x) were ca. 8, 0.2 and -0.03 mmol.m^{-2}.d^{-1} respectively at B14, and ca. 27, 1.0 and 0.14 mmol.m^{-2}.d^{-1} respectively at FF. Both O_2 respiration and NH_4^+ release were maximal during summer (B14: June, FF: June-August), rates being 20 and 0.5 mmol.m^{-2}.d^{-1} respectively at B14, and 40 and 1.7 mmol.m^{-2}.d^{-1} respectively at FF. At B14, NO_x fluxes were directed into the sediment during the first half of 1989 while an efflux was measured from August onwards. At FF an efflux of NO_x was observed throughout the year, however at very low rates during the period January-June. Annually averaged denitrification rates were 0.12 mmol.m^{-2}.d^{-1} at B14 and 0.08 mmol.m^{-2}.d^{-1} at FF, with high rates in April and June and virtual elimination in August and November. Concentrations of NH_4^+ in the upper 120 mm interstitial water were always higher at the silty FF as compared to the sandy B14, maximum values being 250 µM at FF and 75 µM at B14 during August. Pore water data of NO_x showed the occurrence of benthic nitrification mainly from fall to spring, during summer no enhanced NO_x was observed in the oxidized zone of the sediments. On an annual base ca. 50% at B14 and almost all ammonium at FF being produced due to benthic mineralization was regenerated to the watercolumn, and consequently N removal at the silty FF was lower than at the sandy B14. It is argued that this is probably caused by lower O_2 availability due to higer mineralization rates at FF and from this, relatively lower nitrification rates as compared to the organic-poor B14. Annual nitrification was calculated

at ca. 0.1 and 0.2 $mmol.m^{-2}.d^{-1}$ at B14 and FF respectively, supplying > 75% of total denitrifiers demands. From this it is concluded that increased levels of deposition and consumption of organic matter may lead to reduction of benthic N removal. To check for this, the effect of a single addition of Phaeocystis material to the sediments of B14 and FF was studied in mesocosms. Directly after addition of this organic substrate respiration strongly increased, but after ca. 1 month respiration decreased to much lower values. Evaluating the results with a multi-G model (n=3) indicated that the degradable part of the Phaeocystis material (50-75%) had a characteristic decay rate of ca. 30 10^{-3} d^{-1}. According to the model, ca. 80% of the benthic organic C content and ca. 60% of the organic N at FF had decay times > several years, at B14 these numbers were estimated at ca. 55% and ca. 30% respectively. The remaining part was devided into two fractions, the first dominating total mineralization in spring and summer (decay rate ca. 30 10^{-3} d^{-1}) and the second being most important in winter (decay rate 1-5 10^{-3} d^{-1}). Directly after the supply of organic matter to the mesocosms, NO_x interstitial water concentrations and denitrification substantially decreased. Modelling of the pore water NH_4^+ and NO_x profiles indicated that benthic nitrification was eliminated after the the supply. After ca. 2 months when most of the added material was respired, nitrification and denitrification recovered. These findings confirm the importance of nitrification-denitrification coupling in benthic N-cycling.

I.1. INTRODUCTION

Eutrophication of the southern North Sea has been widely discussed (e.g. Folkard & Jones, 1974; van Bennekom et al., 1975; Postma, 1978; Brockmann et al., 1988; van Bennekom & Wetsteyn, 1990; van der Veer et al., 1990). Due to increased nutrient loads from the continental main rivers, winter nitrate concentrations in the Southern Bight off the Dutch coast have increased upto 1.5 times between 1961 and 1978 (van Bennekom & Wetsteyn, 1990). According to van der Veer et al. (1990) annual averages of total nitrogen concentrations at a coastal station increased ca. 4 times in the period 1950-1985. The Dutch coastal zone is dominated by the inflow from the rivers Rhine/Meuse and Scheldt in the south, and obviously nutrient concentrations are highest within and near their plumes. Dutch coastal waters are, however, very turbid and this strongly limits primairy production and accompanying nutrient uptake (Gieskes & Kraay, 1975). Consequently, part of the dissolved nutrients may be transported northwards along the residual current (Brockmann et al., 1988). Due to increased nutrient availability during last decades primairy production increased, and eutrophication probably also stimulated bloomings of nuisance causing algae like Phaeocystis pouchetii (Cadée & Hegeman, 1986; Cadée, 1990). In other areas of the North Sea a relation between eutrophication and bloomings of toxic algae (e.g. Chrysochromulina polylepis) has been suggested (Rosenberg et al., 1988; 1990).

Particularly for shallow systems the importance of the sediments

within overall nutrient cycling is well recognized (e.g. Billen, 1978; Rutgers van der Loeff, 1980; Balzer, 1984; Hopkinson, 1987; Klump & Martens, 1987; Enoksson et al., 1990). Nitrogen cycling in sediments off the Belgian and southern Dutch coast has been studied by Billen (1978) and Rutgers van der Loeff (1980) respectively. However, the quantitative role of sedimentary processes within eutrophication of the North Sea is still not well established (Brockmann et al., 1988). This especially holds for the central parts of the North Sea (Law & Owens, 1990; van Raaphorst et al., 1990), but also for the northern part of the Dutch coastal area.

Basically, sediment-water interactions may be divided into two complementary components. The first is the regeneration to the water-column of organic nutrients deposited on the bottom. This release may sometimes contribute substantially to meeting the primairy producers demands (Aller, 1980; Rutgers van der Loeff, 1980; Hopkinson, 1987; Klump & Martens, 1987). The second component, benthic nutrient removal, probably is more important from a eutrophication perspective, since it largely determines the buffering capacity of the system to changing external nutrient inputs. For nitrogen the dominating removal processes are burial in deeper sediment layers and denitrification (Billen, 1978; Balzer, 1984; Smith et al., 1985; Klump & Martens, 1987; Devol, 1991). Apart from some specific depositional areas, net annual sedimentation and accompanying burial is negligible in the southern North Sea (Eisma, 1981), leaving denitrification as most important nitrogen removal mechanism. In this anoxic proces, nitrate and nitrite are used by microorganisms as terminal electron acceptors for mineralizing organic substrates and are reduced into the gaseous end-products N_2 and N_2O (Payne, 1973; Sørensen et al., 1979; Knowles, 1982; Koike & Sørensen, 1988). An interesting question is to what extent a larger organic deposition on the sediments favoures denitrification. Enoksson et al. (1990) for the Kattegat and Kemp et al. (1990) for Chesapeake Bay conclude that denitrification decreased due to increased organic deposition, making eutrophication a partly self accellerating proces. At the other hand, increased nitrate concentrations in overlying waters may strongly stimulate sedimentary denitrification rates (Christensen et al., 1990; Kieskamp et al., 1991), thus providing an internal mechanism counteracting eutrophication.

In this paper, nitrogen cycling in two sediments off the northern Dutch coast is examined. Direct data on sediment-water exchange rates, denitrification and pore water concentrations are presented for a complete annual cycle (1989-1990). To study the effect of deposition of degradable organic matter on the sediments, specific mesocosm experiments were designed of which results will be presented here also. This research was part of a larger study on North Sea sediment eutrophication. Other aspects of the study for the same stations and mesocosms are described in chapters II to V of the Appendix by van Duyl et al.; Bak et al.; Duineveld et al. and Witbaard respectively.

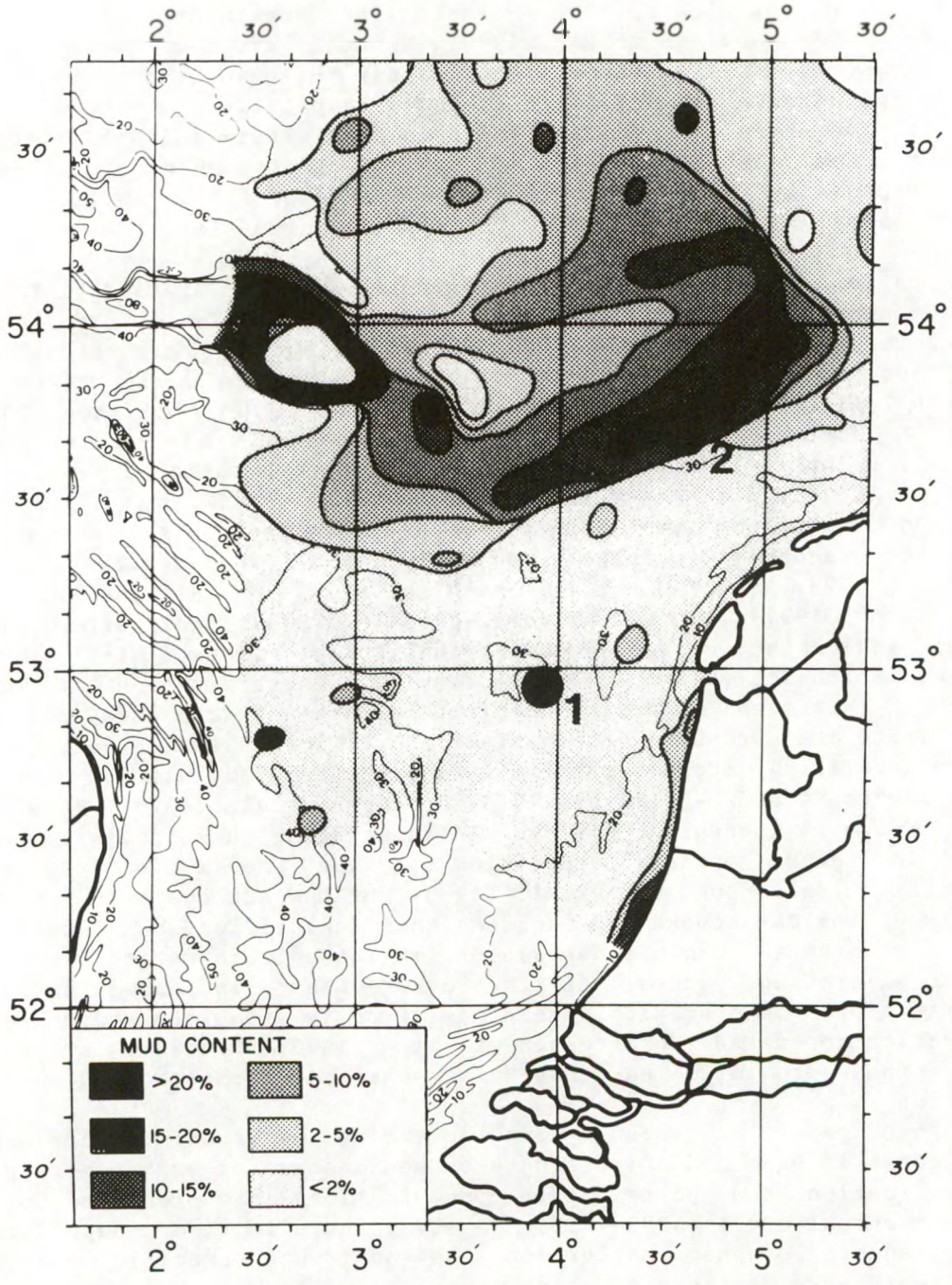


Fig. I.1. Study area in the southern North Sea with sampling stations. 1 is station at the Frisian Front (FF), 2 is station at the Broad Fourteens (B14). Isolines indicate mud content (amount of particles < 50 µm) of the upper 10 cm of the sediments (after Creutzberg et al., 1984).

Acknowledgements. Thanks are due to the crew of R.V. Aurelia for their help during the cruises and to F.C. van Duyl for critically reading the manuscript. P. Verburg contributed by doing part of the oxygen respiration measurements and part of the organic C, N analysis. B. Hofte performed part of the model simulations.

I.2. MATERIALS AND METHODS

Area description: Sediments were collected from two stations in the southern North Sea (Fig. I.1), Frisian Front (FF: 4°30´E, 53°42´N; depth = 39 m) and Broad Fourteens (B14: 3°55´E, 53°00´N; depth = 29 m). Both stations had a vertically well mixed watercolumn throughout the year, and had almost equal temperatures between ca. 5 and 18 °C in winter and summer respectively (Fig. I.2). Station FF is described in detail by Cramer (1990) and corresponds to the enriched benthic zone on the transition between the sandy Southern Bight and the muddy Oystergrounds (Creutzberg et al., 1984; Creutzberg, 1985). The sediments here consist of sands mixed with finer materials. Median grain size is 125-150 µm, the amount of particles < 50 µm is larger than 20 % (Creutzberg et al., 1984). Porosity in the upper 10 cm is 50-60%. Macrobenthic biomass at FF amounts to ca. 30 g-AFDW.m^{-2}, densities being ca. 2500 ind.m^{-2} (Cramer, 1991). Echinoderms (mainly Amphiura filiformis) represent ca. 50% of total biomass and numbers (Duineveld & Moodley, 1991). Station B14 consists of coarse sand with a median grain size of 200-250 µm, the amount of particles < 50 µm being less than 2 % (Creutzberg et al., 1984). Porosity in the top 10 cm of the B14 sediments is 35-40%. In this part of the North Sea macrobenthic biomass is ca. 6 g-AFDW.m^{-2} at densities of ca. 600 ind.m^{-2}, echinoderms (mainly Echinocardium cordatum) dominating here also (Duineveld et al., 1990; Duineveld & Moodley 1991).

Sampling: Intact sediment cores were obtained aboard R.V. Aurelia during the period January 1989 to April 1990. For the collection of sediments needed for installment of the mesocosms 0.25 m^2 stainless steel SCRIPPS corers were applied. All other cores were collected using a modified stainless steel cylindrical Reineck-type boxcorer (Reineck, 1963) with an inner diameter of 0.31 m (75.5 10^{-3} m^2). Each time, 2 cores were used directly for measuring O_2 respiration, while 2 others were subsampled for further use.

Mesocosm installment: At sea the SCRIPPS cores were placed in the dark, continuously supplying fresh North Sea water. After arrival at the laboratory, the cores were accomodated in equally sized plastic containers with as little disturbance as possible. The installment was performed in January and April 1989, sediments were kept at in situ temperature in the dark until December 1989. One set of FF and B14 sediments, installed in January, was starved and the other set, installed in April, received North Sea algal material which was

collected with 50 µm plankton nets during the bloom of Phaeocystis spec. in April-May. Henceforth, the first set will be called "starved mesocosms" (SM), the second set will be refered to as "fed mesocosms" (FM). After homogenizing, freezing and thawing, the organic material was supplied in three portions to the sediments (24 May, 31 May, 7 June) by putting it into the overlying water from where it quickly settled on the sediment surface. The total organic carbon supply was 23 $gC.m^{-2}$, approximately equivalent to the annual metabolic loss of sandy North Sea sediments (de Wilde et al., 1984; Cramer, 1991). The total organic nitrogen supply was 0.21 $mol.m^{-2}$. The starved and enriched sediment cores were maintained separately without any possibility for mutual contamination. During the experiments, North Sea water of constant salinity (29‰) and nutrient concentrations (NH_4^+ = 0.5 µM, NO_3 + NO_2 = 45-50 µM) was supplied continuously to the mesocosms.

Oxygen respiration: Benthic oxygen consumption was measured according to the on-deck bell-jar method described by Cramer (1990). Whole boxcores with fibreglass walls (cf. Cramer, 1989) were placed at *in situ* bottom-water temperature ± 1°C and covered with a plexiglass lid in which a stirring device, two YSI 5739 O_2 electrodes and a temperature sensor were fitted. Respiration was calculated from the change in the O_2 concentration in the chamber during incubation in which O_2 never dropped below 100 µM. In the mesocosms respiration was measured by covering part of the sediment with a plexiglass '*in situ*' bell-jar (i.d. 0.31 m), further equally designed and equipped as the on-deck bell-jars. Both in the field and in the mesocosms usually two replicates were measured.

Benthic DIN fluxes: To measure sediment-water exchange rates of ammonium, nitrate and nitrite 3 to 5 cores of 10 cm i.d. ($7.85\ 10^{-3}\ m^2$) were subsampled from the boxcores with acrylic liners and subsequently placed at *in situ* temperature ± 2 °C. Overlying water was carefully syphoned off and replaced by 750 ml filtered (Sartorius 0.45 µm cellulose acetate) bottom water. The overlying water was kept saturated with O_2 by continuously bubbling air, at the same time introducing turbulence in the water column just below the level of visible bottom material resuspension. During the incubation (ca. 10-20 hr) 5 to 8 samples were taken at regular intervals. The fluxes were calculated from the changes in concentration over time, using the procedure outlined by van Raaphorst & Brinkman (1984). In this procedure, sediment-water exchanges are described by:

$$J = K_m[C_b - C_o] \qquad (1)$$

Where J = benthic flux ($mol.m^{-2}.s^{-1}$)
K_m = overall mass transfer coefficient ($m.s^{-1}$)
C_b = characteristic concentration in the pore water ($mol.m^{-3}$)
C_o = momentaneous concentration in the overlying water ($mol.m^{-3}$)

As the experiment proceeds the concentration in the overlying water equilibrates with that in the pore water, i.e. C_o becomes equal to C_b and the flux J diminishes. Both parameters K_m and C_b are estimated after solving the dynamic mass balance equation for C_o: $dC_o/dt = J.h^{-1}$, where h = depth of the overlying water (decreasing in time due to sampling), and fitting calculated concentrations to those measured during the incubations. The 'in situ' fluxes then follow from eq.1, using the estimated values of K_m and C_b and substituting the in situ bottomwater concentration for C_o.

Denitrification and nitrous oxide fluxes: Four intact cores of 5.5 cm i.d. were subsampled with acrylic liners from the boxcores and mesocosms respectively and placed in the dark at in situ temperature ± 0.5 °C. Sediment surface was adjusted to a fixed position, resulting in 100 ml overlying water and 15 ml headspace. Tubes were closed with gastight polyacetate lids, provided with a sample port and a teflon coated magnetic stirrer as described by Kieskamp et al. (1991). Two cores were treated with acetylene (e.g. Sørensen, 1978; Smith & Delaune, 1983; Koike & Sørensen, 1988) in such a way that in both overlying water and interstitial water acetylene concentrations were ca. 10% (v/v). The 2 remaining cores were used to estimate N_2O fluxes. Total incubation time was 5-10 hr in which headspaces were regularly sampled and analysed for N_2O. Denitrification rates and N_2O fluxes were calculated from the increase of N_2O over time in the cores with and without acetylene respectively, in which the latter ones served as blanks for the first. Essentially the experimental procedure outlined by Kieskamp et al. (1991) was followed and further details may be found there.

Pore water profiles and modelling: Interstitial water was obtained at in situ temperature after slicing 5.2 cm diameter cores (subsampled with acrylic liners) into 5, 10, and 20 mm segments (depending on sediment depth) by processing the segments in teflon Reeburgh-type squeezers under N_2-pressure (Reeburgh 1967). In all cases segments of 3 cores were pooled. It was not well possible to directly measure benthic DIN fluxes in the mesocosms as it was done in the field. We therefore estimated these fluxes from the pore water profiles. For this, the ammonium and nitrate + nitrite (NO_x) profiles were evaluated using the steady state diagenetic N-model described by van Raaphorst et al. (1990). In this model, being a modification of the model of Vanderborght & Billen (1975), the sediment is divided in an upper nitrification and a lower denitrification zone respectively. In the upper layer the coupled equations describing ammonium and NO_x respectively are:

$$0 = D \frac{\partial NH_4^+}{\partial x^2} + R_a - k_n . NH_4^+ \qquad (2)$$

$$0 = D \frac{\partial NO_x}{\partial x^2} + k_n . NH_4^+ \qquad (3)$$

Where D = whole sediment apparent diffusion coefficient for ammonium and NO_x respectively ($m^2.s^{-1}$)
NH_4^+, NO_x = pore water ammonium and nitrate + nitrite concentration respectively ($mol.m^{-3}$)
R_a = zeroth-order ammonification rate ($mol.m^{-3}.s^{-1}$)
k_n = first-order nitrification rate (s^{-1})
x = vertical coordinate, zero at interface (m)

For simplicity diffusion coefficients for ammonium and NO_x are approximated as being equal. The thickness of the nitrification layer (x_n) is assumed to equal the O_2 penetration depth. For the lower zone ($x > x_n$) ammonification is described with a declining exponential term, and a first-order denitrification is assumed:

$$0 = D \frac{\partial NH_4^+}{\partial x^2} + R_a . \exp[-\alpha(x - x_n)] \qquad (4)$$

$$0 = D \frac{\partial NO_x}{\partial x^2} - k_d . NO_x \qquad (5)$$

where α = depth constant (m^{-1})
k_d = first-order denitrification rate (s^{-1})
x_n = thickness of nitrification zone (m)

The applied boundary conditions are $x = 0$: $NH_4^+ = NH_4^+(0)$, $NO_x = NO_x(0)$; $x \rightarrow \infty$: $\partial/\partial x = 0$. Analytical solutions are given by van Raaphorst et al. (1990). Total benthic ammonification, nitrification, and denitrification are calculated from the model by integrating the different terms over appropriate depths. Fluxes at the sediment-water interface follow from Fick's law:

$$\text{Flux}(x=0) = -\varphi . D \left. \frac{\partial NH_4, NO_x}{\partial x} \right|_{x=0} \qquad (6)$$

where φ = volumetric porosity of the sediment (-)

The diffusion coefficient is first calculated from the average molecular diffusion coefficients for ammonium and nitrate given by Li & Gregory (1974), which were corrected for tortuosity according to the method of Ullman & Aller (1982) for coarse sediments (n=2) and for temperature by use of the Stokes-Einstein relation. Hereafter, correction for biologically mediated transport is performed by comparing model results to measured denitrification rates (cf. Billen, 1978).

Sediments: Cores to be used for analysis of sediment porosity and organic C,N content were subsampled with 4.5 cm i.d. PVC tubes, and sliced into segments of 5, 10, 20 an 40 mm (depending on sediment depth). Every time, 3 cores were pooled. Porosity was calculated from loss of weight after drying at 60 °C for at least 24 hr, assuming the specific weight of sediment particles being 2.65 $kg.l^{-1}$. Organic C,N contents were measured on a Carlo Erba NA 1500-2 elemental analyzer following the procedure of Verardo et al. (1990) applying sulfurous acid to remove inorganic carbon. For calibration acetanilide (C_8H_9NO) was used as a standard.

Analytical procedures: Nutrients were determined on a Technicon AA II autoanalyzer. Nitrate and nitrite according to Strickland & Parsons (1972), ammonium following the phenol-hypochlorite method of Helder & de Vries (1979). Gas samples were analyzed for N_2O on a Packard 438a GC with ECD. Carbosorb was used to remove water vapor and CO_2 (Rönner 1983; Law & Owens, 1990). The concentration of N_2O in the waterphase was calculated from that in the gasphase by using the solubility coefficient (F) given by Weiss & Price (1980). The applied methods for chlorophyll-a extraction and determination were according to the procedure described by Peeters & Peperzak (1990).

Modelling mesocosm results with multi-G model: To evaluate the obtained data on O_2 respiration and sedimenatary organic C and N contents in the mesocosms, a simple multi-G model (Berner, 1980) was applied. In this model organic substrate is assumed to consist of n different fractions, each of them being mineralized according to a first-order reaction:

$$\frac{dG_i^C}{dt} = -k_i^* . G_i^C \qquad (7a)$$

$$\frac{dG_i^N}{dt} = -k_i^* . G_i^N \qquad (7b)$$

$$J_{O2} = Y \sum_{i=1}^{n} k_i^* . G_i^C \qquad (7c)$$

where G_i^C, G_i^N = i^{th} fraction of organic C and N respectively in upper 5 cm of the sediments ($g.m^{-2}$)
t = time (d)
k_i^* = first-order decay constant of i^{th} fraction (d^{-1})
J_{O2} = benthic O_2 respiration ($mmol.m^{-2}.d^{-1}$)
Y = coefficient to convert C oxidation into O_2 consumption ($mmolO_2.gC^{-1}$)
i = integer ranging from 1 to n

The parameters k_i^* are corrected for temperature via:

$$k_i^* = k_i . \exp \frac{T - 15}{T^*} \qquad (8)$$

where k_i = decay constant of i^{th} fraction at 15 °C (d^{-1})
T = temperature (°C)
T^* = temperature parameter (°C)

The parameter T^* is fixed at 14.4 °C, resulting in $Q_{10} = 2$ (cf. Hargrave & Philips, 1989). The coefficient γ is assumed to be 100 $mmol O_2$ per gC, equivalent to an atomic O_2:C ratio of 1.2:1. In the present model 3 fractions are distinguished ($n = 3$), from which fraction 3 represents the refractory part of the total organics (consequently $k_3 = 0$), and fractions 1 and 2 the degradable parts with $k_1 >> k_2$. The estimation of the values of these latter two parameters together with the composition of the organic material, i.e. the fractionation into 3 fractions G_i^C and G_i^N, was carried out by fitting model calculations to experimental data (O_2 respiration, organic C,N contents of the sediment), using the least-square criterion for best fit. Simulation and fitting (Simplex routine) is performed with the STEM simulation package (ReMeDy System Modelling, Enschede, NL).

I.3. RESULTS

I.3.1. Field data

Watercolumn

Fig. I.2 shows the temperature and chlorophyll-a, ammonium and nitrate + nitrite (NO_x) concentrations at B14 and FF respectively during 1989-1990. Temperature and nitrogen values are representative for water just above (< 30 cm) the sediment surface (bottom water), chlorophyll-a is measured in the upper part of the watercolumn. The chlorophyll data are obtained from J.C.H. Peeters, Ministry of Transport and Public Works, Tidal Waters Division, Middelburg, NL, and represent locations nearby B14 and FF respectively. At B14 the spring phytoplankton bloom is well recognized in April 1989 and 1991 in which chlorophyll reached concentrations upto ca. 5 and ca. 8 $\mu g.l^{-1}$ respectively. At FF the spring bloom was less clearly present, particularly in 1989 when several small chlorophyll peaks occurred. Chlorophyll concentrations at FF were considerably lower as compared to B14, especially during spring. At both stations chlorophyll concentrations were high (2-3 $\mu g.l^{-1}$) during the summer months, indicating a substantial blooming during that period.

Bottom water concentrations of NH_4^+ and NO_x were relatively high, even during the summer period. Ammonium varied between 1 and 3.5 µM at B14 and between 2 and 10 µM at FF, without a clear seasonal trend.

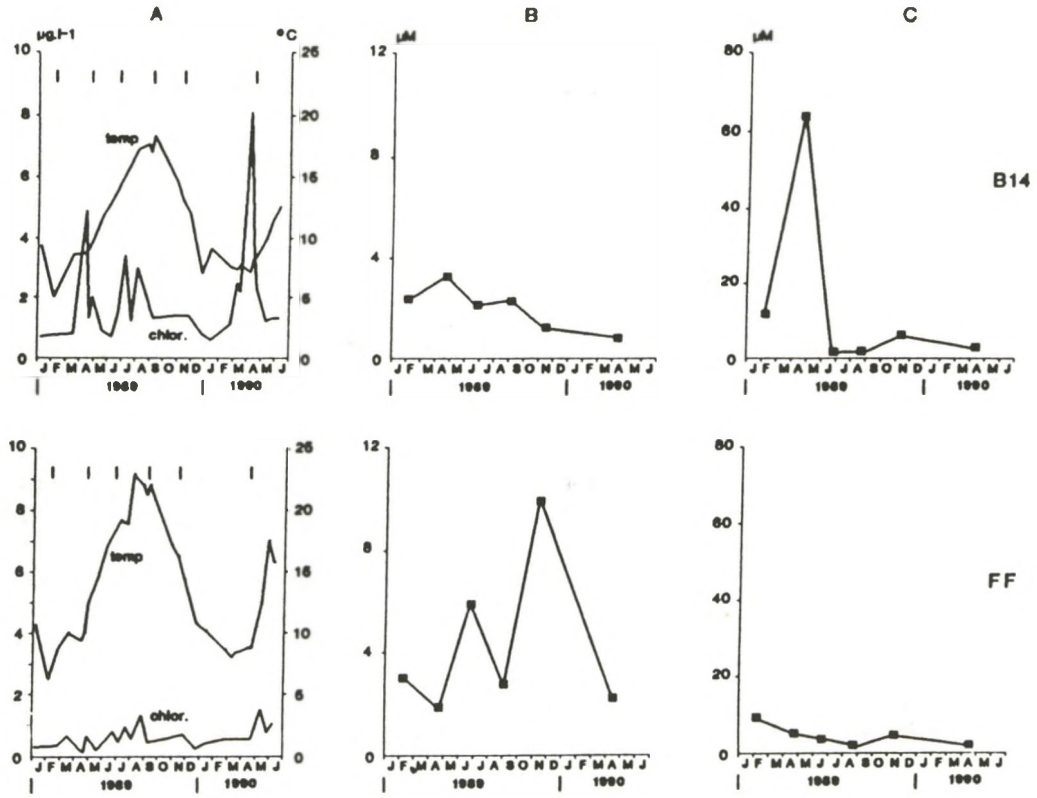


Fig. I.2. A: temperature (^{o}C) and chlorophyll-a ($\mu g.l^{-1}$), B: ammonium and C: nitrate + nitrite (µM) in water overlying B14 (upper part) and FF (lower part) respectively. Chlorophyll-a is measured at the surface of the watercolumn at stations near FF and B14 (data kindly provided by J.C.H. Peeters, Ministry of Transport and Public Works, Tidal Waters Division, Middelburg), all other data are from water < 0.3 m above the sediment surface. Vertical lines in A indicate sampling dates.

NO_3^- + NO_2 (the latter always being < 10% of the first) varied between 1.5 and 70 µM at B14 and between 2 and 9 µM at FF. At both stations highest NO_x values were observed in winter and early spring, the very high concentration at B14 in April 1989 is probably caused by nitrate-rich water coming in from near the southern Dutch coast.

Organic matter content of the sediments

Measured organic C and N contents are given in Fig. I.3. All values are low, particularly those of B14 where org.C varied between 0.4 and 1.0 mg per g of dry sediment and org.N between 0.10 and 0.15 $mg.g^{-1}$. In the FF sediments org.C was almost 10 times higher than at B14, and ranged from 3.7 to 7.8 $mg.g^{-1}$. Org.N was ca. 5 times higher than at

B14: 0.40 - 0.89 $mg.g^{-1}$. Not only the absolute contents, but also the variation with depth differed between the two stations. Though there was a considerable scattering, at the sandy B14 both org. C and N on average decreased with depth. At FF such a regular pattern was not observed. At 3 of the 6 sampling dates (January, April 1989, April 1990) distinct maxima were found at ca. 30 mm depth, while in August a subsurface peak was present at ca. 50 mm, and in November it seemed to be situated even deeper.

At B14, the seasonal pattern to a large extent is restricted to the upper few cm´s. In this layer, particularly in the top 10 mm, organic contents strongly increased in April 1989, i.e. a few weeks after the spring bloom (Fig. I.2). Peak values in the upper 10 mm were reached in June. In August and November, organic C and N contents had decreased again to values only slightly higher than in January. Also in April 1990, i.e. at the end of the spring bloom, high contents were measured. In the Frisian Front sediments, values of the subsurface maxima did not vary much over the year. Just as at B14, seasonal variations were best visible in the upper cm´s, with highest contents in April 1989 and June. Contrary to B14, at FF organic contents above 30 mm were still low in April 1990. This is however consistent with the timing of the spring bloom at FF in 1990 (Fig. I.2) which occurred after the sample collection. Atomic C:N ratios (Table I.1) at FF were approximately twice that of B14, on average ca. 10.5:1 and 5:1 for the two stations respectively. There was little variation in C:N ratios between different months and at different depths.

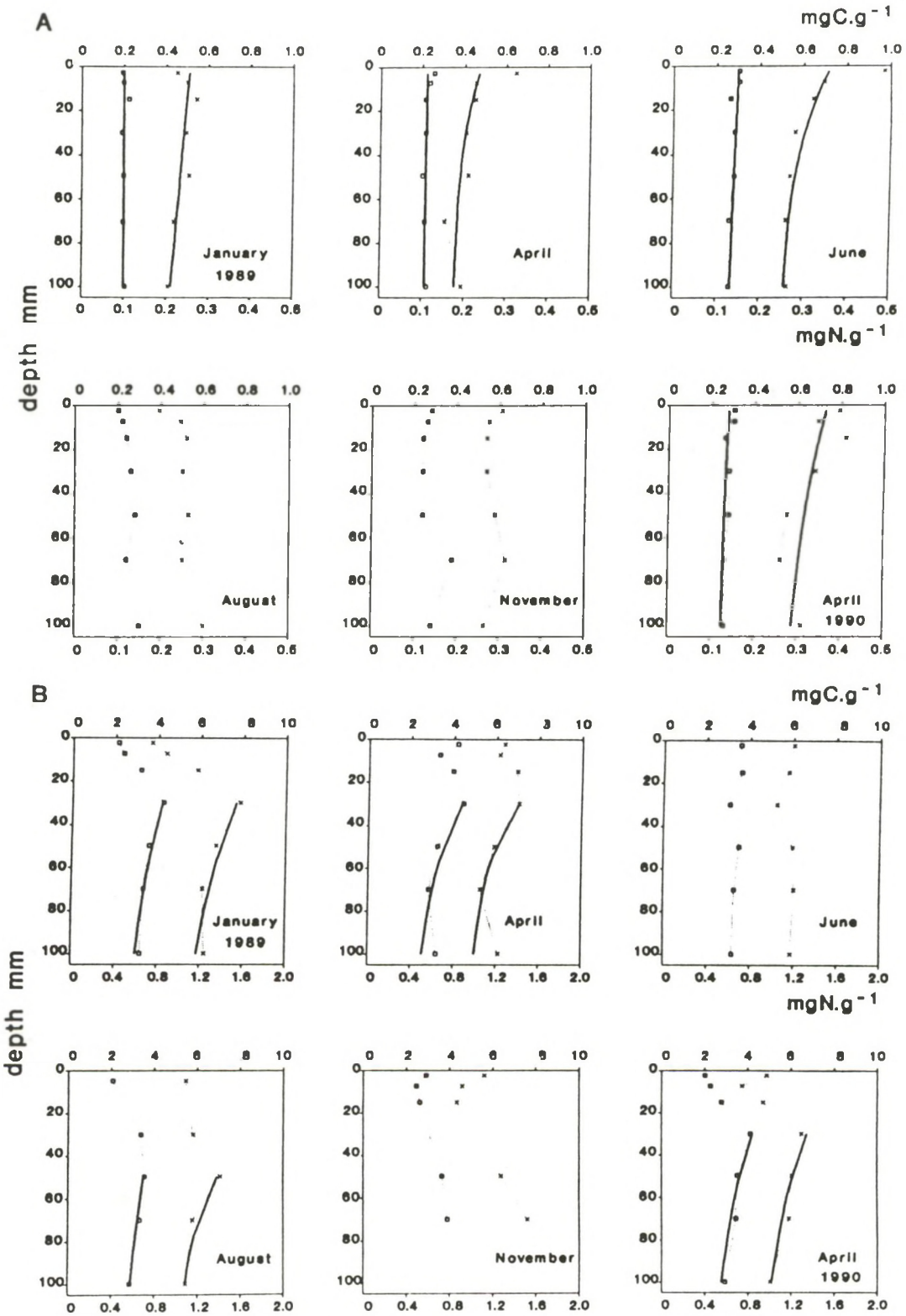


Fig. I.3. Organic C (×) and N (□) contents (mg per g of dry sediment) in the upper 120 mm of sediments at B14 (A) and FF (B). Solid lines are obtained from exponentially declining functions.

TABLE I.1
Atomic C:N ratios in sedimentary organic material of the Broad Fourteens (B14) and the Frisian Front (FF). Layer number 1: 0-10 mm, 2: 10-60 mm, 3: 60-120 mm, nd = no data

Month	Week nr.	Broad Fourteens C:N			Frisian Front C:N		
		1	2	3	1	2	3
1989							
January	5	5.6	6.1	4.9	10.3	10.9	11.0
April	16	5.3	4.6	4.1	9.6	9.9	10.9
June	25	6.5	5.2	4.8	9.9	9.9	10.8
August	35	4.7	4.7	4.5	nd	11.0	10.5
November	45	4.8	5.3	4.4	11.0	9.6	10.7
1990							
April	67	5.1	5.5	5.5	11.7	9.6	10.0

Interstitial water

Oxygen penetration as measured with micro-electrodes typically varied between ca. 4 (summer) and 12 mm (winter) at FF, and between ca. 8 (summer) and 20-25 mm (winter) at B14 (Cramer, unpublished results, Appendix Chap. II and unpublished results). Concentrations of NH_4^+ and NO_x in the upper 120 mm interstitial water of both stations are given in Fig. I.4. Ammonium was always higher at FF than at B14. Maximum concentration at FF was 250 µM, compared to 75 µM at B14 (both in August). At the latter station high subsurface NH_4^+ concentrations were observed at ca. 50 mm depth during the period April-August 1989, at FF such a subsurface maximum was only detected in June (at 15-30 mm depth). After August 1989 NH_4^+ concentrations continuously decreased, at both stations to values < 20-25 µM in April 1990. During summer no subsurface NO_x peaks were observed, suggesting elimination of benthic nitrification during this period. At B14, the high concentration in the overlying water in April 1989 largely influenced the pore-water concentrations in the upper 120 mm of the sediment (NO_x > 30 µM).

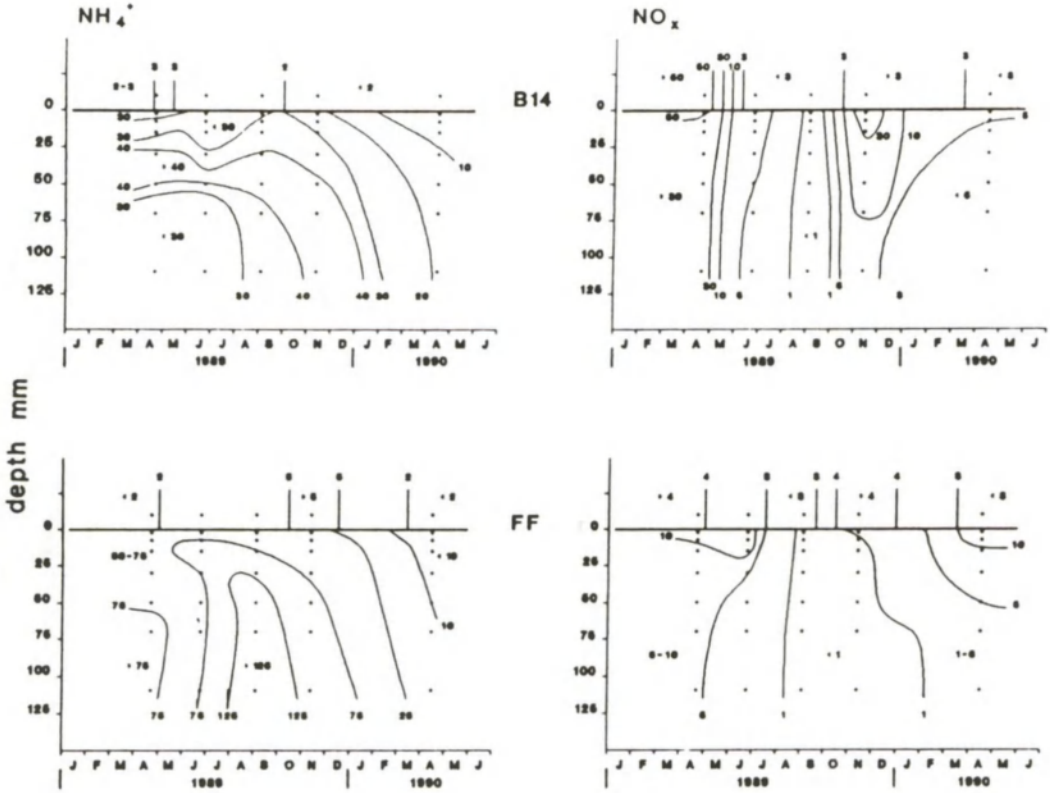


Fig. I.4. Ammonium and nitrate + nitrite (NO_x) concentrations (µM) in pore water from the upper 120 mm of sediments at B14 (upper part) and FF (lower part).

Respiration and sediment-water exchange rates

Benthic respiration and nitrogen sediment-water exchanges are presented in Figs I.5 and I.6 respectively. Fluxes of both O_2 and NH_4^+ were clearly higher at FF compared to B14. Maximum respiration was ca. 40 and 20 $mmol.m^{-2}.d^{-1}$ at FF (August) and B14 (June) respectively. Estimated values for the mass transfer coefficient K_m and the concentration C_b are listed in Table I.2. These parameters represent the effect on benthic fluxes by transport processes (e.g. diffusion, bioirrigation) and enhanced or lowered pore water concentrations (due to e.g. mineralization or nitrification) respectively. Values of K_m vary between 1 and 13 $mm.d^{-1}$ for NH_4^+, and between 1 and 16 $mm.d^{-1}$ for NO_3^-. For NO_x the data suggest a seasonal trend for K_m with maximum values in June and minimum values in winter and early spring. K_m values of NH_4^+ did not show a clear seasonal trend. For ammonium the estimated values of C_b have a maximum in August, while they are very low in April 1990. This result is in good agreement with the measured pore water concentrations (Fig. I.4). Also, the values at FF are substantially higher (1-46 µM) than those at B14 (0-18 µM). For NO_x the high C_b-value at B14 in April 1989 (63 µM) again demonstrates the

effect of the high NO_x concentration in the overlying water (Fig. I.2). Apart from this month, C_b-values for B14 (1-8 µM) do not differ much from those at FF (4-10 µM). At both stations C_b for NO_x is lowest in June-August.

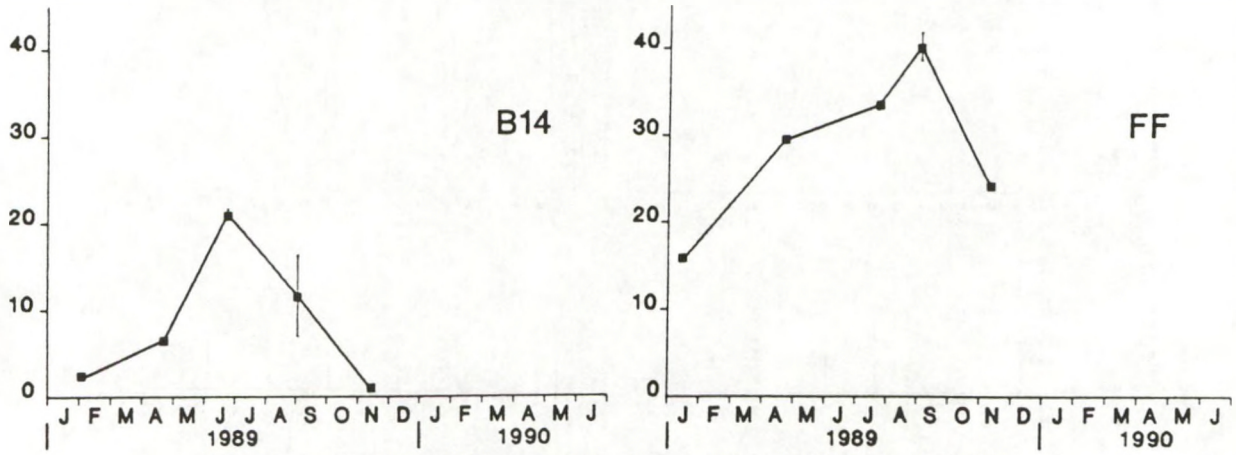


Fig. I.5. Benthic O_2 respiration (mmol.m^{-2}.d^{-1}) measured at B14 and FF.

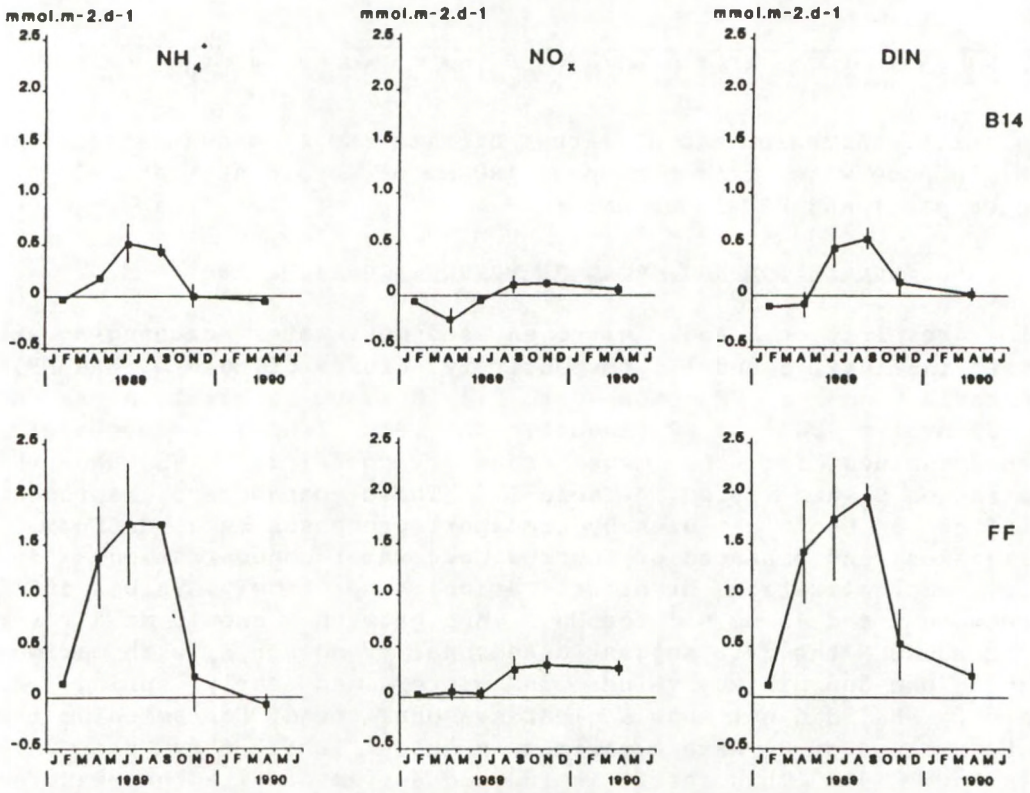


Fig. I.6. Sediment-water exchange rates of NH_4^+, NO_x = nitrate + nitrite, and DIN = NH_4^+ + NO_x (mmol.m^{-2}.d^{-1}) at B14 (upper part) and FF (lower part).

TABLE I.2
Estimated values of the mass transfer coefficient (K_m, $mm.d^{-1}$) and the concentration parameter (C_b, µM) determining sediment-water exchanges of ammonium and NO_x = nitrate + nitrite (see eq. 1). a: Broad Fourteens, b: Frisian Front

a: Broad Fourteens (B14)

Month	Week nr.	NH_4 K_m $mm.d^{-1}$	NH_4 C_b µM	NO_x K_m $mm.d^{-1}$	NO_x C_b µM
1989					
January	5	13 ± 2	2 ± 0	1 ± 0	8 ± 0
April	16	1 ± 0	10 ± 0	3 ± 1	63 ± 3
June	25	4 ± 1	8 ± 2	16 ± 5	1 ± 0
August	35	1 ± 0	18 ± 1	7 ± 2	2 ± 1
November	45	6 ± 3	1 ± 1	4 ± 1	7 ± 1
1990					
April	67	4 ± 1	0 ± 0	4 ± 2	3 ± 0

b: Frisian Front (FF)

Month	Week nr.	NH_4 K_m $mm.d^{-1}$	NH_4 C_b µM	NO_x K_m $mm.d^{-1}$	NO_x C_b µM
1989					
January	2	1 ± 0	8 ± 0	1 ± 0	10 ± 0
April	16	2 ± 1	35 ± 4	2 ± 0	6 ± 3
June	25	2 ± 0	39 ± 12	13 ± 4	4 ± 0
August	35	2 ± 0	46 ± 6	5 ± 1	4 ± 1
November	45	4 ± 2	14 ± 3	6 ± 2	7 ± 1
1990					
April	67	8 ± 1	1 ± 1	5 ± 2	4 ± 0

The highest ammonium fluxes calculated from eq. 1 (Fig. I.6) were 1.7 (FF, June-August) and 0.5 $mmol.m^{-2}.d^{-1}$ (B14, June). In winter the ammonium fluxes were small, sometimes even directed into the sediment. Exchange rates of nitrate + nitrite were low in the first half of 1989, but increased after June, indicating a substantial nitrification activity from this month onwards. The NO_x flux into the sediment at B14 in April 1989 is likely caused by the high NO_x concentrations in the overlying water during that time (Fig. I.2). The absolute values of the NO_x fluxes of B14 did not differ much from those at FF,

compared to the total DIN fluxes (also included in Fig. I.6) the relative contribution of NO_x was however considerably larger at B14 than at FF. Due to the difference in NH_4^+ the total exchange of inorganic nitrogen is 3-4 times higher at FF compared to B14. At both stations maxima were obtained in August.

Denitrification and nitrous-oxide fluxes

Results on denitrification and benthic N_2O fluxes are given in Fig. I.7. At both stations denitrification was low from August to November and relatively high from April to June. The exceptional high value at B14 in April 1989 (0.45 mmol.m^{-2}.d^{-1}) is clearly related to the high NO_x concentration in the overlying water (Fig. I.2). The seasonal trend in the nitrous-oxide fluxes is less clear. In June an influx of N_2O was observed at both stations, the highest effluxes were measured in April. On an annual basis, the average N_2O fluxes were 0.001 and 0.002 mmol.m^{-2}.d^{-1} for B14 and FF respectively.

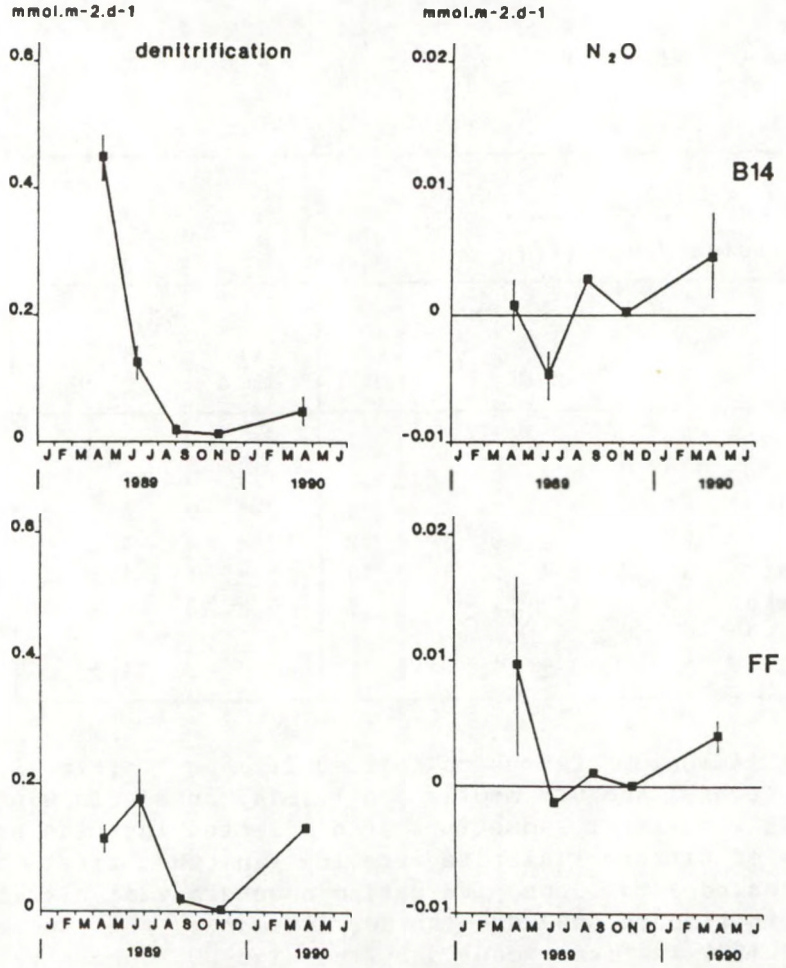


Fig. I.7. Benthic denitrification rate (mmol-N.m^{-2}.d^{-1}) and benthic fluxes of nitrous oxide (mmol-N_2O.m^{-2}.d^{-1}) at B14 (upper part) and FF (lower part).

I.3.2. Mesocosms

Respiration and organic C and N contents

Results on respiration and organic C,N contents are presented in Figs I.8 (B14) and I.9 (FF). The first datapoint in these figures represents the field situation during collection of the mesocosm sediments. Temperatures were approximately equal to those in the field (Fig. I.2), and varied between 6 °C (winter) and 16 °C (summer). Only in the first few weeks after installment of the SM temperature was too high (10 instead of 5 °C), likely stimulating respiration during that period. Since no organic substrate was supplied to the starved mesocosms (SM), organic C and N obviously should decline during the course of the experiments. The data, however, do not clearly indicate

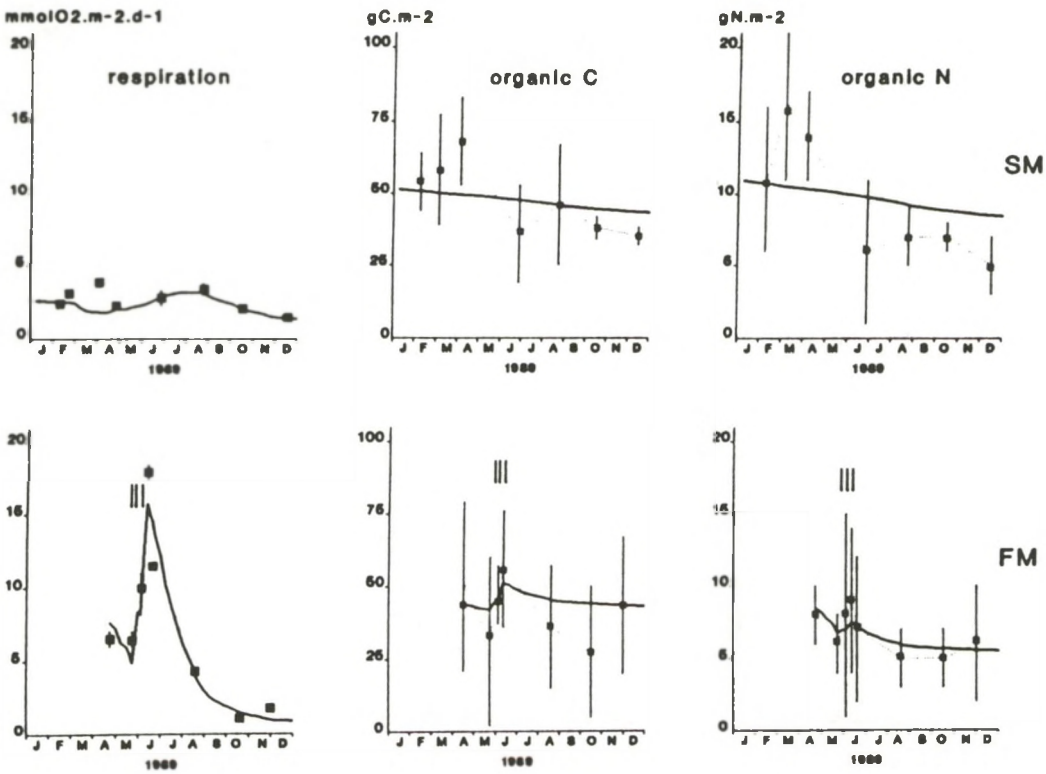


Fig. I.8. Benthic oxygen respiration (mmol.m^{-2}.d^{-1}) and total organic carbon (gC.m^{-2}) and nitrogen (gN.m^{-2}) in the upper 0-5 cm of B14 mesocosm sediments. Upper part: starved mesocosms (SM), lower part: fed mesocosms (FM). The first data point in each graph represents the field situation at the time of the collection of the mesocosm cores. Symbols represent measured values, vertical bars standard deviations. Solid line is calculated using the multi-G model (eqs. 7 and 8). Arrows indicate the addition of Phaeocystis material.

such a decline (e.g. org.C in the FF-SM), so likely a major part was not well degradable. On average, O_2 respiration decreased in the SM during the experiments, confirming a gradual mineralization of initial organic contents. Still, for B14 the highest respiration was found in August (Fig. I.8) when temperature was at maximum. For FF a more steady decrease in respiration was observed, but the effect of temperature was visible here also (Fig. I.9).

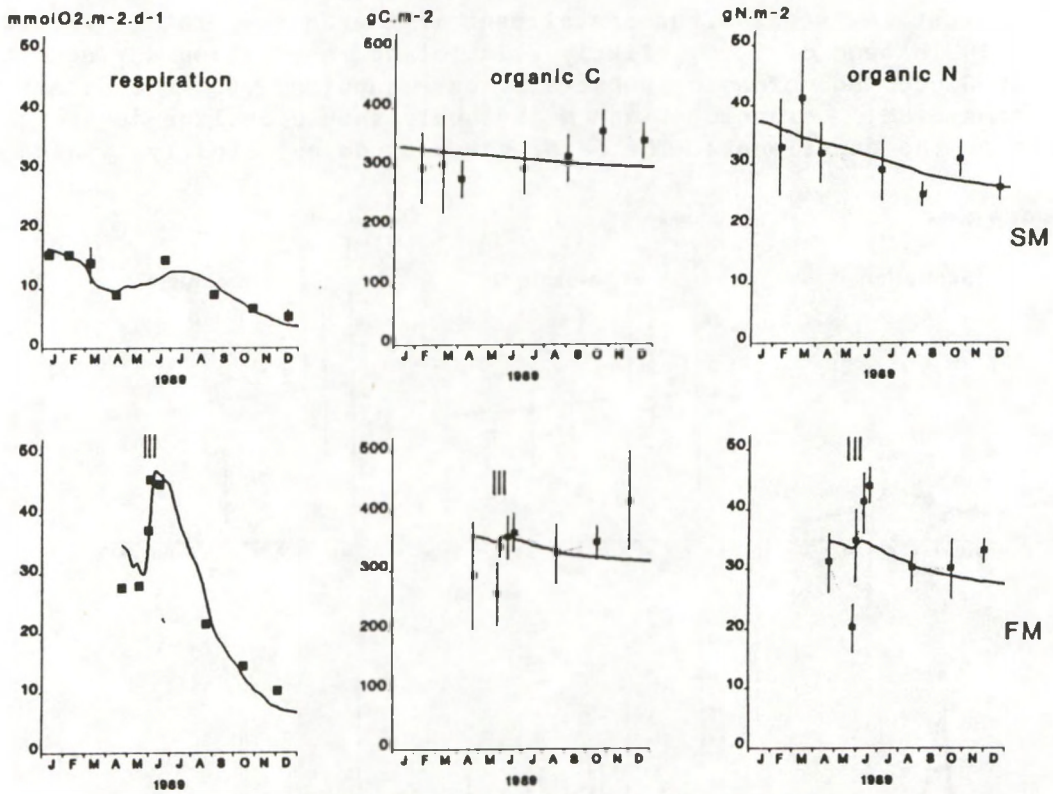


Fig. I.9. Benthic oxygen respiration ($mmol.m^{-2}.d^{-1}$) and total organic carbon ($gC.m^{-2}$) and nitrogen ($gN.m^{-2}$) in the upper 0-5 cm of FF mesocosm sediments. Upper part: starved mesocosms (SM), lower part: fed mesocosms (FM). The first data point in each graph represents the field situation at the time of the collection of the mesocosm cores. Symbols represent measured values, vertical bars standard deviations. Solid line is calculated using the multi-G model (eqs. 7 and 8). Arrows indicate the addition of Phaeocystis material.

In the fed mesocosms (FM) the addition of algal material was not clearly recovered in the sedimentary C and N contents. It resulted however in a large increase of the respiration, upto values encountered in the field during that time (Fig. I.5). In the sediments of both B14 and FF, respiration decreased shortly after the food supply. After ca. 1 month respiration returned to values only slightly

higher than before the addition of organic matter.

To quantitatively evaluate the results on O_2 respiration together with those of the organic C and N contents and to check whether these to sets of data are mutually consistent, a multi-G model was applied with n = 3. For the SM it is assumed that, being collected in winter, the organic material has a relatively low decay constant (Hargrave & Philips, 1989). This is achieved by stating that the sediments of the SM do not contain the most labile organic fraction, i.e. by setting G_1^C and G_1^N = 0 (eq. 7a,b) in the starved mesocosms. Obviously, the Phaeocystis material supplied to the FM had its own characteristics with respect to decay times. For simplicity, for this material we applied the same k_1 values as for the sedimentary organics in the mesocosms. Modelling results are included in Figs I.8 and I.9.

Estimated values of the decay constants k_1, the composition of the supplied Phaeocystis and that of the initial mesocosm sediments are listed in Table I.3. To arrive at these estimates, first the SM were evaluated by comparing O_2 respiration rates and organic C,N contents as measured during the course of the experiments to the corresponding simulation results. Second, the values for k_2 obtained from the SM simulations were used for those of the FM. Results of the parameter estimations indicate that k_1 = 34 and 26 10^{-3} d^{-1} and k_2 = 1 and 5 10^{-3} d^{-1} for B14 and FF respectively. Characteristic decay times (k_1^{-1}) are ca. 1 month for fraction 1 and a few years for fraction 2. In the FM of B14 ca. 47% of the supplied Phaeocystis material, i.e. 11 $gC.m^{-2}$ and 1.4 $gN.m^{-2}$, was recovered in the respiration, carbon and nitrogen data. For the FM of FF this percentage was ca. 68%, i.e. 16 $gC.m^{-2}$ and 2.0 $gN.m^{-2}$. It may be that a substantial part of the algal material is respired within the first days after addition to the mesocosms and that we missed the initial increase in organic C and N content and corresponding O_2 consumption. Pett (1989) reported that 13% of Skeletoma costatum cells were mineralized within 2 days. Also from the regression analysis by Middelburg (1989) on decomposition rates and age of organic matter it may be concluded that fresh material (age < 1 day) is largely decomposed within a few days. The remaining part probably was flushed out or may be converted into dissolved organic C and N (Enoksson, 1987).

The refractory part of the top 5 cm sedimentary organics was estimated at 26 $gC.m^{-2}$ at B14 and 284 $gC.m^{-2}$ at FF, corresponding to average carbon contents of 0.3 and 4.8 mg per g of dry sediment. At B14 this fraction represents ca. 55% of the total organic C content in the upper 5 cm, at FF it represents ca. 80%. Refractory organic N amounts to 3 and 22 $gN.m^{-2}$ at B14 and FF respectively. This corresponds to nitrogen contents of 0.04 and 0.4 mg per g of dry sediment, being equal to ca. 30% of the total org.N content in the top 5 cm at B14 and ca. 60% at FF. The atomic C:N ratios in the refractory material (fraction 3) were calculated at 9:1 and 14:1 for B14 and FF respectively. The C:N ratios in fractions 1 and 2 are much lower (< 8:1). The unrealistically low ratio of fraction 1 of B14 (1:1) probably reflects the relatively large level of uncertainty in the low C,N contents of this fraction.

TABLE I.3

Estimated decay constants k_i (d^{-1}) of sedimentary organic matter in mesocosms, and estimated composition of added Phaeocystis material and of initial sedimentary organic matter in mesocosms (upper 0-5 cm). Estimates based on simultaneously fitting multi-G model (eqs. 7 a,b,c) to measured O_2 respiration rates and measured organic C and N contents. SM: Starved mesocosmms, FM: Fed mesocosms.

	Broad Fourteens (B14)		Frisian Front (FF)	
Decay constants				
k_1 10^{-3} d^{-1}	34 ± 9		26 ± 8	
k_2 10^{-3} d^{-1}	1.4 ± 1.4		4.7 ± 1.4	
k_3 10^{-3} d^{-1}	0		0	
Composition of supplied Phaeocystis material				
fraction 1 %	22		43	
fraction 2 %	0		0	
fraction 3 %	25		25	
not recovered %	53		32	
Composition of initial mesocosm sediments mg/g dry sed.	SM	FM	SM	FM
fraction 1 C	-	0.04	-	0.2
fraction 2 C	0.3	0.2	0.8	1.1
fraction 3 C	0.3	0.3	4.8	4.8
fraction 1 N	-	0.04	-	0.03
fraction 2 N	0.10	0.03	0.3	0.2
fraction 3 N	0.04	0.04	0.4	0.4
atomic C:N ratios				
fraction 1	-	1	-	8
fraction 2	4	8	3	6
fraction 3	9	9	14	14

Interstitial water

Pore water concentrations of NH_4^+ and NO_x (measured only in the FM) are given in Fig. I.10. Oxygen penetration depth as measured with micro-electrodes increased from ca. 8 (FF) and ca. 10 mm (B14) in May-June after the input of algal material to values of ca. 10 (FF) and ca. 15 (B14) at the end of the experiments. In the water overlying the FM, ammonium increased from ca. 6 µM in April-May to 10-11 µM at the time of the addition of Phaeocystis, and subsequently decreased to ca. 0.5 µM from August onwards. The corresponding NO_x concentrations varied between 40 and 50 µM.

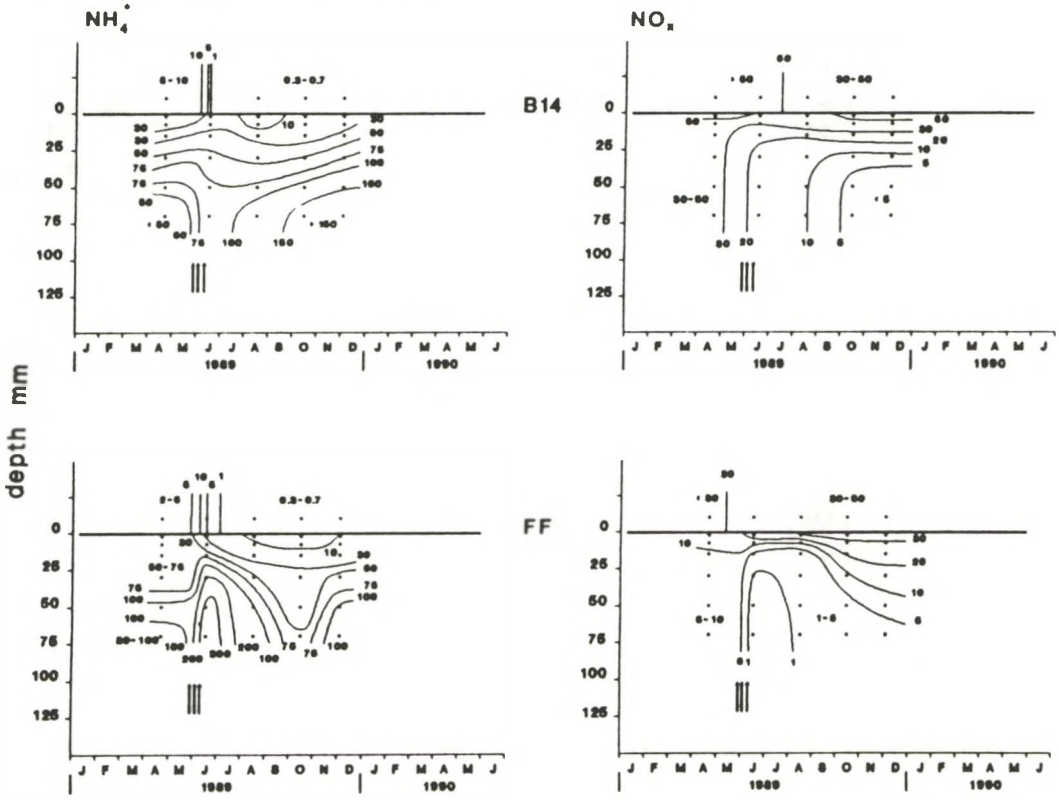


Fig. I.10. Ammonium and nitrate + nitrite (NO_x) concentrations (µM) in pore water from the upper 100 mm of sediments of the fed mesocosms (FM) of B14 (upper part) and FF (lower part). The first data points in each graph represent the field situation at the time of the collection of the mesocosm cores. Arrows indicate the addition of Phaeocystis material.

At FF the interstitial NH_4^+ concentration strongly reacted on the addition of organic substrate, values increased from 50-150 µM to values larger than 300 µM at depths > 50 mm. At the same time NH_4^+ in the upper 20 mm decreased, reaching minimum levels (< 10 µM) in August-October. At B14 the effect of the food supply was not clearly discovered in the ammonium concentrations, only the relatively low

concentrations below 50 mm observed before the addition disappeared. After the initial response, NH_4^+ in the subsurface layers in the FM of both B14 and FF increased, at B14 from August onwards to concentrations > 150 µM, at FF to values > 100 µM in November.

Although NO_x in the overlying water remained well above 40 µM, the initial high NO_x concentration (30-50 µM) below the oxic zone of the B14 sediment gradually decreased to values < 5 µM after the addition of Phaeocystis. At FF, NO_x in the layers below 15 mm immediately responded to the food supply by a decrease to values < 1 µM in June. This decrease of the NO_x concentrations in the deeper layers in both types of sediments indicates subsurface mineralization, probably because part of the added organics is transported from the sediment surface to depths > 20-100 mm within a short time. In the FM of the Frisian Front sediment, NO_x in the anoxic layers increased again 4-8 weeks after the food supply. In the upper 20 mm, the NO_x interstitial water concentration steadily increased from a few weeks after the supply onwards. Particularly at B14 high concentrations well above those in the overlying water developed, suggesting the presence of nitrification in this sandy type of sediment.

Denitrification and sediment-water exchange rates

Measured denitrification rates in the FM are presented in Fig. I.11. In the FM of FF denitrification decreased from ca. 0.11 in April-June to 0.04 $mmol.m^{-2}.d^{-1}$ during August-November. In the B14 sediments the food supply resulted in a substantial decrease of denitrification from 0.45 to 0.05 $mmol.m^{-2}.d^{-1}$ directly before and after the supply respectively. Hereafter denitrification increased again to a high rate in August, and then like in the FF mesocosms decreased to ca. 0.06 $mmol.m^{-2}.d^{-1}$ during October-November. On average, denitrification in the sandy B14 mesocosms was higher than in the silty FF sediments. Compared to the field data, the Frisian Front FM showed almost the same seasonal pattern. The minimum denitrification rate in June and the peak in August in the FM of B14 were however not observed in the field. The low rates in October-November were slightly higher in the mesocosms than in the field, probably due to higer NO_x concentrations in the water overlying the FM.

Included in Fig. I.11 are the sediment-water exchange rates of NH_4^+ and NO_x calculated from the pore water profiles after calibrating the model (eqs. 2 to 6) against the measured denitrification rates. Examples of modelling results are given in Fig. I.12, showing reasonable agreement between calculated and measured profiles. Detailed information on parameter estimates is given in Table I.4. The model outcomes in terms of rates per m^2 per day are linearly dependent on the applied diffusion coefficient. The calibration procedure revealed that in the FM the apparent whole sediment diffusion coefficient D decreased from 4.8 to 0.6 10^{-9} $m^2.s^{-1}$ in B14, and from 13.8 to 0.3 10^{-9} $m^2.s^{-1}$ in FF during the time-course of the experiments, i.e. from values being ca. 8 (B14) and 22 (FF) times molecular diffusion during collection of the cores in April to values

approximately equal to molecular diffusion in October-November. Obviously, these figures are very sensitive to the reliability of the denitrification data and the accuracy of the model in simulating the actual NO_x profiles. Nevertheless, the overall decrease of transport rates to values comparable to molecular diffusion seems clear.

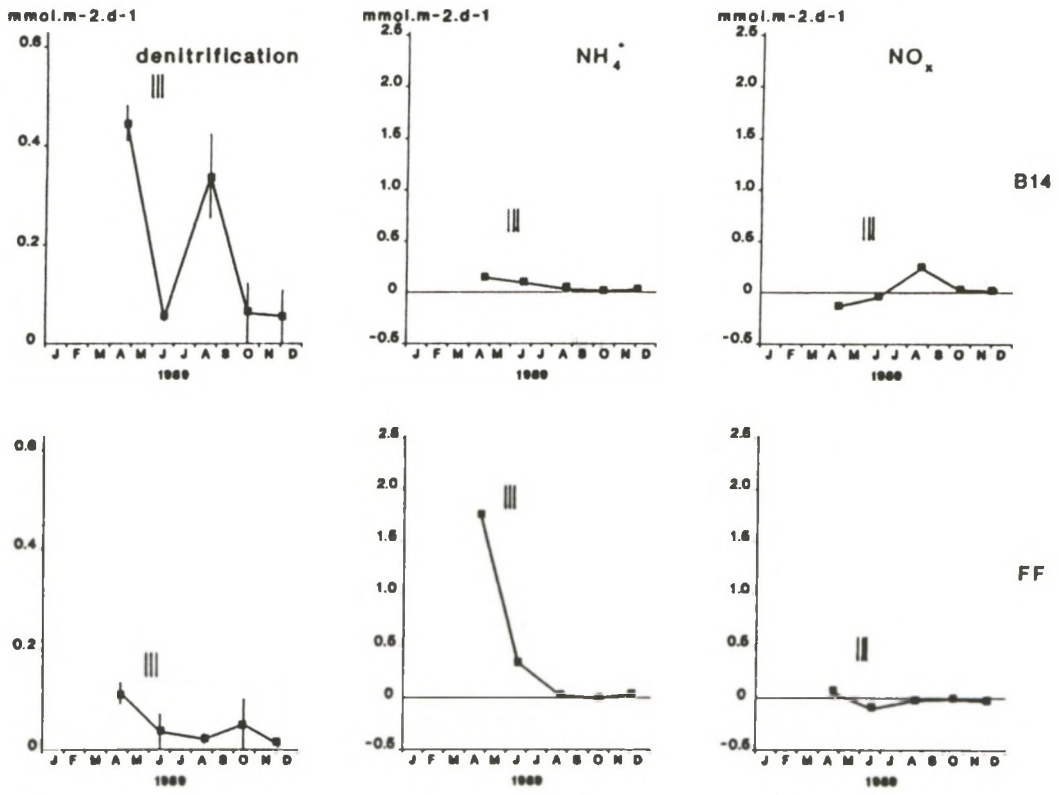


Fig. I.11. Benthic denitrification rate ($mmol.m^{-2}.d^{-1}$), and from pore water gradients estimated NH_4^+ and NO_x sediment-water exchange rates ($mmol.m^{-2}.d^{-1}$) in the fed mesocosms (FM) of B14 and FF. The first data point in each graph represents the field situation at the time of the collection of the mesocosm cores. Arrows indicate the addition of Phaeocystis material.

The ammonification rate R_a as estimated from the models decreased from high values in April to much lower values towards the end of the experiments. This especially holds for the FF sediments. Data obtained from literature indicate that R_a may range from 200 to 3000 $mmol.m^{-3}.s^{-1}$ (Vanderborght et al., 1977; Billen, 1978; Aller, 1980; Berner, 1980; Blackburn & Henriksen, 1983; van Raaphorst et al., 1990). The field data in April are well within this range, the sandy B14 sediments being at the lower end. From June onwards, the FM data are clearly below this range, both for B14 and FF, thus indicating the diminishing ammonification in the mesocosms. The depth parameter α decreases from 30-38 m^{-1} in April-June, to 12 m^{-1} in November in both type of sediments. Consequently, the vertical attenuation of the

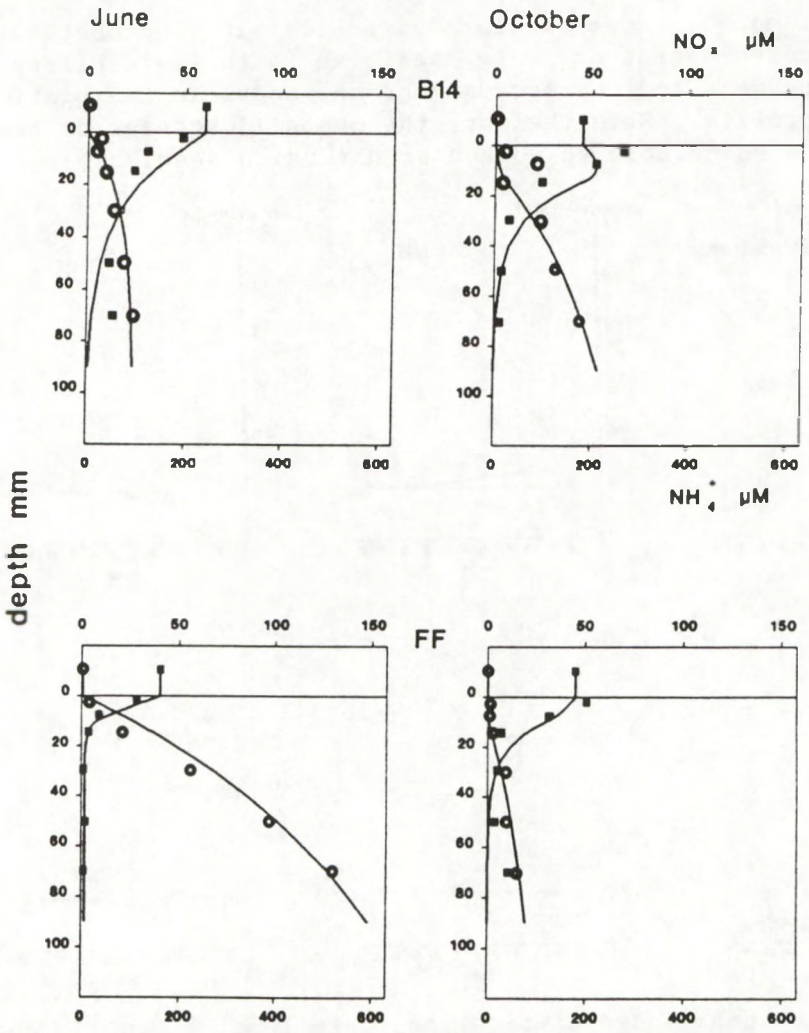


Fig. I.12. Examples of measured (symbols) and calculated (lines) pore water profiles in the fed mesocosms of B14 (upper part) and FF (lower part). Calculations according to the model described in eqs. 2-6. Open symbols: NH_4^+; closed symbols: NO_x.

ammonification rate became less during the experiments. Nitrification rate was very low in June (k_n = 0), directly after the addition of organic matter, and increased to peak values in August and October at B14 and FF respectively. The individual values are slightly lower than those obtained by van Raaphorst et al. (1990) for the Doggerbank area in the North Sea (on average 480 10^{-6} s^{-1}), but are in line with the literature review of Smits (1980) for several benthic systems (10 - 100 10^{-6} s^{-1}). Denitrification rate K_d ranged from 2 to 16 10^{-6} s^{-1}, being at the lower end of the range obtained for other North Sea sediments (0 - 550 10^{-6} s^{-1}, Vanderborght et al., 1977; Billen, 1978; van Raaphorst et al., 1990).

TABLE I.4

Values of apparent whole sediment diffusion coefficient (D, $m^2.s^{-1}$), ratio between D and molecular diffusion ($D:D_0$), zeroth-order ammonification rate (R_a, $mmol.m^{-3}.s^{-1}$), first-order nitrification rate (k_n, s^{-1}) and denitrification rate (k_d, s^{-1}), and depth constant α (m^{-1}) as estimated from fitting the nitrogen pore water model (eqs. 2-6) to the measured NH_4^+ and NO_x profiles in the fed mesocosms. a: Broad Fourteens, b: Frisian Front

a: Broad Fourteens (B14

Month	Week nr.	D 10^{-9} $m^2.s^{-1}$	$D:D_0$ (--)	R_a 10^{-6} $mmol.m^{-3}.s^{-1}$	k_n 10^{-6} s^{-1}	k_d 10^{-6} s^{-1}	α m^{-1}
April	16	4.8	8	320	80	16	30
June	24	1.2	2	58	0	2	38
August	33	4.8	6	216	432	8	25
October	41	0.4	1	38	20	3	15
November	48	0.6	1.5	30	10	3	12

b: Frisian Front (FF)

Month	Week nr.	D 10^{-9} $m^2.s^{-1}$	$D:D_0$ (--)	R_a 10^{-6} $mmol.m^{-3}.s^{-1}$	k_n 10^{-6} s^{-1}	k_d 10^{-6} s^{-1}	α m^{-1}
April	16	13.8	22	1600	230	2	35
June	24	0.7	1	77	0	6	12
August	33	0.2	0.3	8	4	4	12
October	41	0.3	0.6	5	100	3	12
November	48	0.3	0.6	8	1	2	12

The calculated ammonium fluxes across the sediment-water interface of the FM were substantially lower than those measured in the field. Both at B14 and in FF the mesocosms showed continuously decreasing fluxes of NH_4^+ without a clearly visible effect of the food supply. The absence of nitrification directly after the addition of Phaeocystis material (in June $k_n = 0$) together with the high NO_x concentration in the overlying water caused an influx of nitrate + nitrite in the FM sediments in June. In the B14 mesocosms this influx changed into an efflux from August onwards, confirming the influence of nitrification during that period. At FF however, the NO_x fluxes remained directed towards the sediment, suggesting that here nitrification was less pronounced.

I.4. DISCUSSION

O_2 respiration and sedimentary organic C, N contents

The present study deals with a sandy (B14) and a silty station (FF) in the southern North Sea. Between these stations consistent differences were found in organic C and N contents and also in mineralization activity as indicated by O_2 respiration, all of these variables being considerably higher at FF than at B14. Average organic C and N contents of the sediments were ca. 0.6 and 0.12 $mg.g^{-1}$ respectively at B14 and ca. 5 and 0.5 $mg.g^{-1}$ respectively at FF. Integrated over the full annual cycle of 1989, respiration at B14 was ca. 3 $molO_2.m^{-2}$, equivalent to ca. 30 $gC.m^{-2}$. For FF corresponding numbers are ca. 10 $molO_2.m^{-2}$ and ca. 100 $gC.m^{-2}$ respectively, being well in line with the respiration of 95 $gC.m^{-2}$ measured by Cramer (1990) at this station in previous years. Organic contents are determined by the dynamical balance between deposition of organic matter on the sediment surface, decomposition within the sediment and transport down to deeper layers (Froelich et al., 1979; Emerson et al., 1985). Obviously, the deposition of organic substrate is most crucial in benthic nutrient cycling (e.g. Berner, 1980; Klump & Martens, 1987). Both stations had a well mixed watercolumn throughout the entire period of investigation, providing a direct coupling between the productive pelagic part of the ecosystem and the sediment. Consequently, one might expect a relation between phytoplankton dynamics in the watercolumn and benthic activity. Particulary for periods immediately following the phytoplankton spring bloom, this benthic-pelagic coupling is well documented (Graf et al., 1982; Davies & Payne, 1984; Jensen et al., 1990). During these periods the pelagic food chain probably is not well enough established to consume the largest part of material derived from spring primary production, and a substantial part may then reach the sediments (Graf et al., 1982). Chlorophyll-a concentrations in the overlying water were higher at B14 than at FF, especially during the spring bloom, which at FF was hardly recognizable in 1989. Distinct summer peaks of chlorophyll-a were measured, at B14 in July and August, and at the station nearby FF in August. Field observations during previous years indicate that at the Frisian Front local chlorophyll-a maxima as compared to directly adjacent stations may develop at the end of the summer, concentrations being 1-3 $\mu g.l^{-1}$ higher at FF (Creutzberg, 1985). Consequently, the peak at FF in August may be even more pronounced than indicated in Fig. I.2. B14, however, is situated in a large erosive area (Eisma, 1981), where organic material not easily settles, while FF is much more favourable to deposition, at least during calm weather periods (Creutzberg et al., 1984). This means that the sediments of the Frisian Front may trap organic material originating from other areas in the southern North Sea, thus obscuring local benthic-pelagic coupling and explaining higher org. C and N contents of the sediment and higher respiration rates at FF.

For benthic nitrogen cycling not only the amount but also the

quality of the organic substrate is important. At FF the C:N ratio (10.5:1) is twice as high as that at B14 (5:1), suggesting that on average the organic content of FF is of lower nutritional value than that of B14. This is confirmed by the results of the multi-G model applied to the mesocosms. According to the model, ca. 80% of the organic C content and ca. 60% of the organic N content at FF in January and April 1989 was 'refractory' (decay times > several years). At B14 the corresponding numbers are ca. 55% and ca. 30%. The obtained estimates of the decay constants of the degradable fractions were of the same order of magnitude for both types of sediments. For the slowly degrading fraction this constant (k_2) was estimated at 1-5 10^{-3} d^{-1}, for the labile fraction k_1 = 26-34 10^{-3} d^{-1} (T = 15°C). These values are well in line with data from literature. Kristensen & Blackburn (1987) from very similar experiments in microcosms reported average decay constants of 0.5-1.3 10^{-3} d^{-1} (T = 22°C) for sediments collected in a shallow estuary. These estimates, however, represent total sedimentary organic carbon including very slowly degrading and refractory fractions, and thus may underestimate true decay rates (Hargrave & Phillips, 1989). For labile carbon in sedimented detritus in a macrotidal estuary, Hargrave & Phillips (1989) calculated decay times of 20-200 days in August and early September, and of 200-1300 days during winter, corresponding to decay rates of 10-50 10^{-3} d^{-1} and 0.8-5 10^{-3} d^{-1} respectively. This indicates that during summer total mineralization in this estuary was dominated by fastly degrading material well comparable to our fraction 1, while during winter slowly degrading matter comparable to our fraction 2 was most important. Using the multi-G model O_2 respiration as well as organic C, N contents in both the starved and the fed mesocosms were adequately described with Q_{10} = 2 for the decay constants. This Q_{10} value was based on data experimentally obtained by Hargrave & Phillips (1989). An almost tenfold seasonal increase in mineralization rates as indicated by the field data on O_2 respiration, is therefore unlikely explained by variation of temperature during the annual cycle. Middelburg (1989) elegantly demonstrated that multi-G models are equivalent to models considering decomposition of organic matter as a whole, but with first-order rate parameters gradually decreasing with time after deposition, i.e. with age of the material. According to his model (extensively calibrated on literature data) organic matter of which decomposition is dominated by the labile fraction 1 has an age of only a few days to one week, and the presence of this fraction thus indicates recent deposition of fresh material. When fraction 2 is dominating, organic matter would have an age of a few weeks to a few months. This is confirmed by other mesocosm experiments (Kelly & Nixon, 1984; Enoksson, 1987), indicating that the initial increase in benthic respiration after addition of algal material is diminished within a few weeks. Consequently, the high respiration rates during summer are unlikely related to oxidation of organic substrate derived from the spring bloom, but rather reflect deposition of decomposable organics during the summer period it self.

In Fig. I.3 we fitted exponentially decreasing functions to the

measured vertical organic C and N profiles. If steady state is valid these functions are equivalent to the multi-G model combined with random-type mixing of solids (Berner, 1980). The lines in Fig. I.3 suggest that at B14 the *in situ* profiles may adequately be described by the exponential model, suggesting that random mixing probably is the dominating transport mechanism here (Berner, 1980). At FF, the model can only describe the profiles below the subsurface maxima of organic C and N. These maxima point at a dominating transport mechanism other than random mixing. At FF *Amphiura filiformis* is very abundant reaching densities upto 1200 ind.m^{-2} (Cramer, 1991; Duineveld & Moodley, 1991). This echinoderm usually lives at approximately 30 mm depth (Ockelmann & Muus, 1978) transporting food from the sediment surface towards its mouth with one of its arms. As a result of this feeding behaviour a substantial part of the organic matter deposited on top of the sediment is advectively transported downwards, thus explaining the observed maxima.

Interstitial water

The pore water data of NO_x show the occurrence of nitrification mainly from fall to spring. During summer (August) no enhanced NO_x was observed in the oxidized zone of the sediments. The same conclusion may be drawn from the C_b values estimated from the benthic fluxes of nitrate + nitrite, being lowest in summer (Table I.2). This pattern was not observed by Rutgers van der Loeff (1980) in off-shore sediments of the Southern Bight of the north Sea, where he measured only slightly lower peak-NO_x concentrations in summer (5-25 µM) as compared to winter (10-35 µM). His NH_4^+ and NO_x profiles however suggest oxygen penetrations of a few cm´s also in summer, while in our sediments penetration was only 4 (FF) and 8 mm (B14) in August. The discrepancy between the profiles of Rutgers van der Loeff (1980) and ours would then be explained by different O_2 availability to the benthic nitrifiers (Kemp et al., 1990). At the Doggerbank area of the North Sea, van Raaphorst et al. (1990) measured distinct nitrate peaks in the upper 5-10 mm of the sediments in August, while oxygen penetration was 4-15 mm and also O_2 respiration (5-20 mmol.m^{-2}.d^{-1}) was comparable to that at B14 (12 mmol.m^{-2}.d^{-1} in August). Moreover, the benthic NO_x fluxes as measured at both B14 and FF increase from August onwards, thus indicating the presence of nitrification during this entire period. An efflux of NO_x and the absence of a NO_x peak in the pore water was also observed by Kristensen & Blackburn (1987) in microcosm experiments and they concluded that a high nitrate production rate may have been present directly at the sediment-water interface. Since our sampling interval in the upper layers was 5 mm, it is likely that we missed enhanced concentrations in the very top layer of the sediment.

Ammonium in the interstitial waters of B14 and FF showed a distinct seasonal pattern, concentrations at the latter station always being higher than at the former. The maxima in August (125-150 µM at FF and ca. 40 µM at B14) are at the lower end of values reported for many

other marine sediments (10^2-10^4 µM, e.g. Vanderborght et al., 1977; Aller, 1980; Rosenfeld, 1981; Hopkinson, 1987; Klump & Martens, 1987; Mackin et al., 1988; Kemp et al., 1990), but they compare well with concentrations previously being measured in off-shore North Sea sediments (Rutgers van der Loeff, 1980; Law & Owens, 1990; van Raaphorst et al., 1990). Jensen et al. (1990) demonstrated a very significant peak in the ammonium concentration in the upper 0-2 cm of Aarhus Bight sediments in the first two months after deposition of organic material derived from the phytoplankton spring bloom. At B14 concentrations were fairly constant between April and August 1989, particularly in the upper 5 cm´s, while at FF the main increase occurred in June and August. These findings support the hypothesis that in 1989 substantial deposition of degradable matter took place during summer. In both types of sediments ammonium concentrations gradually decreased from August onwards, pointing at a decrease in benthic ammonium production during this period. This pattern is only partly mimicked by the fed mesocosm experiments, where ammonium concentrations in the pore water further increased after the initial response to the addition of Phaeocystis material. Modelling the mesocosm pore water NH_4^+ and NO_x profiles and calibrating the results against measured denitrification rates, indicated that this longer term increase was caused by declining diffusive transports rather than by increasing ammonification rates, which actually decreased during the experiments (R_a in Table I.4). In the FM, ratios between apparent diffusion and molecular diffusion ($D:D_0$) decreased from 8-22:1 in April to 0.6-1.5:1 in October-November. This effect was also observed by Enoksson (1987) in her experimental systems and it is probably caused by inactivation of bioirrigating fauna due to depletion of suitable food (Appendix Chap. IV). This stresses the problems that may be encountered during mesocosms experiments. Several important factors, e.g. hydrodynamics in the overlying water, supply of organic substrate (quantity and quality) and macrofaunal activity probably did not completely mimic in situ conditions. This may have resulted in the observed deviations in the mesocosms as compared to the real world, particularly after several months. In the field, benthic macrofauna probably remained active for a longer period. To check for this we applied the nitrogen pore water model (eqs. 2 to 6) also to the in situ pore water profiles and calibrated the results against measured sediment-water exchange rates and denitrification. Resulting ratios between apparent diffusion and molecular diffusion ($D:D_0$) obtained from these calculations vary from 10-20:1 in April and June to 4-5:1 and 2-3:1 in August and November respectively in both B14 and FF. The seasonal variation is comparable to the observations by Rutgers van der Loeff et al. (1984) in a Swedish fjord. Using the asphyxiation technique they measured much lower $D:D_0$ ratios in winter than in summer. The pattern suggests a general decrease in biologically mediated transport from April/June to November, indicating a diminishing bioirrigation in the second half of the year also in the field, however less severe as in the mesocosms. The obtained $D:D_0$ ratios agree well with literature data on biologically mediated

diffusion. According to Callender & Hammond (1982); Rutgers van der Loeff et al. (1984); Aller & Yingst (1985); Helder & Andersen (1987) and Hofman et al. (1991) sediment-water exchanges may be enhanced 2-10 times when bioirrigating fauna is abundant compared to situations where macrofauna is absent. From the work of Billen (1978) an average apparent diffusion coefficient of 1 10^{-8} $m^2.s^{-1}$ is concluded for North Sea sediments off the Belgian coast, implying $D:D_0 \approx 15:1$ for NH_4^+ and NO_x. At B14 and FF apparent diffusion was only this high in April and June. During other months $D:D_0$ was almost equal to ratios obtained by van Raaphorst et al. (1990) for the Doggerbank area during August 1988 ($D:D_0$ = 1-6:1). These findings demonstrate that within a large area as the southern North Sea regional differences may exist in apparent diffusion. Moreover, the present results show that apparent diffusion is not constant for a given sediment, but that it may vary considerably over the year.

Sediment-water exchange rates and denitrification

Benthic fluxes of ammonium and nitrate + nitrite showed a clear seasonal pattern. Exchange rates of NH_4^+ covaried with O_2 respiration with highest values in June (B14) and August (FF) and lowest values in winter. At B14, NO_x fluxes were directed into the sediment during the first half of 1989, pointing at an important denitrification activity during this period. The large influx of NO_x at B14 in April 1989 is most likely caused by the high NO_x concentrations in the overlying water at that time. At FF an efflux of NO_x was measured throughout the year, however at very low rates during the period January-June 1989. Annually averaged exchange rates of ammonium were ca. 1.0 and 0.2 $mmol.m^{-2}.d^{-1}$ at FF and B14 respectively, those of NO_x were ca. 0.14 and -0.03 $mmol.m^{-2}.d^{-1}$ for the same stations respectively. The fluxes at B14 are lower than release rates obtained by Rutgers van der Loeff (1980) for well comparable stations in the Southern Bight of the North Sea. Based on pore water gradients and an apparent diffusion coefficient of 1 10^{-8} $m^2.s^{-1}$ he calculated average ammonium fluxes of ca. 0.9 $mmol.m^{-2}.d^{-1}$ and NO_x release rates between 0.04 (winter) and 0.35 $mmol.m^{-2}.d^{-1}$ (summer). Obviously, the fluxes of Rutgers van der Loeff (1980) are directly dependent on the applied diffusion coefficient and this may have lead to overestimating average release rates (van Raaphorst et al., 1990). The present data of B14 are well in line with fluxes measured in the sandy Doggerbank area (van Raaphorst et al., 1990), being 0.0 to 0.7 $mmol.m^{-2}.d^{-1}$ for NH_4^+ and 0.1 to 0.3 $mmol.m^{-2}.d^{-1}$ for NO_x during August 1988.

In Aarhus Bight Jensen et al. (1990) observed large benthic effluxes of ammonium (up to 1.5 $mmol.m^{-2}.d^{-1}$) and large influxes of nitrate (ca. 0.8 $mmol.m^{-2}.d^{-1}$) directly after the phytoplankton spring bloom. After a few months ammonium release rates decreased to a much lower level and nitrate influxes turned into effluxes. They explain this pattern by the occurrence of high rates of mineralization and denitrification immediately after deposition of material derived from the spring bloom. At the same time nitrification was virtually absent,

probably due to reduced O_2 penetration depths. Basically, the same pattern was observed at B14 and FF, although less dramatic. Here, major deposition of organic matter probably occurred in April-June and during these months ammonium release rates strongly increased while NO_x fluxes were negative (B14) or very small (FF). The seasonal cycle obtained for B14 and FF is very similar to that measured by Kemp et al. (1990) in Chesapeake Bay, who also found increasing ammonium effluxes from April onwards reaching maximum values in August and nitrate consumption by the sediments in the first half of the year. They conclude from separate measurements that both nitrification and denitrification were small during summer, when O_2 penetration was small. According to their results total nitrogen regeneration to the watercolumn may largely depend on the existence of benthic nitrification-denitrification coupling, particularly in periods when nitrate concentrations in the overlying water are low. The present data with high ammonium release rates in summer (minimal O_2 penetrations) seem to support this conclusion.

Measured denitrification rates are in accordance with the above statements. The seasonal pattern reveals high rates in April and June and virtual elimination of denitrification in August, but also in November. Similar patterns were found by Kemp et al. (1990) and Kieskamp et al. (1991) in estuarine sediments. Obviously, the high rate at B14 in April 1989 was related to the high NO_x concentration in the overlying water. Averaged over the full anual cycle of 1989, denitrification rates were ca. 0.12 and 0.08 $mmol.m^{-2}.d^{-1}$ at B14 and FF respectively. This means that sedimentary nitrogen removal was higher at the sandy B14 than in the silty Frisian Front. These rates compare well with values given by Billen (1978); Law & Owens (1990) and van Raaphorst et al. (1990) for several other North Sea sediments (0.005 - 1.4 $mmol.m^{-2}.d^{-1}$). Denitrification rates were based on the acetylene block method. Recent publications indicate that this method may systematically underestimate true denitrification rates (Kemp et al., 1990; Devol, 1991), and consequently absolute values presented here should be regarded with caution. We believe, however, that the seasonal pattern is correct (Kemp et al., 1990), and so probably will be the measured order of magnitude.

The seasonal patterns of sediment-water exchange of nitrous oxide show distinct consumption by the sediment in June and effluxes in in April and August. From data of Jørgensen & Sørensen (1985) for the Kysing Fjord and of Kieskamp et al. (1991) for the western Dutch Wadden Sea it may be concluded that maximum effluxes of nitrous oxide are coupled to maximum denitrification rates. The present data demonstrate that this may partly hold for the North Sea also (April), but that substantial influxes may occur at relatively high denitrification rates (June). This influx may be related to the utilization of nitrous oxide as a terminal electron acceptor as suggested by Kieskamp et al. (1990), particularly during increase of denitrification activity as a result of a decrease in oxygen penetration in the sediment (Jensen et al., 1984) and at the same time limited NO_3^- availability to the denitrifiers.

Nitrogen cycling

Numerous studies have dealt with nitrogen cycling and exchange processes in marine sediments, particularly in coastal and estuarine systems (e.g. Balzer, 1984; Smith et al., 1985; Enoksson, 1987; Klump & Martens, 1987; Jørgensen & Sørensen, 1988; Jensen et al., 1990; Kemp et al., 1991; Lomstein et al., 1990; Devol, 1991; Kieskamp et al., 1991). Many of these sediments have large organic contents and high dissolved nitrogen concentrations in the overlying water as compared to off-shore areas. Part of these studies stress the importance of the sedimentation of fresh algal material, triggering benthic ammonification and denitrification, and they also indicate that due to high nitrate concentrations in the overlying water the coupling between nitrification and denitrification (Jenkins & Kemp, 1984) may be weak or absent (Jørgensen & Sørensen, 1988; Jensen et al., 1990; Lomstein et al., 1990; Kieskamp et al., 1991). In these sediments oxygen consumption is high and oxygen penetration depth is less than a few millimeters. Consequently, nitrification is relatively unimportant and denitrification may largely be fuelled by nitrate diffusing into the sediments from the bottom water. On the other hand, results of Kemp et al. (1990) for Chesapeake Bay sediments, also having low oxygen penetration depths, confirm the importance of direct nitrification-denitrification coupling. Investigations on benthic nutrients in the North Sea (Billen, 1978; Rutgers van der Loeff, 1980; Law & Owens, 1990; van Raaphorst et al., 1990) suggest that in the off-shore areas nitrogen cycling may differ substantially from estuarine and coastal sediments. Off-shore, sedimentary organic contents are low, particularly in the sandy sediments (at B14 < 0.8 mgC per g of dry matter), and also dissolved nutrient concentrations in bottom water and interstitial water are considerably lower than near the coast (Rutgers van der Loeff, 1980). These sediments may have larger oxygen penetration depths and thus nitrification may be the dominating nitrate source for denitrifiers (Law & Owens, 1990; van Raaphorst et al., 1990). This statement is supported by the benthic N-fluxes and denitrification rates measured at B14 and FF as discussed in previous sections.

To further elaborate benthic N cycling at B14 and FF and to calculate net ammonification and net nitrification, we applied the same basic conceptual model depicting N pools, transformations and fluxes across the sediment-water interface as considered by Kemp et al. (1990). In this simplified concept net ammonification = net nitrification + ammonium release to the watercolumn, and net nitrification = denitrification + NO_x-flux towards the overlying water. Resulting values are presented in Fig. I.13. Net ammonification rates were only slightly higher than measured DIN exchanges to the watercolumn, particularly at FF. On an annual base, net ammonification was 0.4 and 1.2 $mmol.m^{-2}.d^{-1}$ at B14 and FF respectively, while anually averaged DIN releases were 0.2 and 1.2 $mmol.m^{-2}.d^{-1}$ at these stations.

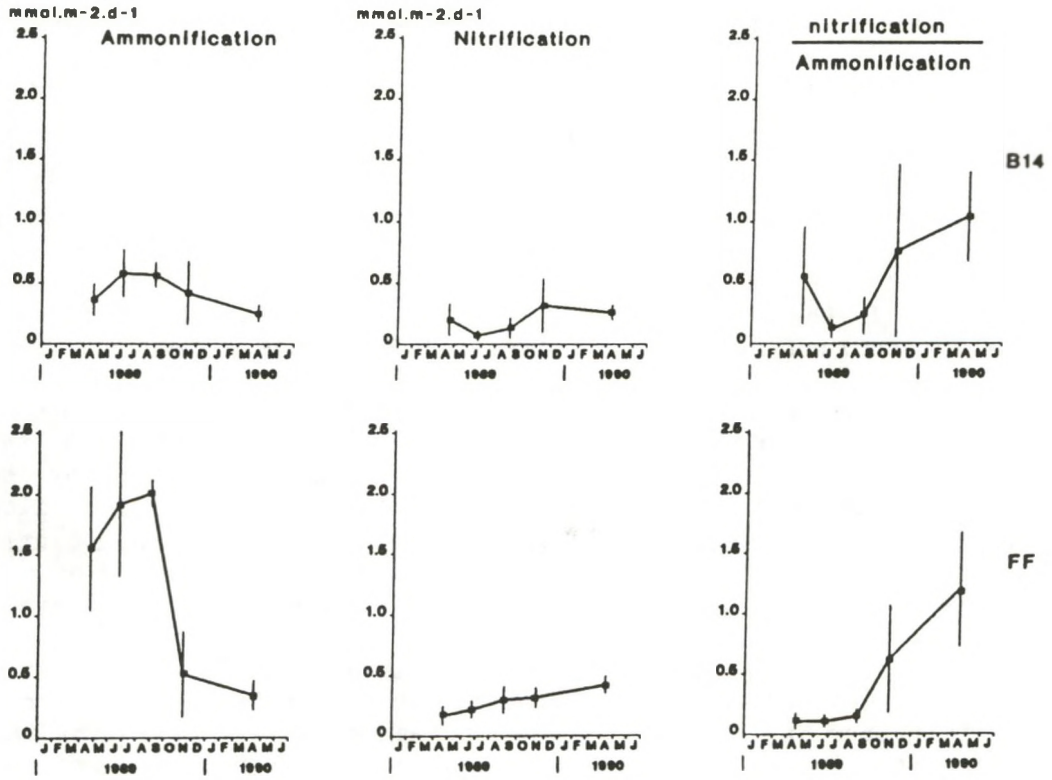


Fig. I.13. Calculated net ammonification and net nitrification rates (mmol.m^{-2}.d^{-1}), as well as the ratio between net ammonification and net nitrification in sediments of B14 (upper part) and FF (lower part).

Consequently, ca. 50% of the ammonium produced in the sediment of B14 and almost all NH_4^+ being produced at FF was regenerated to the watercolumn. This seems to support the conclusions of Enoksson (1990) for the Kattegat and Kemp et al. (1990) for Chesapeake Bay that increased levels of deposition and consumption of organic matter may lead to reduction in rates of benthic N removal. The patterns of net nitrification show minimum values during summer at B14 when ammonification was highest, and gradually increasing values from April 1989 onwards at FF. Major factors controlling nitrification are temperature (equal at B14 and FF) and the availability of oxygen and ammonium. The average rates in 1989 were ca. 0.1 and 0.2 mmol.m^{-2}.d^{-1} at B14 and FF respectively. Consequently, on an annual base ca. 3 times higher net ammonification rates together with lower oxygen availability at FF as compared to B14, apparently resulted in twice as high net nitrification rates. Especially during summer at high temperatures and extended mineralization rates, the effect of enhanced ammonium availability was counteracted by lesser O_2 penetration depths. This statement becomes more evident from the ratio net ammonification to net nitrification (Fig. I.13). During summer and at FF also in April 1989 this ratio is low at both stations (0.1-0.2).

From november onwards this ratio increases to values > 1, indicating that during this period with low mineralization rates and consequently larger O_2 penetration, all sedimentary organic nitrogen being mineralized was nitrified. Comparing anually averaged denitrification rates (0.12 at B14 and 0.08 mmol.m^{-2}.d^{-1} at FF) with corresponding NO_x releases (-0.03 at B14 and 0.14 mmol.m^{-2}.d^{-1} at FF) and with the above net nitrification rates reveals that on an annual base nitrification is the far most important nitrate source for denitrification, supplying > 75% of total denitrifiers demands. Only at B14 in April 1989 when NO_x in the overlying water was very high, direct diffusion from the watercolumn largely contributed (54%) to these demands.

The above discussion suggests that benthic nitrogen removal due to denitrification may be reduced at increased organic inputs to the sediment. More evidence for this conclusion may be gained from the mesocosm experiments, particularly from the fed mesocosms to which Phaeocystis material was supplied. The total amount of Phaeocystis that was recovered in the mesocosms was 11 gC.m^{-2} at B14 and 16 gC.m^{-2} at FF, representing ca. 35% and 15% of the *in situ* annual carbon mineralization at B14 and FF respectively. In both mesocosms the initial response to the input of Phaeocystis material was a dramatic increase in oxygen consumption and a decrease in denitrification rate. Interstitial water NH_4^+ concentrations increased immediately after the addition, particularly at FF. Ammonium concentrations in the overlying water increased in the first week after the supply, pointing at stimulated NH_4^+ exchange rates, but decreased hereafter to lower values. In the FF mesocosms low denitrification rates remained towards the end of the experiments, in those of B14 denitrification recovered in August but decreased again thereafter. From pore water modelling (Table I.4) it was concluded that in both types of sediments no nitrification occurred in June (k_n = 0 d^{-1}), i.e. shortly after the supply of organic substrate. In the FM nitrification recovered in August (B14, k_n = 432 d^{-1}) and October (FF, k_n = 100 d^{-1}) when benthic O_2 respiration had decreased to much lower levels as compared with June. This would mean that the food supply caused a temporary elimination of benthic nitrification activity, probably due to reduced oxygen availability. The high nitrification rate in the B14 mesocosm in August coincided with a high denitrification rate and a large efflux of NO_x to the water column as estimated from the pore water data. This again confirms the importance of nitrification-denitrification coupling in benthic N cycling (cf. Kemp et al., 1990), even at NO_x concentrations in the overlying water as high as 40-50 µM as being present in the mesocosms. Enoksson (1987) concluded from similar experiments that the addition of algal material induced a substantial raise in oxygen consumption and release of inorganic nitrogen, and a decrease in the ratio denitrification to net ammonification. The present results fit well within this conclusion, but she also found that dissolved organic nitrogen was a significant part (30-50%) of the nitrogen released from her experimental systems, both before and after the addition of algal material. This likely also

occurred in our mesocosms, and probably also in the field. Comparing the organic N loss rates in the FM as estimated from the multi-G model to the measured denitrification rates and calculated fluxes of inorganic nitrogen, it follows that a substantial part of this organic N loss is not recovered as inorganic nitrogen. This may partly be explained by possible changes in intracellular and adsorbed ammonium and nitrate pools in the sediment (Lomstein et al., 1990), but substantial releases of DON may not be excluded. Clearly, this subject needs further attention in future research on benthic N cycling.

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APPENDIX II

Mesocosm experiments: mimicking seasonal developments of microbial variables in North Sea bottoms

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II. MESOCOSM EXPERIMENTS: MIMICKING SEASONAL DEVELOPMENTS OF MICROBIAL VARIABLES IN NORTH SEA BOTTOMS*

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ABSTRACT

We investigated the applicability of mesocosms with respect to seasonal development of microbial variables in the benthic system of the North Sea. From 2 North Sea locations, a sandy (28 m depth) and a silty sediment (38 m depth), undisturbed sediment cores were taken and installed in mesocosms in Januari-April 1989. Cores were kept at *in situ* temperature in the dark until December 1989. One set of sandy and silty sediments was starved and the other set received an organic matter supply in May-June, simulating the settlement of the spring bloom of *Phaeocystis* spec. Seasonal developments in bacterial production (methyl ^{3}H-thymidine incorporation), abundance and biomass of bacteria and nanoflagellates and oxygen consumption were compared between the mesocosms and the field in surface sediments every 1.5 to 2.5 months. Effects of seasonal temperature variations (range 6 - 17.5 ^{0}C) on microbial variables in starved mesocosms were limited, which possibly indicates a subordinate role of temperature in microbial processes in North Sea sediments. Organic matter supply elicited a direct response of bacterial production and oxygen consumption in mesocosms. Bacterial and protozoan abundance also increased. The effect of the organic input was extinguished within 2 months and values of enhanced variables declined to initial levels. The organic matter enrichment in mesocosms apparently did not provide sufficient energy to keep the microbenthos active at field levels through summer. Our results suggest that in the silty sediment in the field organic matter was available for bacterial production throughout summer. In sandy sediments the major organic matter input which set the seasonal pattern, appears to be in June. Apparently the seasonal development of microbial variables can be mimicked in mesocosms with organic matter supplies. Differences between field and mesocosms are further illustrated by carbon budgets.

II.1. INTRODUCTION

Mesocosm systems were introduced to study natural processes which are not readily studied *in situ* (Lalli, 1990). Systems meet the requirements of size in which the components of interest for a

particular research behave "naturally" and are not restricted by the containment per se. As such, systems are realistic, replicable and manipulable (Grice & Reeve, 1982). Benthic mesocosms are now in use at several institutes (de Wilde, 1990) and the field resemblance of systems tested appears acceptable (Berge et al., 1986; Santschi, 1985). So far, performance of bacteria and protozoa in benthic mesocosms in comparison to the field received little attention.

Field studies on the seasonal development of microbial variables in marine sediments suggest that organic matter input is an important stimulus for benthic microbial activities (Meyer-Reil, 1983, 1987; van Duyl & Kop, 1990). Microbial activities appear to be enhanced by organic enrichment of the sediment following a sedimentation event. Particularly the settlement of the spring phytoplankton bloom arouses a strong response of microbial activities in Kiel Bight sediment (Meyer-Reil, 1987) and bacterial production and protozoan abundance in Wadden Sea sediments (Bak & Nieuwland, 1989; van Duyl & Kop, 1990). Indications are also available that temperature may be a controlling factor in bacterial activity in sediments (Findlay et al., 1989; Boström et al., 1989; van Duyl & Kop, 1990). Cramer (1990) found significant relations between temperature and benthic oxygen consumption in North Sea sediments in a depositional area. Causal relationships between microbial activities, input and temperature could not be assessed in these, mainly descriptive, studies. The actual input of organic matter to the benthos is difficult to determine particularly in estuarine and subtidal shelfsea areas where reliable organic matter sedimentation measurements are prevented by turbulence and resuspension. If seasonal temperature variation covaries with organic matter input in the field, the controlling factor for benthic activity is difficult to delineate. In benthic mesocosms organic matter input in the sediment and temperature can be controlled separately.

The present study was conducted to test the performance of North Sea bottoms in mesocosms and to assess the possibility of mimicking the seasonal development of North Sea sediments in mesocosms. In the attempt of mimicking we intend to increase our insight in the controlling factors of seasonal development in North Sea benthic systems. Emphasis lays on the microbial processes in sediments and aspects of nutrient regeneration are treated elsewhere (Appendix Chapter I). The development of microbial processes in the field was compared with those in the mesocosms. We focused on the following questions:

1. is temperature or organic carbon supply the major factor controlling aerobic microbial processes in North Sea sediments?
2. is a single fresh organic carbon supply in May-June (simulating the settlement of the spring bloom of Phaeocystis) sufficient to generate seasonal patterns in microbial processes occurring in the field?

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II.2. MATERIAL AND METHODS

Experimental set-up

Undisturbed sediment cores (0.25 m^2) were collected with Scripps corers at 2 sites in the North Sea, a sandy sediment from Broad Fourteens (median grain size 200-250 µm, 28 m depth) and a silty sediment from the Frisian Front (median grain size 125-150 µm, 38-40 m depth). In Fig. II.1 the geographical position of the sites is indicated together with additional bottom characteristics. At sea Scripps corers containing intact sediment cores were hoisted up the deck and placed in wooden crates with lids on top in which they were transported to the laboratory. During the boatride cores were supplied with North Sea water. After arrival crates were immediately transported to the mesocosms, where the cores were lifted out of the crates, bottom spades and sides of corers were removed and the sediment cores were accomodated in equally sized containers with as little disturbance as possible. Installments in mesocosms took place in Januari-April 1989 and sediments were kept at *in situ* temperature in the dark until December 1989.

North Sea water in two 60 m^3 reservoirs was used for the water circulation in the mesocosms. The reservoirs were refilled with "fresh" North Sea water twice during the experiment. One set of sandy and silty sediments was starved (installed in January) and the other set, which was installed in April, received algal material which was collected with 50 µm plankton nets in the field during the spring bloom of *Phaeocystis pouchetii* in April-May. The material was scraped from the nets, froozen, subsequently thawed and divided in equal portions, which were supplied to half of the sediment cores at regular intervals in 2 weeks time (24 May, 31 May and 7 June). The organic matter supply was put into the overlying water and settled quickly on the sediment surface. The water circulation was resumed after 24 h.

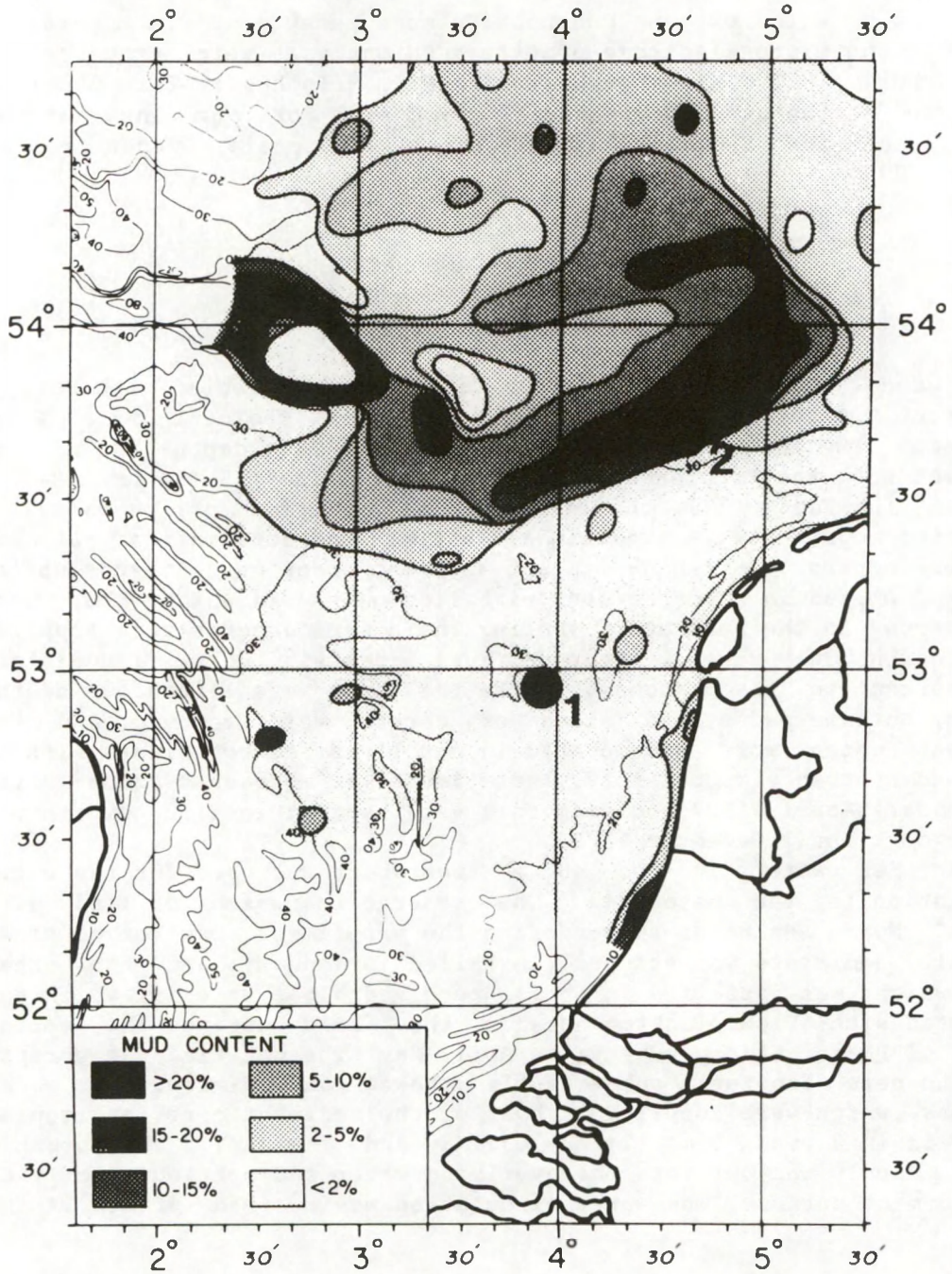

Fig. II.1. Location of sample sites Broad Fourteens, sandy sediment (1) and Frisian Front, silty sediment (2) in the southern North Sea. Shading indicates mud content (particles < 50 µm) of bottom sediments (adapted from Creutzberg et al., 1984).

The total carbon supply was 23 g $C.m^{-2}$, equivalent to the annual metabolic loss of sandy North Sea sediments (de Wilde et al., 1984; Cramer, 1990). Losses of the organic matter supply in the waterphase were 32% (7 $gC.m^{-2}$) for the silty sediments and 53% (12 $gC.m^{-2}$) for the sandy sediments. The starved and organically enriched sediment cores were maintained separately, with separate water circulation systems. Before organic matter supply and/or sampling the water level was lowered until ca 10-20 cm above the sediment surface which excluded the 0.25 m^2 sediment cores from the water circulation. Field measurements were carried out 5 times in 10 months time (every 1.5 to 2.5 months) to compare the development of microbial variables in the mesocosms with the developments in the field.

Measurements

Oxygen consumption was measured with PVC bell jars according to Cramer (1989). Two replicates were usually measured. The thickness of the oxygenated zone of the sediment was determined with micro-electrodes (van Duyl & Kop, 1990).

Sediment samples were taken in fresh bottom cores collected with modified Reineck-type boxcorers from the field and in mesocosms. The 0.5 cm surface layer was taken of ca 10 cores (diameter 2.5 cm) from a sediment surface of 0.25 m^2. Unless a bottom section in the mesocosm was completely sacrified by sampling, the sediment was prevented from collapse after sampling by placing an outer tube around each 2.5 cm core. After removal of the core sample the outer tube was left behind in the sediment. The required sediment was pooled in a glassjar, gently stirred with a spatula and immediately subsampled for bacterial production (12 replicas to determine isotope dilution) and bacterial abundance and biomass (one slide determination per sample).

Bacterial production was measured with (methyl-^{3}H) thymidine and the bacterial abundance and biomass with epifluorescence microscopy (van Duyl & Kop, 1990). For bacterial biomass production the following conversion factors were used: $1 * 10^{18}$ cells formed. mol^{-1} thymidine incorporated in DNA (Bell, 1986; Moriarty 1988) ; bacterial carbon content $2.2 * 10^{-10}$ mg $C.\mu m^{-3}$ (Bratbak & Dundas, 1984).

The efficiency of DNA recovery from the sandy and silty sediments was estimated using a ^{3}H-thymidine labelled batch culture of a mixed natural bacterial population. After filtration of 0.5 l seawater over 5 µm nuclepore filters 0.5 g glucose and 0.1 g yeast extract were added and the sample was incubated at room temperature with a stirring device. In the exponential growth phase ^{3}H-thymidine was added and the sample was incubated with a surplus of ^{3}H-thymidine for ca 1.5 h. The labelled bacteria were collected with centrifugation. The supernatant was aspirated and the pellet resuspended in filtered seawater with tetraborate buffered formaldehyde (2%). This wash procedure was repeated once.

For determination of recovery of DNA from sediments 100 µl sediment was mixed with 50 µl labelled bacteria suspension and processed according to the standard procedure (Moriarty, 1990) and a comparable procedure in which ethanol washes were replaced by 0.2 µm filtered sea water washes. Results are listed in Table II.1. Washes with ethanol reduced the recovery efficiency of the labelled batch, particularly in sandy sediments (with ca 10%, see Table II.1). Recovery efficiencies determined in the series which were washed with 0.2 µm filtered sea water were applied to estimate actual bacterial production as presented in Figs II.4a, b.

TABLE II.1
DNA recovery from sediments of a batch culture with labelled (^{3}H) thymidine with the standard procedure (Moriarty 1990) and with a procedure in which ethanol washes were replaced by filtered seawater washes.

DNA recovery	SILTY SEDIMENT	SANDY SEDIMENT
with ethanol washes	24 ± 2% (n=12)	48 ± 6% (n=12)
with filt. seaw. washes	27 ± 4% (n=10)	57 ± 7% (n=12)

Heterotrophic nanoflagellate (< 20 µm) densities were sized and counted in the surface layer of 3 mm of the sediment according to Bak & Nieuwland (1989), Appendix Chapter III. On each sampling date 5 tubes (2.5 cm diameter) per 0.25 m^2 sediment were taken and analysed. A mean carbon content of $2 * 10^{-10}$ mg C.μm^3 was used (Fenchel, 1982; Børsheim & Bratbak, 1987).

Total organic carbon content of the sediment surface layer (0-5 mm) was determined by HCN-analyser. Samples were dried at 60 ^{0}C, grinded and analysed according to Verardo et al. (1990). Two replicates were analysed per sediment sample.

II.3. RESULTS

Temperature

Fig. II.2 shows the temperature curves as measured during earlier cruises to the Frisian Front and the Broad Fourteens. The temperature regime in mesocosms was established weekly according to the mean of these curves. Field temperatures measured in 1989 fitted well on the curve. Highest water temperatures were always reached in August.

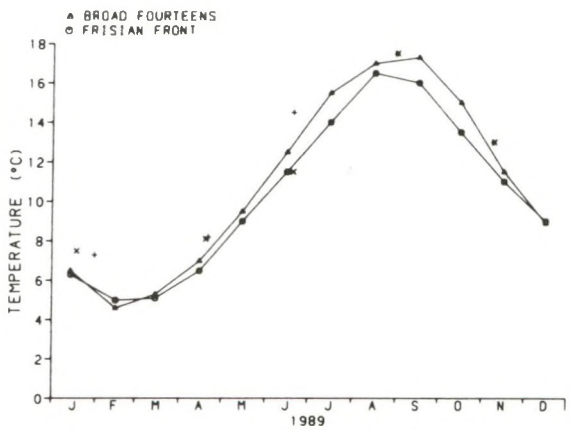

Fig. II.2. Seasonal variations in temperature at the Broad Fourteens and the Frisian Front. Temperature measurements in 1989 in the field are shown as single data points (+ Broad Fourteens; * Frisian Front).

O_2 consumption

In the field the oxygen consumption rates were higher in the silty sediments, ranging from 15-40 $mmol.m^{-2}.d^{-1}$ than in the sandy sediments ranging from 2-22 $mmol.m^{-2}.d^{-1}$ (Figs II.3a, b). In silty sediments the seasonal pattern was characterized by enhanced uptake rates from April until October. Highest rates coincided with the highest water temperatures in August. In sandy sediments the oxygen uptake pattern was dominated by an early summer peak followed by slightly reduced uptake rates. In the starved mesocosms oxygen consumption gradually decreased during the experiment. The temperature increase from 8 to 17.5 ^{0}C in April-August had no effect on the oxygen uptake rate. The organic input of Phaeocystis in May-June stimulated the oxygen uptake within days. Highest values were measured at the the end of the "feeding" period and equalled field values in both sediment types. Rates after the input in the silty sediment exceeded the rates in the

sandy sediment more than 2 times, while the amount supplied was similar for both sediment types. The enhanced response declined within 2 months to levels slightly higher (silty sediment) or comparable to the low level found in the starved mesocosms (sandy sediment). The characteristic differences in oxygen consumption between sediments in the field remained present in the mesocosms until the end of the experiment.

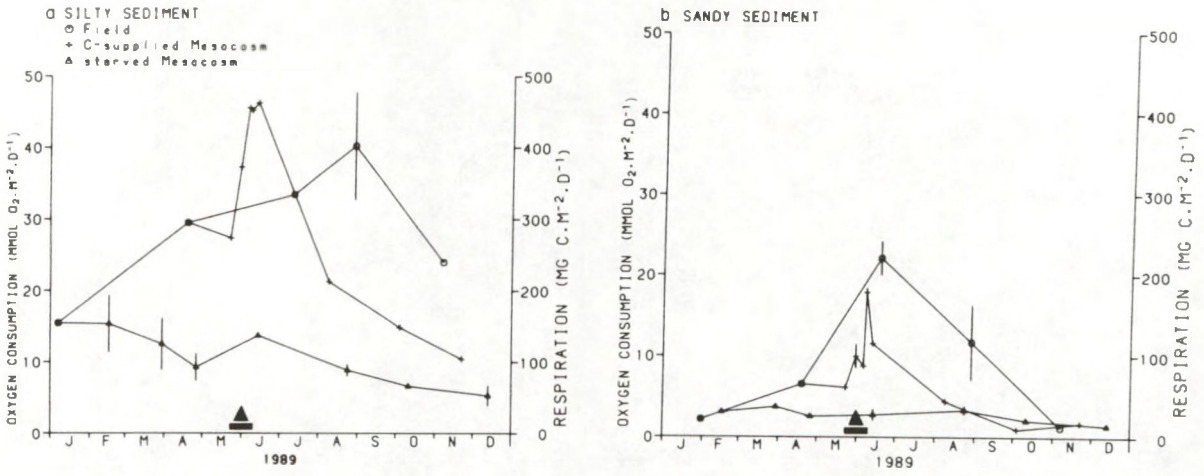

Fig. II.3. Seasonal variations in oxygen consumption. a: silty sediments, b: sandy sediments. Arrow indicates the timing of the Phaeocystis supply in C-supplied mesocosms.

Oxygen penetration depths were generally more than 5 mm. The thickness was usually around 7 mm in silty sediments and around 12 mm in sandy sediments.

Bacterial production

The bacterial production in the field was up to 5-fold higher in silty than in sandy sediments (Figs II.4a, b). The seasonal pattern in silty sediments was characterized by a high production plateau in summer-autumn followed by relatively low winter values. In April-May an increase in bacterial production was observed in both sediment types. In sandy sediment the highest production level was also reached in early summer, but then dropped within 2 months to values intermediate between early summer and winter values. In the starved mesocosms bacterial production remained about constant at low levels in both sediment types with an overall 4-fold higher production in silty sediments (Figs II.4a, b). In the mesocosms supplied with Phaeocystis bacterial production increased more than 4-fold in comparison to the starved mesocosm and approached values found in situ in June. In silty sediments an increase of 230 mg $C.m^{-2}.d^{-1}$ was found

and in sandy sediments an increase of 50 mg $C.m^{-2}.d^{-1}$. Within 2 months after the organic input the production dropped to values as low as the starved mesocosms. Onwards the bacterial production in C-supplied and starved mesocosms was comparable for the respective sediments.

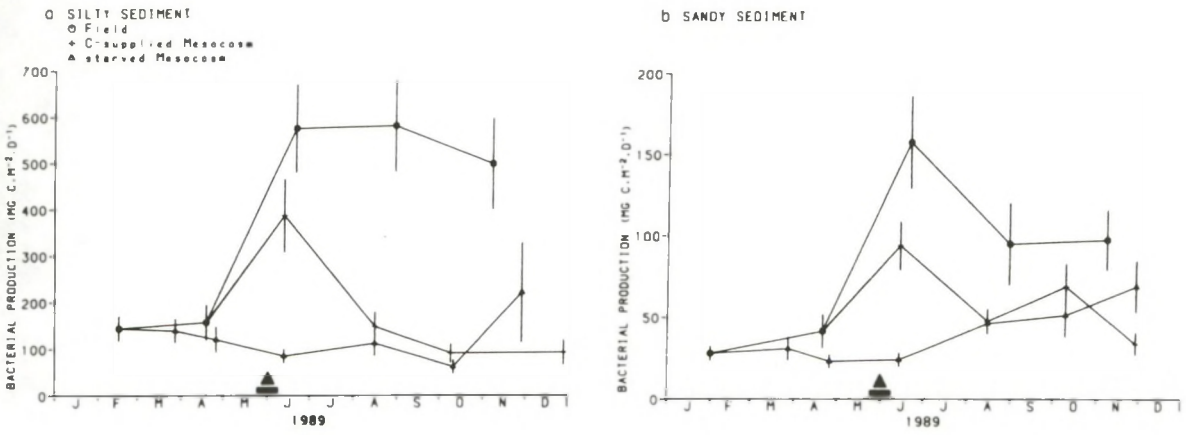

Fig. II.4. Seasonal variations in bacterial production. a: silty sediments, b: sandy sediments. Arrow indicates the timing of the Phaeocystis supply in C-supplied mesocosms.

Differences between the values in sediment types remained throughout the experiment. Production in the mesocosms was lower than the production rates as measured in the field.

Bacterial abundance and biomass

In the field the average bacterial abundance was more than 2.5 fold higher in silty than in sandy sediments (Figs II.5a, b). Abundance patterns were comparable in silty and sandy sediments. Lowest values for bacterial abundance were found in April (Figs II.5a, b). An increase of bacterial abundance in May-June was observed, maintaining high summer-autumn levels until September-November. Variations in bacterial abundance were irregular in mesocosms (Figs II.5a, b). In mesocosms starvation nor Phaeocystis input had a distinct effect on the development of the bacterial abundance in silty sediments. Here the bacterial abundance declined from April (ca $2.5 * 10^9$ cells.ml^{-1}) until November (ca $1 * 10^9$ cells.ml^{-1}), irrespective of the temperature rise in summer or the organic matter input. In sandy sediment mesocosms the organic input had a pronounced effect. Bacterial abundance doubled at least and exceeded the values found in the field in June. Within 2 months the abundance dropped to initial values. Biomass patterns were not essentially different from abundance patterns as shown in Figs II.5a, b. The biomass in the 5 mm surface

layer varied from 200 to 600 mg $C.m^{-2}$ in silty sediment and from 100 to 300 mg $C.m^{-2}$ in sandy sediment. Mean biovolume per sampling date varied from 0.130 to 0.229 μm^3 in silty and from 0.121 to 0.219 $\mu m^3.cell^{-1}$ in sandy sediments.

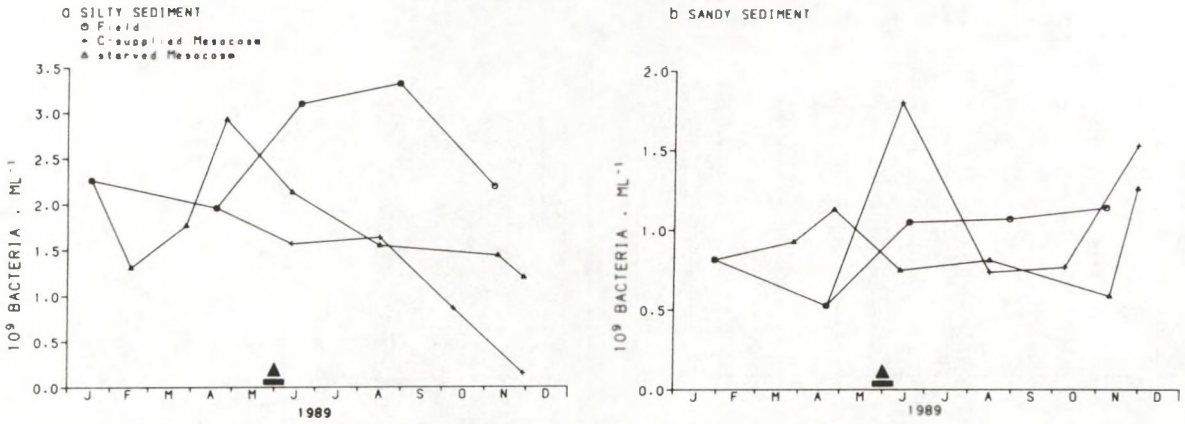

Fig. II.5. Seasonal variations in bacterial abundance. a: silty sediments, b: sandy sediments. Arrow indicates the timing of the Phaeocystis supply in C-supplied mesocosms.

Protist abundance and biomass

In the field the protist abundance (heterotrophic nanoflagellates < 20 µm) was up to 10 times higher in sandy than in silty sediments (Figs II.6a, b). The seasonal patterns were comparable except for the autumn increase in abundance in silty sediments. An increase in abundance in May-June was found in both sediments. In sandy sediments the abundance dropped from August to November. Starved mesocosms were not sampled for heterotrophic nanoflagellates. In silty sediments no clear response to the Phaeocystis input was observed (Fig. II.6a). The 2-fold increase here was dwarfed by the 8-fold increase in numbers in sandy sediments after the organic input (Fig. II.6b). Size class distribution analysis just after the input learned that the increase was due to the 2-5 µm size class protists. Within 2 months after the organic matter supply was discontinued protozoan numbers dropped to initial values in the sandy sediments in mesocosms. In the silty sediment a different pattern was found. The abundance remained constant after feeding until the end of the experiment, around 20.000 protists.ml^{-1}. The abundance in silty sediments remained low (10-fold) as compared to the abundance in sandy sediments, in the mesocosms as

well as in the field. Biomass patterns of heterotrophic nanoflagellates were not much different from abundance patterns. Biomass extrapolated from the surface layer values, varied from 0.8-3 mg $C.m^{-2}$ in silty sediments and from 2-23 mg $C.m^{-2}$ in sandy sediments.

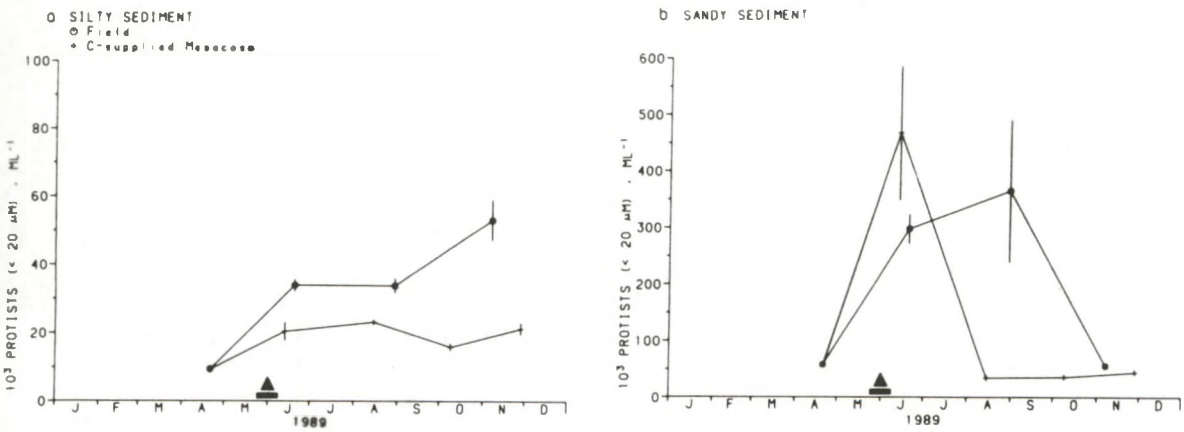

Fig. II.6. Seasonal variations in protist abundance (< 20µm). a: silty sediments, b: sandy sediments. Arrow indicates the timing of the Phaeocystis supply in C-supplied mesocosms.

Organic matter content

Silty sediment organic matter content varied from 3.7-7.8 mg C.g dry $sediment^{-1}$ in the upper 10 cm layer of the sediment with atomic C:N ratios in the upper cm layer ranging from 9.6 to 11.7. Sandy sediments contained 0.4-1.0 mg C.g dry $sediment^{-1}$ in the upper 10 cm layer of the sediment with atomic C:N ratios in the upper cm layer ranging from 4.7 to 6.5. The porosity (v/v) of silty and sandy sediments was respectively 50-60% and 35-40%. The atomic C:N ratio of the Phaeocystis material was 6.8.

Carbon budget

Annually integrated benthic oxygen consumption measurements, as expressed in C-equivalents (RQ=0.85, Hargrave 1973) of annual C respiration rendered estimates which lay in the same order of magnitude as annually integrated bacterial production measurements in the 5 mm surface layer of the sediment (Table II.2). This implicates that the C-demand of bacteria (with a growth efficiency of 50%, e.g. Moriarty et al. 1985) exceeded the respiration by 2-fold on an annual basis. Annual production and respiration yields in the field exceeded yields in the mesocosms. Compared to the starved sediments the

addition of 23 g $C.m^{-2}$ in mesocosms of which only 16, 11 $C.m^{-2}$ was recovered, resulted in an annual increase of bacterial production of 13, 5 g $C.m^{-2}$ and a benthic respiration increase of 46, 8 g $C.m^{-2}.y^{-1}$ in silty, sandy sediments respectively. Considering the relatively deep oxygen penetration in both sediment types (> 5mm) it is assumed for the budget that all organic carbon is oxidized in the sediment surface under oxic conditions.

TABLE II.2
Annually integrated respiration (g $C.m^{-2}.y^{-1}$) as derived from benthic oxygen consumption measurements and bacterial production for each field and mesocosm scenario. The bacterial carbon demand was estimated on basis of a 50% growth efficiency of bacteria.

g $C.m^{-2}.y^{-1}$	SILTY SEDIMENT			SANDY SEDIMENT		
	Comm. Resp.	Bact. Prod.	Bact. C-demand	Comm. Resp.	Bact. Prod.	Bact. C-demand
FIELD	104	143	286	31	34	68
C-supplied MESOCOSM	85	60	120	18	20	40
starved MESOCOSM	40	47	94	10	15	30

II.4. DISCUSSION

Suitability of mesocosms for North Sea sediments

This pilot study examined the suitability of mesocosms to maintain North Sea sediment in a realistic and lively condition. The test was applied to 2 North Sea sediment types, extremes with respect to mud content. Experience showed that it is possible to collect intact cores of these sediment types at sea and to transport them submerged in with sea water filled crates to the laboratory and to install them in mesocosms without major disturbances. Differences between sediment types in the field with respect to magnitude of microbial variables were reflected in the mesocosms. Irrespective of the treatment in mesocosms differences in microbial variables between sediment types remained almost constant as long as the experiment lasted. Considering the fact that this was approximately 10 months, we conclude that it is indeed possible to study seasonal patterns of microbial variables in intact sediment cores in mesocosms.

Seasonal field patterns of microbial variables

Seasonal variations were observed in all microbial variables studied in the field. The most pronounced patterns in both sediment types were found in benthic oxygen consumption and bacterial production. Enhanced rates of oxygen consumption extended from May to September. Oxygen uptake patterns in silty sediments aligned with patterns descibed by Cramer (1990) for the the Frisian Front. Rates were also in a comparable order of magnitude. The pattern in sandy sediment was slightly different during the production season. Peak values were restricted to early summer after which values dropped to intermediate summer-autumn values. The level of consumption rates in August-September lay in the same order of magnitude as de Wilde et al. (1984) found at the Oyster Grounds in 1980/1981.

The bacterial production rates presented here are the first for North Sea sediments as far as we know (see also Billen et al. 1990). Rates were unexpectedly high compared to rates measured on the intertidal flats in the Wadden Sea (van Duyl & Kop, 1990). Particularly the rates in the sandy sediment equalled productions on sandy intertidal flats. Rates measured at the Frisian Front were higher than the rates in the sandy Wadden Sea sediments, but considerably lower than at the silty intertidal flat.

Bacterial production patterns in North Sea sediments broadly aligned with oxygen consumption patterns for both sediment types. This can be attributed to the fact that most oxygen is consumed by bacteria in the oxic layer, which was usually more than 5 mm thick in both sediment types. Chemical oxidation of reduced compounds hardly contributed to the oxygen consumption considering the fact that sulphide could not be detected until 4 cm depth (unpubl. results). The subtile differences in seasonal patterns of oxygen consumption and bacterial production found between silty and sandy sediments are possibly structural. At the Frisian Front, a "depositional" area (Creutzberg et al., 1984), the input of organic matter is not exclusively determined by the sedimentation of organic matter from the water column but also by the horizontal transport and accumulation of allochthonous organic matter. This might influence seasonal patterns. The benthic system at the Broad Fourteens will be more dependent on the occasional sedimentation of phytoplankton blooms from the water column. A major phytoplankton sedimentation event in spring may have set the seasonal pattern here.

Patterns in bacterial abundance in the field are for both sediment types characterized by enhanced summer values and relatively low April values. Comparable patterns were found in intertidal sediments of the Wadden Sea (van Duyl & Kop, 1990). Bacterial abundances are 2-3 fold higher in silty than in sandy sediments.

Protozoan abundances demonstrated obvious seasonal variations in sandy sediments. A 7-fold increase was observed from April to August. Densities in the sandy North Sea sediments were comparable to the densities found in the intertidal flats in the Wadden Sea (Bak & Nieuwland, 1989). The much lower densities of protists in silty North Sea sediments are difficult to interprete (Appendix Chapter III).

The seasonal variations in microbial variables are often suggested to be the controlled by temperature and organic matter input (e.g. Meyer-Reil, 1987; van Duyl & Kop, 1990; Cramer, 1990). Causal relations between these factors and microbial activity in sediments can usually not be deduced from field measurements.

Mimicking seasonal developments

The possibilty of mimicking seasonal development in mesocosms was evaluated by concentrating on the factors controlling the seasonal development in North Sea benthic systems. Therefore the effect of temperature and organic input was analysed in mesocosms. We determined the effect of temperature separate from the effect of organic matter input. Seasonal variations in temperature alone did not generate seasonal variations of microbial variables in the mesocosms. There was no response of benthic oxygen consumption, bacterial production or abundance of bacteria and protozoa in mesocosms to temperature increase. Mesocosm results show that in agreement with the suggestions of Cramer (1990) significant covariations between benthic oxygen consumption and temperature in the North Sea do not necessarily represent a causal relationship. In Kiel Bight sediments the organic matter deposition appeared to be the controlling factor in stead of temperature (Meyer-Reil, 1983; Graf et al. 1984). In such a system the sequence of sedimentation events will determine the seasonal pattern (Meyer-Reil, 1987). In the North Sea the spring sedimentation event appears to be the most important event for the benthos (Fransz & Gieskes, 1984). Therefore we supplied both sediment types in the mesocosms in May-June. The organic matter consisted predominantly of dead Phaeocystis pouchetii material. Phaeocystis pouchetii is a common autotrophic flagellate which develops large blooms in spring in the coastal zone of the North Sea from Belgium up to the German Bight (e.g. Veldhuis, 1986; Cadée, 1986). Within days such a bloom can disappear from the watercolumn and considering the rapid sinking rates of the material it is purported to settle on the bottom becoming available for the benthos. The actual input of organic matter in North Sea sediments is unknown. Based on benthic oxygen consumption measurements estimates have been made of the minimum amount of degradable organic matter annually available to benthic organisms in the North Sea. Dependent on location 23 to 95 g $C.m^{-2}.y^{-1}$ was found to be the metabolic loss of the benthos in the North Sea (de Wilde et al., 1984; Cramer, 1990). We supplied the minimum of the range found, 23 gr $C.m^{-2}$, to both sediment types. The Phaeocystis supply elicited a direct response of microbial variables which disappeared within 2 months. For oxygen consumption similar results were obtained in microcosms in Narragansett Bay, Rhode Island (USA) after an organic matter supply (Kelly & Nixon, 1984). The rapid response implicates that the bulk of organic matter is rapidly assimilated by bacteria and that the initial activity is the largest. The almost 5 times larger increase in bacterial production in silty than in sandy sediments in response to the input might be attributed to the importance of the

pool of organic matter present in the sediment (Appendix Chapter I). The determined organic matter content in silty sediments was almost 10 times higher than in sandy sediments. The fresh material added might function as a katalysator for degradation of more refractory organic matter. The 23 g $C.m^{-2}$ added in May-June was insufficient to generate seasonal patterns and yearround values of microbial variables as found in the field. The pulse itself was large enough to mimic the increase in oxygen consumption and bacterial production in the field in early summer, but considering the quick reponse a higher sampling frequency is recommended than was held in this experiment. To mimic seasonal patterns in mesocosms more organic matter sedimentation events are required. Particularly for silty sediments, where activity levels remain high throughout summer-autumn a more continuous organic matter input of easily degradable quality is required. Results indicate that in sandy sediments the major organic matter sedimentation events to which the benthos responds, indeed occur in early summer after which some minor events maintain moderately enhanced summer-autumn values. Seasonal patterns in microbial variables apparently can be mimicked in mesocosms by administering balanced and easily degradable organic matter amounts, taking the organic matter content of the sediment into account and supply the amounts at the right time to the sediments, taking the rapid responses to certain organic matter fractions into account. Microbial activities tend to be easier to mimic than abundances of bacteria.

It is evident that an organic matter input in North Sea sediments is required to enhance microbial activities and to induce the seasonal patterns of microbial variables.

Carbon budgets

Carbon cycling through microzoobenthos in the field and the mesocosms can be illustrated by carbon budgets. Before constructing a budget it should be mentioned that conversion factors for bacterial biomass production are still in discussion (e.g. Moriarty 1989; Bell, 1990). Our conversion factor for thymidine incorporation, $1 * 10^{18}$, lays at the lower end of the average range in factors in the waterphase 0.4 to $2 * 10^{18}$ $cells.mol^{-1}$ thymidine incorporated in DNA (Riemann & Bell, 1990). For the conversion of cell volume to cell carbon a range of 1.21 to $5.80 * 10^{-10}$ mg $C.\mu m^{-3}$ is in use, in which we used the factor of $2.2 * 10^{-10}$ suggested by Bratbak & Dundas (1984). Considering our conservative factors for bacterial production and the assumption that the bacterial production is nil below 5 mm depth in the sediment, bacterial production is assumably underestimated in the budget. The accurateness of benthic oxygen consumption as measured with benthic chambers (bell-jars) is discussed by e.g. Lindeboom & Sandee (1984). Such measurements might well be underestimates of total benthic respiration (de Wilde & Beukema, 1984; de Wilde et al. 1984). So both bacterial production and respiration might be underestimated in the budget.

The budgets are based on the information presented in Table II.2. In the field, community respiration or carbon loss will equal the carbon input into the sediment on an annual basis. In both sediment types already the bacterial carbon demand is more than double this carbon input. Apparently the carbon input into the sediment is insufficient to fuel the bacteria apart from all the other benthic organisms. Other sources of carbon must be available to bacteria. Utilization of the pool of organic matter in the sediment by bacteria might be a solution in the mesocosm. This is however unlikely to occur in the field as it would result in a net reduction of the bulk of sediment organic matter, which mainly consists of refractory organic material (Appendix Chapter I). In addition patterns in annual benthic respiration do not support such a reduction in sediment organic matter.

The paradox is solved by invoking the characteristics of secondary production. Strayer (1988) clearly showed that the summed carbon demand and the summed production of consumers both may easily exceed the organic carbon inputs to the ecosystem. Only the summed respiration of consumers cannot exceed the organic input in consumers. So all carbon produced by secondary producers remains available to consumers. Recycling might provide in the rest of the bacterial carbon demand. Indeed there are indications that recycling of bacterial biomass forms an important flow in benthic systems (Moriarty, 1989; Alongi, 1989). Such an internal loop greatly increases the gross efficiency of the C-cycling and reduces the external C-demand of bacteria considerably.

In the budgets (Fig. II.7) bacterial mortality and subsequent recycling is the most important carbon source beside external input. Losses of bacterial carbon are due to respiration and grazing by protists. The part of the bacterial production, which was not grazed, was assumed to be available for recycling. Grazing rates of 50 bacteria. protozo^{-1}.h^{-1} are possible considering available literature values for the plankton (e.g. Sherr et al., 1989; Davis & Sieburth, 1984). Based on protist abundances we calculated the percentage of the bacterial production that was grazed in the field, respectively 1.4% in silty sediments and 32% in sandy sediments. In the mesocosms supplied with organic matter this was 3% for silty sediments and 19% for sandy sediments. We assumed similar percentages for grazed bacterial production in starved mesocosms in which protist densities were not determined. These percentages were converted into carbon (g C.m^{-2}.y^{-1})(Fig. II.7). Recycling drastically depressed the external carbon demand of bacteria, resulting in better correspondence between external input and community metabolic carbon loss in all budgets. In mesocosms a strong reduction of the carbon flow in the microbenthic community was found compared to the field situation for both sediment types. The supply of carbon in the mesocosms suppressed this reduction in both sediments, however 23 g C.m^{-2} (of which 16 g C.m^{-2} was recovered for silty sediments and 11 g C.m^{-2} for sandy sediments) was far too low to maintain the microbenthic community at field levels.

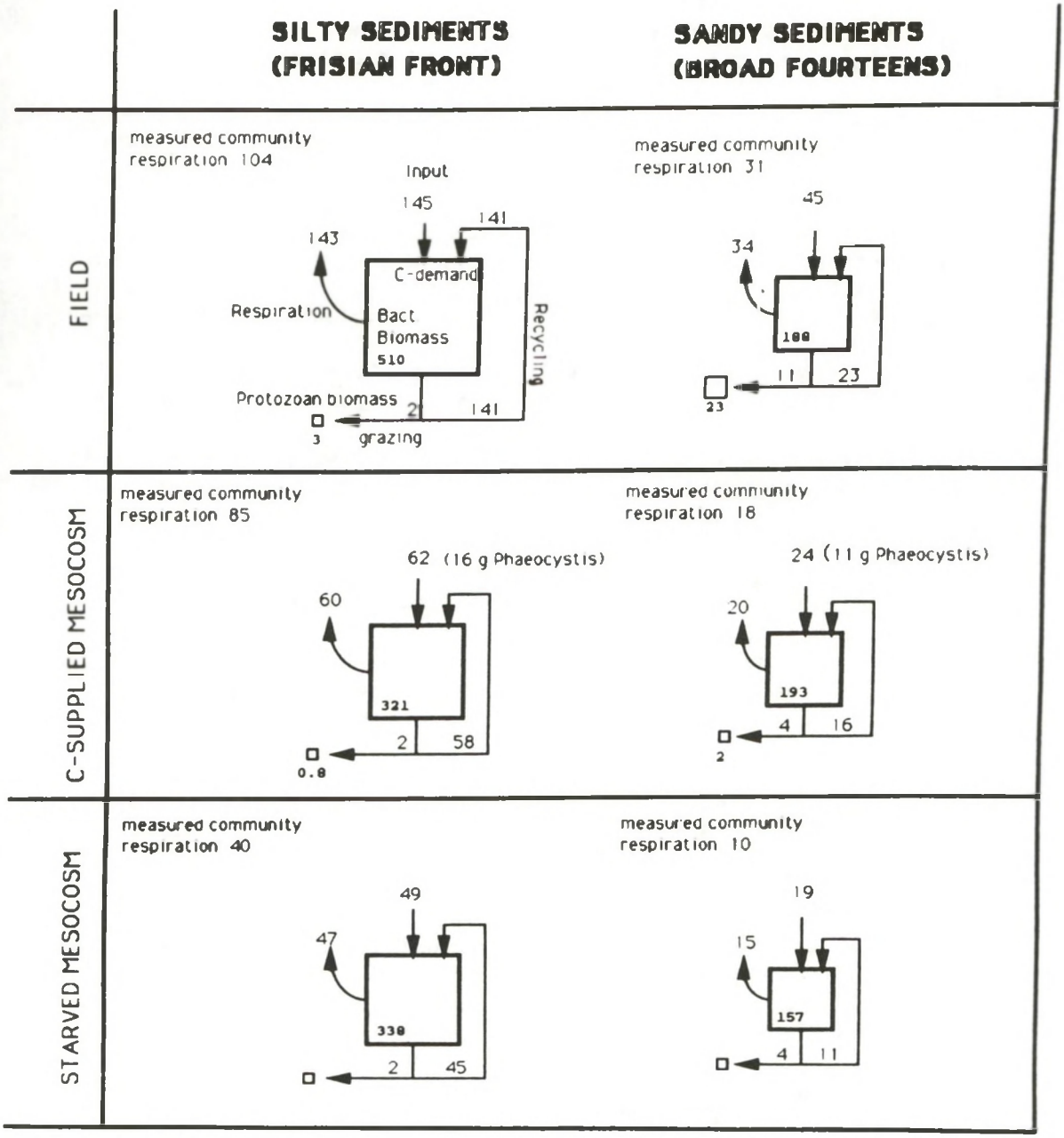

Fig. II.7. Annual carbon cycling through the microzoobenthos in g $C.m^{-2}.y^{-1}$. Separate budgets are given for each scenario in the field and in the mesocosms. Input and recycling of bacterial biomass together is equal to the bacterial C demand. Size of the boxes refers approximately to biomass (also given in mg $C.m^{-2}$ in 5 mm surface layer).

These budgets clearly show that "new" input of organic matter is required to explain the level of microbial activity in the field. The input in bacteria in the field and in mesocosms is in the same order of magnitude as the measurd community metabolic carbon loss.

In further carbon budget studies it is essential to get a better insight in the role of recycling of bacterial biomass in sediments in relation to season, location and sediment type.

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APPENDIX III

Benthic heterotrophic nanoflagellates in North Sea field/mesocosm bottoms and their response to sedimentation

(R.P.M. Bak, F.C. van Duyl, G. Nieuwland & A.J. Kop)

III. BENTHIC HETEROTROPHIC NANOFLAGELLATES IN NORTH SEA FIELD/MESOCOSM BOTTOMS AND THEIR RESPONSE TO SEDIMENTATION*

Rolf P.M. Bak, Fleur C. van Duyl, Gerard Nieuwland & Arjen J. Kop

* Publication no. 25 of the project Applied Scientific Research Netherlands Institute for Sea Research (BEWON)

ABSTRACT

We studied densities of heterotrophic benthic nanoflagellates (<20 µm) in relation with densities and productivity of bacterial communities in North Sea bottoms. Experimental mesocosm bottoms, collected at a sandy (depth 28 m) and a silty (depth 38 m) locality, received one substantial input of organic matter (23 g C dead phytoplankton.m^{-2}) simulating spring phytoplankton sedimentation. Flagellate densities and bacterial productivity/numbers were measured in the surface layers of the mesocosm sediments and in fresh sediment cores from the field.

A clear pattern emerged for the top layer in the field sandy sediments: flagellate densities were low in April (50 x 10^3 cells.cm^{-3}), high during June and August (300 x 10^3 cells.cm^{-3}), and low again in November. In the mesocosm sandy sediments numbers had increased from low to high after the simulated algal bloom in June, but they were again low in August and through the remainder of the season. There were no variations in the flagellate densities deeper in the sediment. In the silty sediments flagellate densities were low in the field and in the mesocosm.

High densities of these benthic flagellates were invariably parallelled by high benthic bacterial production, but not vice versa. Flagellate bacterivory could have considerable impact on bacterial production in the sandy North Sea bottom but not in the muddy sediment.

III.1. INTRODUCTION

That small heterotrophic nanoflagellates are an important component of pelagic communities is a major new insight recently gained in marine biology (e.g. Andersen & Fenchel, 1985; Wikner & Hagström, 1988; Bernard & Rassoulzadegan, 1990; Riemann et al., 1990). In their role as predators on bacteria and other small cells nanoflagellates, 2-20 µm, appear as a new trophic level, essential in the functioning of the small food web at the base of the Eltonian pyramid (Azam et al., 1983). The consequences for theories on the passage of carbon along the trophic structures through pelagic marine systems are significant (Fenchel, 1987).

The possible occurrence of nanoflagellates as a similarly active component of benthic marine systems remained unstudied. Larger

phagotrophic protists such as ciliates can be abundant and dominant predators in some marine sediments (Fenchel, 1967; 1968). The smaller flagellates appeared logistically more difficult to study (Alongi, 1986; 1991). However, it has been shown that nanoflagellates occur in high and fluctuating densities in marine bottoms (Bak & Nieuwland, 1989). It seems reasonable to assume that benthic flagellates may be as important as consumers of bacterial production as their pelagic counterpart.

In the North Sea benthic fauna must basically be fuelled by pelagic production even though locally there may be an influx of horizontally transported organic detritus (Creutzberg et al., 1984). An important segment of total pelagic production is the carbon resulting from primary production during the spring algal bloom (Fransz & Gieskes, 1984). Sedimentation of this material, cells and phytodetritus, stimulates microbial activity (Graf et al., 1982) and is related to seasonal fluctuations in characteristics of bacterial populations (Meyer-Reil, 1983).

We wanted to study the response of the microbial community to such an input of organic material and in particular the response of the nanoflagellates in relation to bacterial production. Bacterial production is by far the most relevant parameter with respect to bacterivory of small protists (van Duyl et al., 1990). The marine habitats where these phenomena occur, North Sea bottoms, are however very difficult to manipulate in situ. Therefore we decided to do our experiments in North Sea mesocosms while relating the experimental results at each data point with freshly collected North Sea bottoms.

Acknowledgement. We thank all all participants in the NIOZ North Sea mesocosm experiment, in particular G.J. van Noort, and including the crew of our research vessel Aurelia, for their support.

III.2. MATERIALS AND METHODS

North Sea bottoms vary locally in sediment characteristics (Creutzberg et al., 1984) and we included two distinct sediment types in our experiments, a sandy sediment: Broad Fourteens, mud content <2%, depth 28 m, and a muddy sediment: Frisian Front with a mud content >20%, depth 38 m (Fig. III.1). The undisturbed North Sea bottoms, i.e. in tact with macrofauna, were collected with 0.5 x 0.5 m boxcores from January to April 1989. Ten boxcores of each sediment type were put adjacently in a 20 m^2 mesocosm and kept in the dark at in situ North Sea temperatures until December 1989. The systems were exposed to circulating seawater.

To study the effect of sedimentation after the algal spring bloom half of the bottoms of each sediment type received an substantial input of organic matter, 23 g C m^{-2}, over a two week period in May/June. To facillitate sedimentation of this material, dead phytoplankton, water circulation was stopped after each addition for 24 hours.

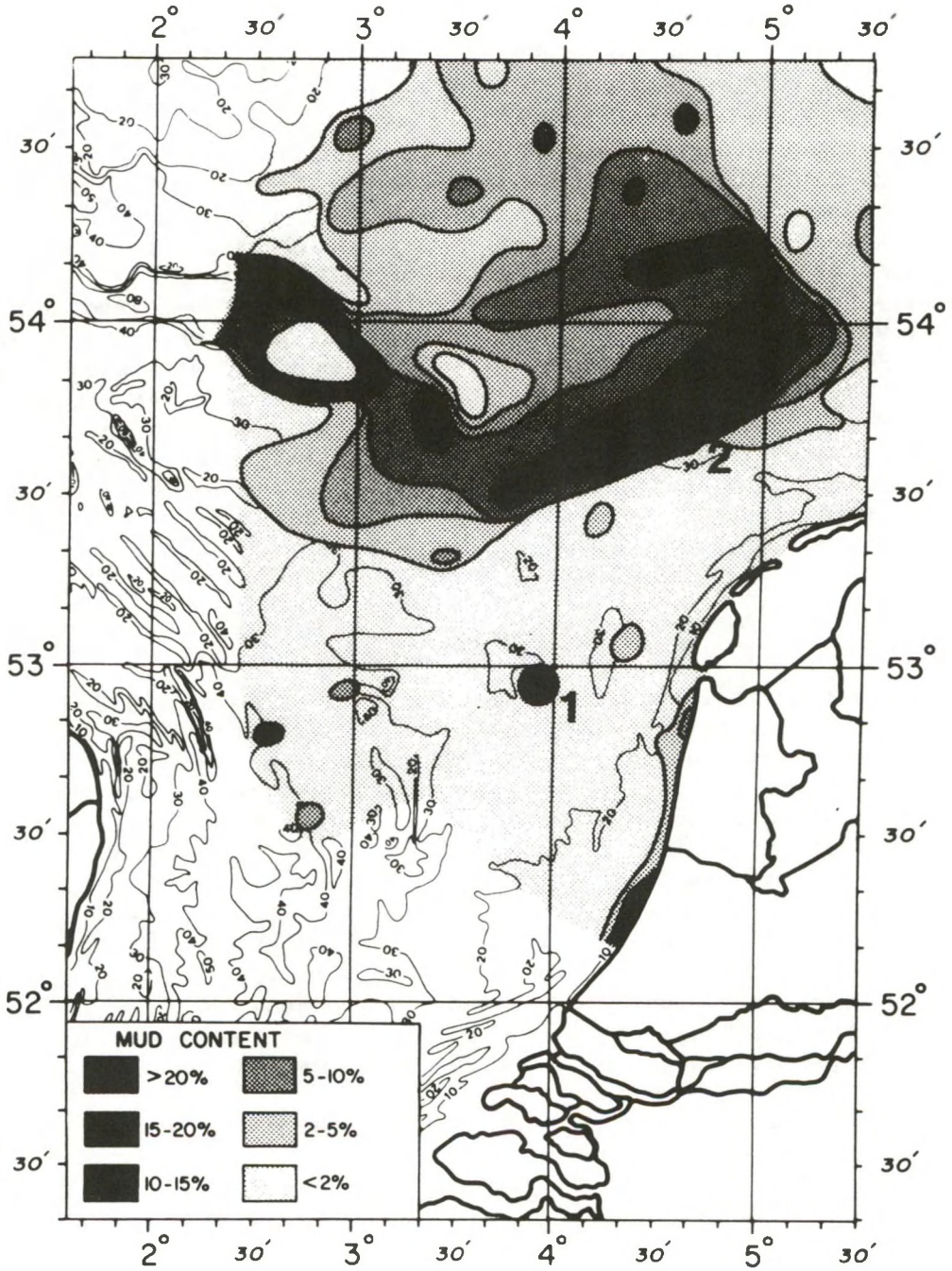

Fig. III.1. Location of sample sites Broad Fourteens (1) and Frisian Front (2) in the southern North Sea. Shading indicates mud content (particles < 50 µm) of bottom sediments (adapted from Creutzberg et al. 1984).

At roughly two month intervals we sampled the top layer (0-5 mm) of the sediment for characteristics of the bacterial community. We obtained bacterial numbers with epifluorescence microscopy and acridine orange stained samples (van Duyl & Kop, 1990). Bacterial production was measured with the (methyl-^{3}H) thymidine method (Moriarty, 1990). Procedures follow van Duyl & Kop (1990). These data were collected in the mesocosm bottoms which received the carbon supply, the starved control mesocosm bottoms and in fresh North Sea bottoms collected at the Broad Fourteens and Frisian Front locality at each data point. The inclusion of fresh North Sea bottoms in the experiment meant that weather conditions and availabilty of ships to a large degree decided the possible number of data points.

Densities of heterotrophic nanoflagellates were obtained in the mesocosms which received the carbon input and in the fresh North Sea bottoms. For each sample we took 5 small cores which were subsampled at sediment depths of 00-03, 30-33 amd 60-63 mm. Procedures followed Bak and Nieuwland (1989) except for fixing and staining (final concentration sterile seawater wash 1% glutaraldehyde and 0.0015% proflavine, staining/sedimentation time 1 hour). The flagellates were counted in size classes, <2 µm, 2-5 µm, 5-10 µm and 10-20 µm, using epifluorescence microscopy (Hobbie et al., 1977).

III.3. RESULTS

In the field the densities of the nanoflagellate communities showed a clear pattern in the sandy sediments at Broad Fourteens (Fig. III.2). Densities were low in April, much higher after the pelagic bloom in primary production, still high in late summer but were down at the low initial level again in November. These fluctuations were restricted to the top layer of the sediment. Deeper, at 30 and 60 mm, densities remained at the same low level throughout the year (17-48 x 10^3 cm^{-3}).

In the Broad Fourteens mesocosm bottoms densities were low in April but high in June after the simulated algal sedimentation (Fig. III.2). In contrast to the field, values were low again in August and remained at this low level in October and November. Again density fluctuations were restricted to the surface sediment layer and densities remained low and unchanged at depths of 30 and 60 mm in the sediment (11-22 x 10^3 cm^{-3}).

Densities of flagellates were much lower in the muddy Frisian Front bottoms than in the sandy Broad Fourteen ones. Also there was a general lack of variation or pattern. This was true for the field samples as well as for the mesocosm (Fig. III.2).

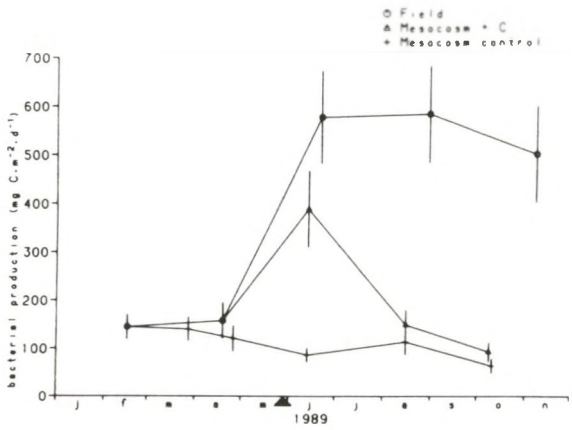

Fig. III.2. Densities of small protists in the top layer (00-03 mm) of sandy (Broad Fourteens) and muddy (Frisian Front) North Sesediments in the field and in experimental mesocosms. Arrow indicates carbon input in experimental mesocosm.

Looking closer at the flagellates, at the contribution of the different size classes to the total numbers, it appeared that in all bottoms and at all data points the size class of 2-5 µm cells was the most numerous. This is illustrated by the size class distribution in the field and mesocosm Broad Fourteen bottoms (Figs III.3a, b).

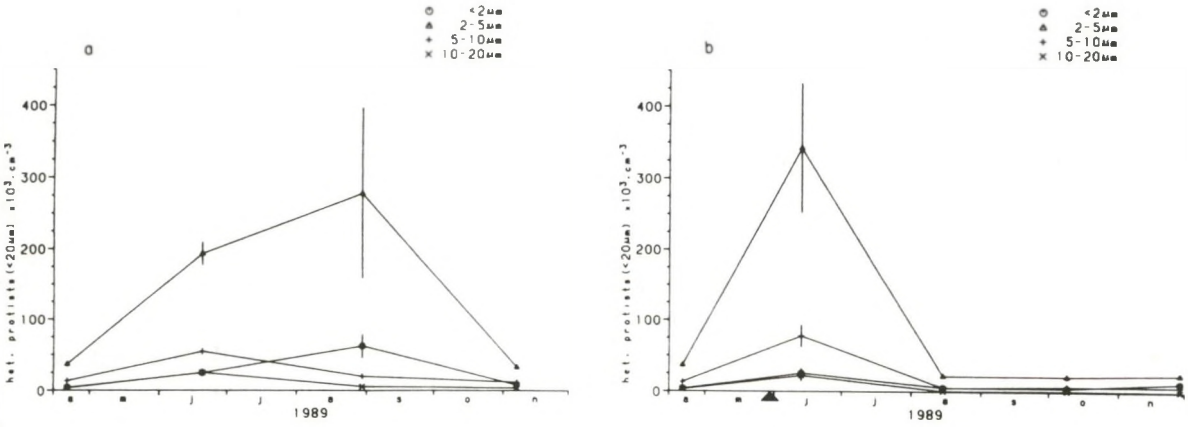

Fig. III.3. Densities of small protists in different size classes in the top layer (00-03 mm) of sandy North Sea Broad Fourteens sediment, a: in the field and b: in mesocosm. Arrow indicates carbon input in experimental mesocosm.

Flagellates varied in terms of carbon between 0.1 to 7 µg C cm^{-3} throughout the experiment in the sandy bottoms.

The abundance of potential bacterial prey of the flagellates varied haphazardly between 0.5 - 3.0 . 10^9 cm^{-3} but bacterial productivity showed a clear pattern (Fig. III.4a). Production was low in winter and early spring. In the field, at sandy Broad Fourteens, there was an sharp increase after the pelagic spring bloom and values remained high at intermediate levels. In the mesocosm there was a sharp increase in production after the simulated spring bloom followed by a decrease to the background levels of the control. The starved control mesocosm showed low production levels throughout this period. A similar pattern, but with higher production values, emerged for the three different sets of muddy Frisian Front bottoms (Fig. III.4b).

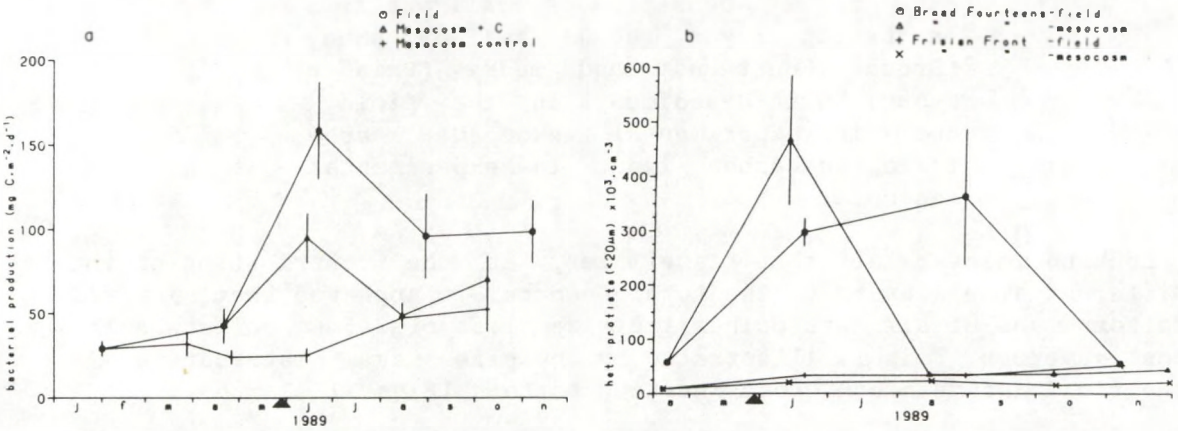

Fig. III.4. Bacterial production in the field and experimental mesocosms of a: sandy Broad Fourteens and b: muddy Frisian Front. Arrow indicates carbon input in experimental mesocosm.

III.4. DISCUSSION

We cannot relate our findings to other data sets on benthic nano-flagellate numbers and the relation with bacterial production in bottoms of temperate seas because there are no comparable data.

The only other data available on variation in marine benthic flagellate densities (Bak & Nieuwland, 1989) are from intertidal flats in the Wadden Sea. Abundances show a pattern similar to that in the North Sea sandy sediment, low winter levels and increased abundances during summer. During the summer period of high abundance flagellate densities vary. But the large variations in abundance, due to predator-prey oscillations, which appear to characterize communities of pelagic flagellates have not yet been observed in the marine sediments (Bak, Nieuwland unpubl.; Hondeveld, pers. comm.). Therefore we suggest that a high flagellate density is maintained between our data points in June and August.

Bacterial production appears to be a limiting factor for the flagellates in these sediments. Flagellate number is only increased when bacterial production is increased (i.e. in spring and following experimental sedimentation) and when bacterial production has decreased flagellate densities are reduced (August, Broad Fourteens mesocosm, Figs III.2, 4a). High flagellate densities occur only at relatively high levels of bacterial production. The opposite is not necessarily true. Bacterial production can be high while flagellate densities are low. This is obvious at Broad Fourteens in the autumn and during the year in the muddy Frisian Front sediments. In these muddy bottoms there is no clear relation between bacterial production or numbers and nanoflagellates. Fenchel (1987) mentions the mechanical properties of sediments, such as grain size and interstitial space, to be important in the occurrence and abundance of protozoa. However, it remains to be studied how very small phagotrophic nanoflagellate exploit the interstitial habitats and how the characteristics of such habitats vary between different sediments (Patterson et al. 1989).

The difference between sandy and muddy sediments is not only reflected in the low abundance of the flagellates in muddy sediments and their lack of seasonal variation. It is emphasized when we compare the number of potential prey, bacterial cells produced per hour, available per flagellate in the different sediments. Comparisons within a type of sediment are consistent. In sandy sediments, fresh from the field or in mesocosms, half to a few hundred bacteria are available each hour for a flagellate (Table III.1). In muddy sediments the figures are also similar when we compare the field samples with the experimental systems, numbers are consistently in the low thousands. It is clear, of course, that there is a consistent order of magnitude difference between the availability of prey in these

TABEL III.1

Bacterial production (cells h^{-1}) available per flagellate h^{-1} in the sandy sediments of Broad Fourteens and the muddy sediment of the Frisian Front. Data points for field and mesocosm: 1=April, 2=June, 3=August, 4=November

	Sandy sediment	Muddy sediment
Field 1	126	4961
,, 2	110	3683
,, 3	75	3869
,, 4	310	1803
Mesocosm 1	50	3130
,, 2	393	1227
,, 3	427	1173
,, 4	170	1073

sediments. Data on the predation rates of pelagic nanoflagellates range from about 50 to 210 (Davies & Sieburth, 1984). It seems reasonable to presume that similar rates up to 100 to 200 bacteria flagellate^{-1} hour^{-1} could exist in benthic flagellates but rates of thousands of bacteria flagellate^{-1} hour^{-1} seem preposterous.

Consequently it appears that bacterivory potentially can be a controlling factor in our sandy North Sea sediments but not in the muddy ones.

The congruous patterns of bacterial production in both sediment types and for flagellate densities in the sandy sediment, are indicative for the difference between the effect of a single period of organic sedimentation to the benthos and the situation in the field. High activity through the summer period in the field, low activity in the controls and the single peak after experimental sedimentation suggest that the spring sedimentation of organic material to the benthos may be insufficient to support microbial population in the field throughout the season.

We conclude:

1. that experimental mesocosms, in parallel to field observations, can be used to study microbial communities in North Sea sediments and
2. that our experiment shows that the relationship between flagellate and bacterial communities appears to be very different in sandy versus muddy North Sea sediments.

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APPENDIX IV

Metabolic responses of soft-bottom benthic mesocosms to enrichment and food deprivation

(G.C.A. Duineveld, P. Verburg, E.M. Berghuis, A. Kok, G.J. van Noort & P.A.W.J. de Wilde)

IV. METABOLIC RESPONSES OF SOFT-BOTTOM BENTHIC MESOCOSMS TO ENRICHMENT AND FOOD DEPRIVATION

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ABSTRACT

This paper discusses an experiment whereby intact boxsamples from the open North Sea were transferred to a mesocosm in order to study the effect of food addition and food deprivation on the aerobic metabolism of the community. The samples were collected from two sites in the southern North Sea: one with a sand bottom and the other with a muddy sand bottom. While the mesocosm experiment was run, field measurements of community metabolism were performed for comparison of the actual situation with the experimental situation. Community metabolism in the food-deprived cores declined steadily during the experiment irrespective of temperature variation which was closely fitted to the field temperature. A concurrent reduction of the macrofaunal and meiofaunal abundance was observed. Some differences in the resistence of the different faunal taxa to starvation were noticable. Food addition (23 g $C.m^{-2}$ of dead Phaeocystis) evoked an immediate but short-lasting metabolic response in both sediment types. The amount that was added was apparently not enough to keep the metabolism at a level found in the field situation. Changes in the carbon, nitrogen and pigment content of the sediment in the field and experimental samples are discussed in relation with changes in the community metabolism. Furthermore some comments are added with respect to the feasibility of doing mesocosm research with intact boxsamples.

IV.1. INTRODUCTION

One aspect of the present experiment was to investigate the feasibility of introducing and maintaining large-scale subtidal soft-bottom benthic communities in a mesocosm facility. So far this was only done succesfully with intertidal communities (de Wilde & Kuipers, 1977). Positive results with subtidal communities in mesocosms concern intact boxcores, that were held separately in a large basin (de Wilde, 1990). An advantage of having a large surface area at ones disposal, is that edge effects induced by the artificial casing are reduced. Larger mobile animals, for instance, can exhibit their natural behaviour and their effects on the community can be studied under more natural conditions (Appendix Chapter V). The effort put in constructing a large surface area, on the other hand, requires that one refrains as much as possible from destructive sampling procedures (de Wilde, 1990).

Two sediment types were selected for the experiment: a sand bottom representing a large part of the Broad Fourteens, and a silty-sand bottom characteristic for the Frisian Front (see Appendix Chapter III Fig. III.1 for regions). Large surface areas of each sediment type were constructed by uniting series of neatly aligned boxcores in the mesocosm. In an early phase of the experiment a decision was made not to feed the large surface areas, but to keep them for the purpose of monitoring the fate of a community in response to food deprivation. The reactions by the different faunal categories (micro-, meio, and macrofauna) were expected to be a mirrorimage of their response to food input. This effect of food input was studied in an additional series of boxcores that were taken into the mesocosm before the onset of the spring bloom. Since this concerned a short-term experiment, the boxcores were held separately.

In order to follow the wax and wane of the mesocosm communities in comparison to the in situ communities, regular measurements of benthic respiration, carbon-nitrogen content, and meiofaunal abundance were made in the two habitats (field/mesocosm). Because in both situations, algal material consitutes the principal fuel for benthic activity, regular analyses were made of types and amounts of pigment in the sediment. The fate of macrofauna in the mesocosm was monitored by means of counting dead animals and of surface photographs on which burrow patterns were counted.

VI.2. METHODS

Some biological characteristics and sediment parameters of the two sediment types have been summarized in Table IV.1.

TABLE IV.1

	Broad Fourteens	Frisian Front
Waterdepth (m)	28	38-40
Median grain size (µm)	250	120
% mud (particles < 50 µm)	<1	25
Biomass macrofauna g afdw.m^{-2}	5-10	25
Macrofaunal community	Venus	Amphiura

For the field measurements of benthic respiration, undisturbed samples were taken with a modified Reineck boxcorer equipped with a circular, polyester box (diameter 31 cm)(Cramer, 1989). The major modification of the boxcorer consists of a valve which is open during penetration in order to reduce a bow-wave effect, but after penetration tightly seals the top of the box. This prevents the loss of the in situ bottom water and the fine top layer of the sediment. On deck the box containing the sediment sample with overlying water was removed from

the corer, sealed with a cover plate containing a stirrer and electrodes, and placed in a thermostatted incubator. Data from the electrodes were continuously read and stored on a PC. The electrodes were calibrated with a winkler titration at the end of each measurement and, moreover, compared to blank readings without a sediment core. The same technique was used in the mesocosm with the only difference that the polyester box was manually inserted in the sediment and left in this position for the duration of the whole experiment.

For the feeding experiment, algal material was collected during the Phaeocystis bloom in the Wadden Sea. This material was divided in portions and deep-frozen. The carbon and nitrogen content of the algal material was measured in a series of samples.

Pigment analyses were performed by means of HPLC. Not all the peaks could be identified to a pigment species, hence no actual weight concentrations could be calculated. For the purpose of comparing the pigment content in different samples, the counts belonging to a set of major peaks were summed.

IV.3. RESULTS AND DISCUSSION

IV.3.1. Sandy sediment (Broad Fourteens)

IV.3.1.1. Field situation

During a series of cruises in 1989, two boxcores (0.06 m^2) were incubated on deck for the measurement of community respiration. Additionally, meiofauna numbers were counted in subcores, and the carbon, nitrogen and pigment content of the sediment was analysed. The results are shown in Table IV.2.

TABLE IV.2

Date	Temp	Resp	%Corg		%N		Pigments[a]		Meio
		µmol/m^2/h	0-1cm	1-5cm	0-1	1-5	0-1	1-5	*10^6/m^2
31-01	5.0	95/95	0.09	0.06	.015	.015	19	6	0.39
20-04	8.5	260/285	0.24	0.10	.015	.010	132	24	0.64
19-06	14.2	860/985	0.13	0.08	.020	.020	32	51	1.22
29-08	17.0	350/625	0.09	0.11	.011	.017	6	33	0.65
07-11	13.0	50/50	0.05	0.04	.005	.004	15	8	0.63

[a] in counts.g dry sediment^{-1}; values correspond with first column of respiration values

All the values in Table IV. 2 show a distinct seasonality. In April a sharp increase in pigment content was found as a result of the spring bloom; at the same time the carbon content of the top 1 cm reached its

highest value. Noticable is the increase of the C:N ratio in the top 1 cm accompanying the deposition of the spring bloom. The greatest increase in benthic respiration and meiofaunal abundance takes place in the period April-June. This is due to the combined effect of an increased amount of organic matter in the sediment and a rise in temperature. By June the pigment content of the top 1 cm has been reduced considerably, but now the deeper layer (1-5 cm), contains the highest pigment content.

A further rise in temperature in summer, is neither accompanied by an increase in meiofaunal abundance nor in benthic respiration because the food becomes depleted. Nevertheless the pigment and carbon values in the deeper layer are still elevated.

On the basis of the O_2-uptake values in Table IV.2, a total annual carbon demand of 31 g C/m^2 is estimated for the Broad Fourteens sand community. This figure agrees with the earlier estimate by Cornelis (1984) for the same station, and with value found by Cramer (1990) at the more northern fine-sand station. Also the present observation that the respiration in June is higher than in August, corresponds with the results of Cornelis (1984). At a fine-sand station 30 miles north of the present Broad Fourteens station, Cramer (1990) found highest respiration values in August and lower values in June. The maximum values at both stations, however, show a close agreement.

IV.3.1.2. Starved Mesocosm

In January 1989 12 boxcores (0.25 m^2 each) were taken into the mesocosm and arranged to form a surface area of 3 m^2. This bottom sections was kept in the mesocosm without addition of food during a one-year period. At regular intervals the community respiration was measured and cores extracted for meiofaunal counts and carbon-nitrogen analysis. Dead animals appearing at the surface were collected and counted and regular surface photographs were taken. The temperature cycle of the water was closely fitted to the temperature in the open North Sea. The results from the measurements are shown in Table IV.3. Not included are the pigment contents in the sediment, because they were near or below the detection limit.

Table IV.3 shows that respiration remained more or less constant throughout most of the period, irrespective of the increase in temperature. Only during the last months of the experiment respiration decreased significantly, probably in response to food depletion. The carbon content of the sediment decreased steadily during the first half year and remained more or less constant thereafter at very low levels.

Remarkable is the very early reduction of the meiofauna (all taxa) in comparison to the macrofauna. It is not clear whether this is due to the experimental conditions or to a rapid depletion of suitable (fresh labile?) food. Bacteria, being a potential food source for meiofauna, did not show any dramatic reduction in terms of numbers or production during the experiment (Appendix Chapter II).

Larger animals are able to sustain for longer periods on the energy

stored in their tissues or on the organic matter stored in the sediment. But also among the different macrobenthic species, differences exist in their ability to cope with food deprivation. The population of the suspension-feeding bivalve Donax vittatus was the first to collapse, whereas the surface-depositfeeding Echinocardium cordatum and Tellina fabula managed to survive over a longer period.

TABLE IV.3

Date	Temp	Resp	%Corg		%N		Meio	Ec	Ev	Tf
		µmol/m²/h	0-1cm	1-5cm	0-1	1-5	x10⁶/m²	*	*	*
16-02	10.0	134/125	0.15	0.08	.035	.028	0.82	133	22	11
30-03	6.5	155/-	0.09	0.10	.020	.025	0.17	-	-	-
26-04	6.0	106/108	-.-	-.-	.-	.-	0.18	105	19	11
10-05		-/-	-.-	-.-	.-	.-	-.-	93	5	10
14-06	10.8	136/95	0.03	0.03	.000	.000	-.-	86	3	-
24-08	16.6	155/123					0.09	25	1	1
11-10	14.8	83/89					0.05	3	0	0
13-12	10.5	58/59					0.05	0	0	0

* Ec= Echinocardium cordatum in no. ind./m²
Dv= Donax vittatus
Tf= Tellina fabula

IV.3.1.3. Enriched mesocosms

In April 1989, 8 boxcores (0.25 m² each) were collected for feeding experiments in the mesocosm. Four cores were collected from the Broad Fourteens sand station and the other four from the silty sand station at the Frisian Front (see section 3.2.3). The intact cores were transferred to the laboratory where they were put in the temperature controlled mesocosm. Temperatures were kept at the ambient North Sea values. In order to study the effect of sedimentating phytoplankton on benthic respiration and meiofauna, each core received a ration of 23 g C/m² in the form of dead phytoplankton (mainly Phaeocystis). This ration was administered in three portions over a two-week period.

Benthic respiration was measured in two replicate cores. The results from these measurements as well as those from the analysis of carbon, nitrogen, pigments, and meiofauna density in the other two replicates, are shown in Table IV.4.

TABLE IV.4

Date	Temp	Resp µmol/m²/h	%Corg 0-1cm	%Corg 1-5cm	%N 0-1	%N 1-5	Pigments[a] 0-1	Pigments[a] 1-5	Meio x10⁶/m²
23-05	9.0	262/284	0.05	0.02	.002	.002	12	17	0.65
24-05									[1]
31-05	9.8	372/459	0.07	0.06	.000	.000	110	38	0.46
01-06									[2]
06-06	9.5	375/355	-.-	-.-	.-	.-	-	-	-.-
07-06									[3]
08-06	10.2	728/761	0.12	0.06	.040	.020	124	37	-.-
13-06	10.4	486/476	0.10	0.03	.030	.010	79	30	0.27
09-08	15.5	183/-	0.04	0.04	.004	.005	-	-	-.-
04-10	15.0	40/-	0.03	0.03	.005	.006	5	4	0.13
22-11	10.8	72/-	0.05	0.05	.007	.007	-	-	0.06

[a] in counts.g dry sediment^{-1}; values correspond with first column of respiration values. $_{1,2,3}$ time of addition of one food portion

The respiration measurements that were made immediately after the input of the third portion suggest that each portion induces a quick, but short-lasting, increase of benthic respiration: one day after administering the food, respiration rose to a level comparable to the the field (see Table IV.1), but within five days this level had been reduced by roughly 40%. Table IV.4, moreover, shows that shortly after the third portion had been added, the total pigment content of the sediment was similar to that in between the first and second feeding, which suggests that little algae had accumulated prior to the addition of the third portion.

Assuming that each portion was largely utilized during the intervals between feeding it is then possible to make a rough estimate for the respiratory effect of the three portions. Because after the first two portions several days elapsed before respiration was measured, the actual peak values may have been missed. The respiration measured during the six days following the third feeding event are therefore taken as a representative response. With an average respiration rate of 620 µmol $O_2/m^2/h$ in this period, the gross carbon demand would be about 1100 mg C/m^2. Assuming that the same response occurred in the preceeding two intervals, total gross consumption over the entire reactive period would amount to roughly 3.5 g C/m^2. Taking the pre-treatment respiration, i.e. 273 µmol $O_2/m^2/h$, as a standard metabolic level, the additional consumption as a result of the input of 23 g C/m^2 would be 1.7 g C/m^2.

In contrast to the pigment levels, carbon and nitrogen values suggest that some accumulation had occurred during the course of food addition. Table IV.4 shows that the carbon content in the top 1 cm had increased from an initial value of 0.05% (date 23-05) to 0.10% (date 13-06), which equals a net addition of roughly 8 g C/m^2. This value is

probably far too high, because the mineralization of this amount in the period up to 09-08 when the overall carbon level was back at its low pre-treatment value again, would require a respiration rate of appoximately 585 µmol $O_2/m^2/h$ throughout the whole 57 day period. These rates were neither found at the beginning (13-06) nor at the end (09-08) of this period.
It is clear that a substantial amount of carbon that was introduced cannot be easily accounted for. Part of this 'missing' carbon will have been washed from the surface as dissolved carbon, whereas another (minor) part may have been stored in larger metazoan animals which were excluded from the carbon measurements.

Although the meiofauna was not so frequently sampled, their decreasing numbers indicate that they didn't benefit from the food input. This observation in combination with the short lasting metabolic reaction, illustrates that the actual amount of food available to the community, was too small to induce a natural reaction.

IV.3.2. Silty sand station (Frisian Front)

IV.3.2.1. Field Situation

Table IV.5 shows the results from the field measurements (cf. section IV.3.1.1.) at the silty sand station.

TABLE IV.5

Date	Temp	Resp	%Corg		%N		Pigments[a]		Meio
		µmol/m^2/h	0-1cm	1-5cm	0-1	1-5	0-1	1-5	*10^6.m^2
07-01	5.0	645/-	0.38	0.54	.035	.060	285	318	-.-
20-04	8.0	1230/-	0.46	0.54	.035	.063	991	599	4.02
12-07	16.0	1380/1407	-.-	-.-	.-	.-	418	377	8.90
29-08	17.0	1900/1450	0.55	0.72	.041	.059	182	357	5.27
07-11	13.0	1000/-	0.61	0.77	.052	.064	415	352	6.50

[a] in counts.g dry $sediment^{-1}$; pigment values correspond with first column of respiration values

Already in April high benthic respiration rates were found at this station. At the same time an increase in pigment content and carbon content was observed resulting from the spring bloom. Benthic respiration continued to rise in the proceeding period until August, accompanied by an increase in the organic carbon and nitrogen levels. Van Duyl et al. (Appendix Chapter II) report a concommittant increase in bacterial production and biomass in the period April-June, with constantly high levels up to August. Contrasting with this pattern, is the apparent decrease of the pigment content, notably in the top layer. Unlike the initial rise in benthic respiration at the time of

the spring bloom, the subsequent rise in summer seems to be unrelated to the pigment content of the sediment. A clear explanation for this discrepancy is lacking.

A further enhancement of the carbon and nitrogen levels, and this time also of the pigment content, was found in November. Bacterial production remained relatively high (Appendix Chapter II) but benthic respiration had decreased by this time, partly as a result of the decreasing temperature.

The meiofaunal counts didn't produce any clear seasonal pattern. Earlier meiofauna work on the same station (Groenewold, unpubl.), showed growing numbers during the course of the year, culminating in maximum densities in autumn and followed by a decrease in winter.

The high levels of benthic respiration, bacterial production (Appendix Chapter II), as well as the pigment content, indicate that this station must receive an additional input of organic material in comparison to the sandy Broad Fourteens station. The estimated annual carbon demand of the silty station amounts to 104 g C/m^2 which agrees with the earlier estimate by Cornelis (1984) and a more recent one by Cramer (1990).

IV.3.2.2. Starved mesocosm

The results from the measurements in the starved mesocosm (cf. section IV.3.1.2) are shown in Table IV.6a.

TABLE IV.6A

Date	Temp	Resp	%Corg		%N		Pigments		Meio
		$\mu mol/m^2/h$	0-1cm	1-5cm	0-1	1-5	0-1	1-5	$*10^6/m^2$
16-02	10.0	758/522	-.-	-.-	.-	.-	286	318	-.-
06-03	6.5	-/-	0.43	0.53	.060	.070	304	273	3.08
30-03	6.5	630/415	-.-	-.-	.-	.-	119	284	3.20
26-04	6.0	444/331	-.-	-.-	.-	.-	-	-	1.84
14-06	10.8	568/577	0.37	0.53	.055	.050	-	-	-.-
24-08	16.6	395/345	0.45	0.54	.034	.044	35	104	0.59
11-10	14.8	284/270	0.60	0.69	.052	.037	-	-	0.51
13-12	10.5	264/179	0.51	0.59	.036	.045	6	155	0.31

Benthic respiration rates showed a gradual decrease irrespective of the rise in temperature. Throughout the duration of the experiment, benthic respiration remained at a higher level than in the starved sandy sediment. This is explained by the larger pool of organic carbon (biomass, buried carbon) present in the silty sediment. The carbon levels in the sediment did not show any consistent change during the experiment, probably because of the high background levels of refractory carbon. Nitrogen levels, however, are clearly depressed at the end of the experiment, indicating a reduction of the labile fraction of the organic material. The total pigment content showed the

most distinct reduction during the experiment, and the closest correlation with the respiration rate. Especially in the top layer, where most of the feeding activity by macro and meiofauna is concentrated, was almost cleared of pigments by the end of the experiment.

From March onwards, the meiofauna (mostly nematodes) showed a steady decrease in numbers, but the final densities were still considerably higher than in the starved sandy mesocosm (section IV.3.1.2).

Table IV.6b summarizes some results from the analysis of surface photographs that were regularly taken from two bottom sections (0.25 m^2 each).

TABLE IV.6B

	Amphiura arms	Callianassa mounds	Burrows
February	591/409	12/39	15/11
April	377/288	16/29	10/27
May	481/245	13/17	16/41
June	414/265	6/15	38/39
August	587/269	10/25	36/51
September	344/173	7/16	21/45
October	766/299	9/24	18/49

Table IV.6b shows no consistent decline of the surface activity by the resident macrofauna. Because the initial abundances are not exactly known and the patchiness of the fauna is rather high, it is hard to make accurate statements about the reduction of the macrofauna in the starved mesocosm. At the end of the experiment two samples (0.06 m^2) from the mesocosm were sieved over a 1 mm sieve. When compared to density estimates derived from field samples taken in January, the density of species retrieved from the mesocosm appeared to be invariably lower. Some abundant species in the field samples were even absent from the mesocosm samples. The species with the best survival rate was the ophiuroid Amphiura filiformis (900 ind./m^2 vs. 1300 in January). Considerable reductions were found with Callianassa subterranea (18 ind./m^2 vs. 104) and Mysella bidentata (28 ind./m^2 vs. 1000). It is remarkable that also in this case a representative of the echinoderms showed highest survival.

IV.3.2.3. Enriched mesocosms

Table IV.7 shows the results from the measurements in the enriched silty sand mesocosms (cf. section IV.3.1.3).

TABLE IV.7

Date	Temp	Resp µmol/m²/h	%Corg 0-1cm	%Corg 1-5cm	%N 0-1	%N 1-5	Pigments[a] 0-1	Pigments[a] 1-2	Meio x10⁶/m²
23-05	9.0	763/1141	0.50	0.42	.035	.030	240	623	1.22
24-05									[1]
31-05	9.8	803/1554	0.52	0.59	.048	.060	157	286	1.05
01-06									[2]
06-06	9.5	820/1900	-.-	-.-	.-	.-	-	-	-.-
07-06									[3]
08-06	10.2	909/1885	0.61	0.60	.075	.065	261	193	-.-
13-06	10.4	765/1930	0.59	0.72	.075	.090	165	280	2.05
09-08	15.5	-/887	0.44	0.86	.040	.052	-	-	-.-
04-10	15.0	-/620	0.64	0.57	.055	.051	33	47	2.51
22-11	10.8	-/435	0.55	0.74	.050	.057	-	-	3.16

[a] in counts.g dry sediment^{-1}; pigment values correspond with second column of respiration values. $_{1,2,3}$ time of addition of one food portion

The benthic respiration measurements show that feeding induced an enhancement of the respiration rate. In contrast to the enriched sand mesocosms (see Table IV.3), the responses by the two replicates in this case differ both in magnitude and character. In both replicates, however, respiration rates did increase after the first portion, and continued to rise after the second portion had been administered. In the more reactive core, the third portion did not elicit a further increase of the respiration rate, which remained at the same, apparently maximum, level until 13-06. This pattern suggests that food in this stage was not a limiting factor, possibly as a result of accumulated food in the sediment. It should be stressed beforehand that because of the high background values of refractory carbon (see section IV.3.2.2.) in comparison to the moderate amounts that were introduced, it is risky to draw conclusions from the carbon values. Nevertheless, both the rising carbon and nitrogen contents in the period 23-05 to 13-06 seem to substantiate the supposition that material had accumulated during the addition of food.

The pigment values in Table IV.7 tell, however, a different story. At the start of the experiment the core, which had been collected in April, had an elevated pigment level compared to the pigment level in January in the field (Table IV.5). Apparently some fresh organic matter had settled on the bottom between 7 January and 20 April. Already shortly after the introduction of the first portion, pigment levels showed a significant decrease and two additional portions did

not bring the pigment back near pre-treatment levels. Thus instead of an accumulation, pigment values point at a fast reduction of the food stock, which was however still sufficient to maintain an enhanced respiration rate at least until 13-06. The logical next question is why the initial pigment content didn't give rise to maximum rates? An hypothetical explanation could be that the fresh material introduced acted as a catalyst for the benthic system to utilize the older residing material.

The response by the less reactive core displays a different pattern. Immediately after the third portion, the respiration rate surpassed the preceeding levels, but at 13-06 the respiration rate had again decreased to a pre-treatment level. In this respect the less reactive core resembles the enriched sandy mesocosms, where feeding induced a quick, but short-lasting effect on benthic respiration.

Because of this differential response, total mineralization as a result of food input is estimated separately for the two cores. The period over which this quantity is estimated for the more reactive core starts with the first portion and ends approximately 2 months later as soon as respiration had resumed its pre-treatment level. Gross total mineralization in this period amounts to 29 g C/m^2. Assuming a standard (pre-treatment) level of 1141 $\mu mol/m^2/h$, net mineralization due to food input would be 8 g C/m^2. It is impossible to account for the remaining carbon in the reactive core because of the problems associated with the high background values in relation to the moderate amounts that were introduced. For the less reactive core a similar approach was applied as with the enriched sand mesocosms (see section IV.3.1.3.). The resulting figure for gross mineralization in this case is 4.3 g C/m^2, with a net mineralization of 0.4 g C/m^2 when one assumes a standard respiration of 765 $\mu mol/m^2/h$.

The first meiofauna sample (23-05) yielded extremely low numbers compared to the field density (see Table IV.4). This rapid decline apparently took place in the month that the experimental cores were allowed to adapt prior to the introduction of food. Such an initial reduction did not seem to have taken place in the enriched sand mesocosms (see Table IV.4). In both the starved mesocosms (Table IV.3 and 6a), however, the meiofauna populations also collapsed in an early stage, which was ascribed to either the effect of experimental conditions or the lack of suitable food. The steady increase of meiofauna density after the food had been introduced in the present case, points at improved conditions despite the apparent decrease in food stock as indicated by the pigment values.

IV.4. Some additional remarks regarding practical matters

- The present experiment has shown that it is possible to introduce benthic communities in the available mesocosm facility without immediate detrimental effects. A reservation should perhaps be made for the meiofauna, which in several cases showed a initial decline after the boxes had been transplanted into the mesocosm.

- It is obvious that food is a necessary prerequisite to maintain the communities in their original state. Especially the sand community, which contains only a small buffer of organic material, shows a rapid dying of the larger metazoan fauna if it is kept devoid of food. Non-destructive techniques (visual observations, photography) meant to follow the fate of the larger macrofauna didn't produce adequate results in the silty sediment with its large fraction of subsurface fauna. In the sand community, dying individuals of several dominant species appeared on the surface and could therefore be easily counted.
- It is not possible to make any statements about effect of the size of the experimental surface area on the well-being of the macrofauna. Surface photographs showed that in the large (3 m^2) unfed section with silty sediment, Callianassa migrated beyond the dimensions of the smaller containers (0.25 m^2). Whether these migrations were a reaction upon the absence of food input is however unknown.

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APPENDIX V

Measurement on burrows of *Callianassa subterranea* made in a mesocosm

(R. Witbaard)

V. MEASUREMENTS ON BURROWS OF CALLIANASSA SUBTERRANEA MADE IN A MESOCOSM

Rob Witbaard

ABSTRACT

Burrow casts were made from a semi-natural population of Callianassa subterranea, accomodated in a mesocosm (3 m^2). Strict relations between animal size, burrow volume, surface wall area and burrow depth were found, upon which a total bottom surface area enlargement of at least 50% was calculated.

V.1. INTRODUCTION

In benthic research resin casting has become a popular technique for studying burrows of subsurface dwelling organisms. In most cases Thallassinid shrimps, like Callianassa or Upogebia are taken as object in these studies.

These crustaceans significantly influence the benthic community by their enormous capacity of sediment reworking and burrow construction. Vaugelas (1985) splits their effects into physical and biological consequences. Among the physical consequences, sediment reworking with a turnover of the surface sediment layer, lateral transport of expelled sediment and sediment sorting are most conspicuous. Less obvious are the changes in the effects on sediment water exchange or the consequences for larval setting. A schematic representation of the processes taking place is given in Fig. V.1.

Most studies in which the resin casting technique was used, were confined to intertidally living species (Atkinson, 1984; Suchaneck, 1985). Much less attention has been paid to subtidal burrowing organisms, probably due to the great difficulties caused by working at great depth with scuba divers (Atkinson, 1984).

Callianassa subterranea is such a deep burrowing subtidally living shrimp. In the North Sea densities of more than 50 individuals per square meter are found in the Oyster Grounds (Witbaard & Duineveld, 1989). Laboratory studies revealed that this species, as other Callianassids, must exert a great influence on the community by its burrowing and pumping behaviour. For instance; nutrient concentrations and oxygen concentration within the burrow deviates strongly from the overlying water (Witbaard & Duineveld, 1989).

To unravel the burrow structure, its function and its possible effect on the community, resin casts were made in the laboratory between 1985 and 1988. Although much problems arose, some idea of burrow morphology and size was established (Witbaard & Duineveld, 1989). Laboratory reared burrows consist of several vertical shafts

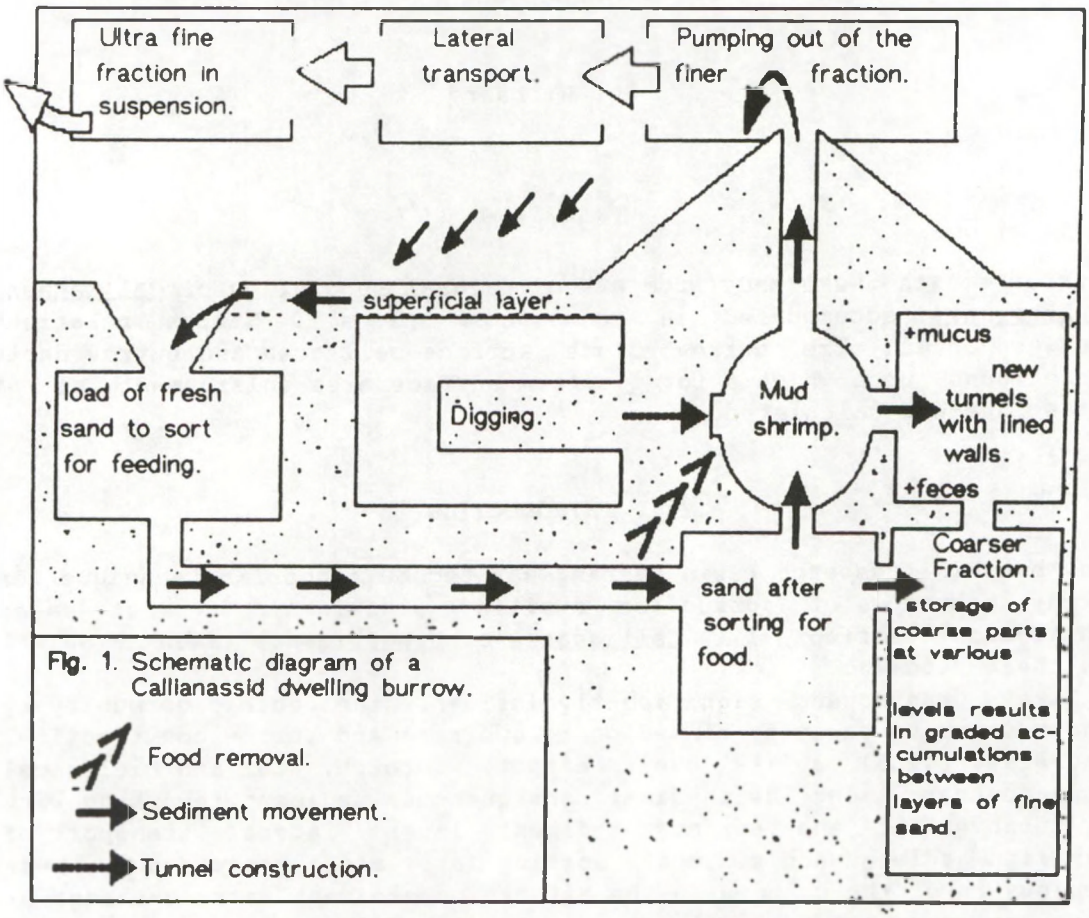

Fig. V.1. Schematic diagram of influences Callianassa species exert on their environment (after Vaugelas, 1985).

descending from the surface which start branching in a more or less horizontal plane (=penetration depth, depth of first floor) at a depth which is dependent of the inhabitants size. That this structure resembles in situ constructed burrows is confirmed by observations on "dissected" boxcores (Foster, personal communication) and by cast fragments from the field (Atkinson, 1985). In the laboratory, space limitations however could significantly have influenced burrow size.

The 1989 mesocosm experiment offered an opportunity to work "subtidally" without dealing with the technical problems encountered in the field, but still offering the advantages of field work. Working in these mesocosms could probably enlight the problem of space limitations dealt with in the previous experiments. Also the starting point differed from the former experiments. Here already established burrows were taken with a Scripps core and maintained in the laboratory while animals had to excavate new burrows in the earlier experiments.

V.2. METHODS

A mesocosm was filled with a surface area of 3 m^2 with 12 intact sediment cores of 0.25 m^2 each from the Frisian Front (53.42´50 N; 4.30´00 E). Collection took place between 4 and 18 January 1989. After collection, the 0.25 m^2 cores were accomodated in slightly larger sized boxes filled with seawater and transported to the laboratory. There the cores were put into the mesocosm side by side. After removal of the bottom lids and core mantle open spaces between different cores were filled with lose sediment. Some parts of this sediment surface were sampled regularly other parts were photographed at monthly intervals. Two time series of photographs were roughly analysed. Of the undisturbed parts resin casts were made in December 1989.

Uralam 94/43 resin (DSM) and bezoeperoxide as catalyst were used. To slow down the reaction 0.75% Hobilon M was added before adding the catalyst. Furthermore the resin was precooled in ice. At the time of casting water level was lowered to about 15 cm above the sediment level. The resin was carefully poured into all burrow entrances, but leaving one opening open as long as possible. This was done on a total surface area of about 0.75 m^2.

After overnight hardening of the resin, water level was lowered further down and casts were excavated by washing the sediment away with water.

Burrow displacement volume and total burrow wall surface were measured for each burrow separately. For the surface measurements the burrow was simplified into cylinders, knotted cylinders and balls. For each cast the length of every individual shaft and its diameter (average value) were measured as well as the diameters of the "balls" from which a surface could be calculated. Summation of these separate surfaces gave total burrow wall surface for that specified burrow.

V.3. RESULTS

Burrow entrances were small shortly after the photographs were taken, but as the year progressed they grew in size and gave the sediment surface a rougher appearance. There was however no trend found in the number of openings or mounds (Appendix Chapter IV). From the photographes the wandering position of the burrows could be confirmed. New entrances were formed while others collapsed. From 19 casts made (0.75 m^2) 11 were more or less "complete". An overview of their volume and surface area is given in Table V.1. Morphology of the burrows was in concordance with observations made earlier (Witbaard & Duineveld, 1989) and those in natural systems (Foster, personal communication).

Based on these data a relation between burrow volume and wall surface and between shaft diameter (animal size) and penetration depth could be established. Furthermore good relations were found between shaft diameter and volume or wall surface. Above mentioned relations are illustrated in Figs V.2, 3, 4 and 5.

TABLE V.1

Given is the estimated volume, the burrow wall surface, the minimum depth at which the vertical shafts start branching (penetration depth) and the estimated carapax length of its inhabitant (Dworschak & Pervesler, 1988).

burrow	volume (ml)	surface cm^2	depth of 1st floor	estimated carapax length
A	171	411	27	9.4
B	151	303	18	7.3
C	17	95	9	4.2
D	26	82	8	3.1
E	30	130	6	4.2
F	75	309	16	7.3
G	58	150	13	7.5
H	8	34	9	3.1
I	88	28	14	6.2
J	155	462	27	7.3
K	133	428	12	8.3

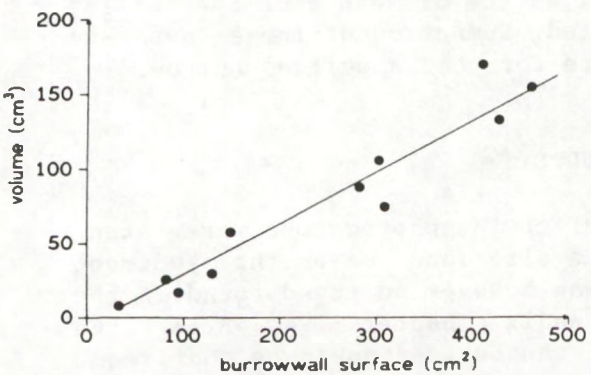

Fig. V.2. Relation between burrow volume and burrow wall surface.

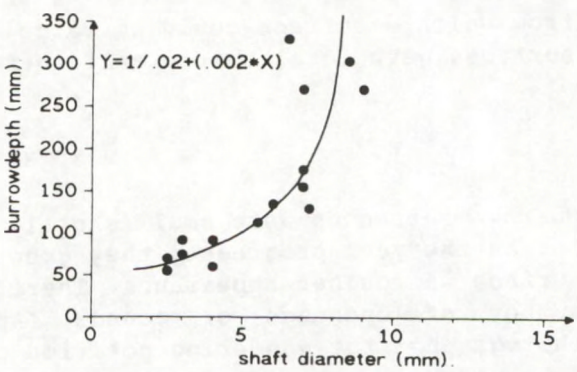

Fig. V.3. Penetration depth of the burrow as a function of the diameter of the vertical shaft, which is related to animal size.

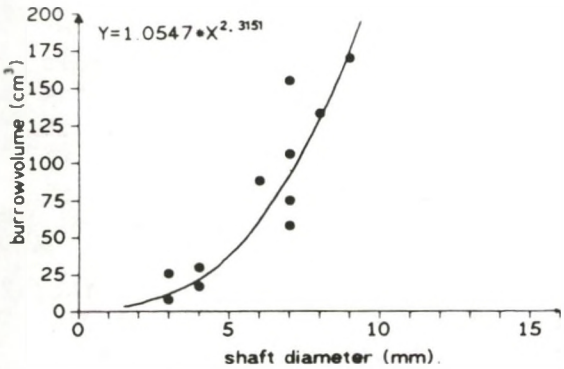

Fig. V.4. burrow volume in relation to shaft diameter.

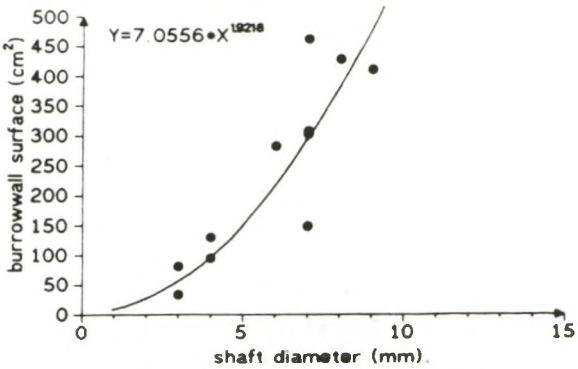

Fig. V.5. burrow wall surface in relation to shaft diameter.

V.4. DISCUSSION

The change in size of the burrow openings is probably caused by a lack of bottom current and large bottom dwelling organisms which probably smooth the surface by their action.

No difference was found in the morphology between burrows made in this mesocosm and those made earlier or by other observers (Foster, pers. comm.), although the starting point from which the burrows evolved differed markedly. In this mesocosm experiment it was however illustrated that the burrows can have very widely spaced (> 40 cm) openings from which it must be concluded that space limitation must have played a role in the primer experiments. Although there was no horizontal oriented space limitation in the mesocosm there must have been a space limitation in the vertical plane for at least one animal, which had excavated its burrow down to the bottom of the basin, which was about 50 cm below the sediment surface. From this it can be concluded that even in the field it will be very difficult to sample 1 complete burrow.

The relation that was found between burrow wall surface and burrow volume of these newly made casts fits perfectly with the relation established from the earlier made casts. This illustrates the geometrical constancy between surface and volume (Fig. V.2) eventhough burrow history differed. Furthermore it illustrates the reliability of estimating burrow volume and surface.

Of much greater importance for field work are the relations between shaft diameter and penetration depth and burrow size expressed as volume or wall surface. As shaft diameter is animal dependent, an indirect relation between animal size and burrow size and depth is established. Assuming a relation between shaft diameter (SD) and carapax length (CL) (CL:SD=1.04:1) as found for C. bouvieri (Dworschak & Pervesler, 1988) results in the estimated lengths as given in Table V.1. That this relation fits well for C. subterranea is collaborated

by A. Rowden (Plymouth Marine Laboratory) (pers. comm.).

Based on these newly made casts a more detailed depth separation of different length classes could be made (Fig. V.3). Smallest animals (Cl 3 mm) which construct shafts of about 3 mm diameter live in the upper 10 cm of the sediment. Medium sized animals which construct vertical shafts of 4 to 6 mm in diameter are living roughly between 10 and 20 cm and the biggest animals (Cl 10 mm) are living at a depth of 20 cm or more. There is however much overlap between these last two categories.

The presence of the smallest animals in the toplayers of the sediment suggests that juveniles settle at the sediment surface. Probably the young, just hatched, larvae are expelled from burrow by the exhalent water current generated by the parent which is in accordance with literature in which the pelagic status of the larvae is confirmed (Lutze, 1938).

Based on the calculations made, the total burrow wall surface and burrow volume in the mesocosm at the end of the year, must have been about 1.5 m^2 and 5.4 liter respectively. These values are one third of the estimations made for the field (Witbaard & Duineveld, 1989). This decrease is probably caused by mortality. It should however be stressed that, although densities were low, the influence on the total of the mesocosm must have been great. Realize for instance that at a total bottom surface of 3 m^2 an extra burrow wall surface of about 1.5 m^2 is present. So actual bottomsurface in the mud part of the mesocosm was not 3 m^2 but 4.5 m^2 or even more as other burrowing animals are not taken into account. It should also be realized that in the burrows, oxygen levels are very low (0.5 to 84% of the O_2 concentration of the overlying water, Witbaard & Duineveld, 1989) and thereby strongly influencing denitrification, phosphate fluxes and speciation of metals.

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