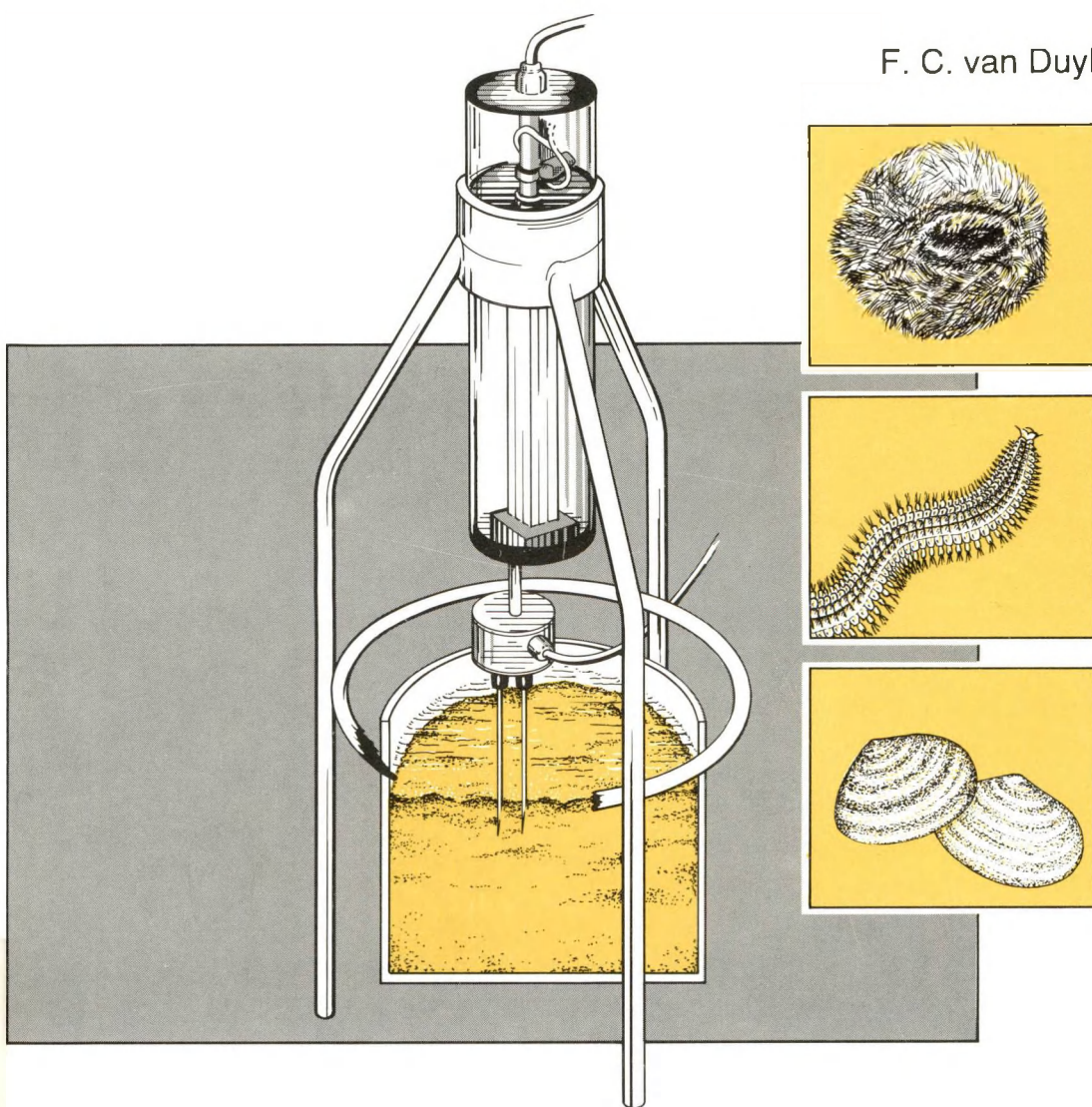


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BENTHIC RESPONSE TO EUTROPHICATION IN MANIPULATED MARINE SANDY SEDIMENTS

- MESO/BOXCOSM RESEARCH 1990 -

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BENTHIC RESPONSE TO EUTROPHICATION IN MANIPULATED MARINE SANDY SEDIMENTS

- MESO/BOXCOSM RESEARCH 1990: INFLUENCE OF ORGANIC MATTER LOADINGS -

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1. INTRODUCTION

North Sea benthic meso/boxcosm studies were initiated in 1989 to increase our understanding of benthic metabolism and nutrient regeneration in relation to eutrophication, i.e. an increasing input of particulate organic matter into the sediment. Major questions are: what organisms benefit or suffer from increasing or decreasing eutrophication and what is the threshold for oxygen depletion? So far, the consequences of the increase in organic matter input for benthic metabolism and nutrient cycling have received limited attention. Increase of organic matter input may result in enhancement of sediment organic matter content and benthic metabolism, decreasing oxygen penetration depths in the sediment and decreasing oxygen concentrations at the sediment-water interface. When the oxygen supply to the sediment is insufficient to sustain aerobic mineralization, anaerobic mineralization processes will predominate and suboxic or anoxic conditions at the sediment surface and in the bottom water may develop. The deleterious effects of low oxygen concentrations on bottomfauna and water quality are widely recognized (mass mortalities, poisoning). Complete deoxygenation of the sediment surface layer reduces nitrification, denitrification (the removal of nitrogen) and the buffer capacity (sorption) of the sediment for phosphate. Consequently, benthic nutrient regeneration and sediment-water exchange processes may be influenced also. The bottom can suddenly change from a sink for nutrients (burial of organic matter, adsorption, benthic biomass increase, denitrification) to a supplier of nutrients. Inorganic phosphate release from the sediment is known to increase due to anoxia.

In the German Bight, along the Danish coast, in the Kattegat and in the Baltic anoxic sediments and bottom waters have been found to occur regularly during summer. The extension of black patches (FeS) on the sediment surface of intertidal flats in the Wadden Sea has been reported. Extension of hypoxia in the North Sea and Wadden Sea due to eutrophication cannot be reconciled with a durable management of the marine environment. The processes underlying the nutrient release and development of the oxygen demand of the sediment and the thickness of the oxic zone in response to organic matter input need to be understood. With meso/boxcosm research aspects of these problems have been addressed.

Our mesocosm experiments in 1989 (NIOZ Report 1991-5) showed that the NIOZ-systems are suitable for eutrophication studies. The systems meet the requirements for the study of benthic responses to inputs of organic matter, particularly with respect to the faunistic components. It was possible to mimic the benthic microbial response to the sedimentation of the spring bloom in the field by supplying organic matter to mesocosms with intact sediment cores. Patterns in nutrient regeneration could not so easily be mimicked and the role of macrozoobenthos in benthic metabolism remained unclear. This urged us to develop better defined systems with more emphasis on the study of processes than on mimicking the field situation. The present study focussed on the response of different faunistic groups and the response of phosphate sediment-water exchange to algal inputs in manipulated sediments. Our research topics were: 1. the processes regulating sediment uptake and release of phosphate. 2. the response of benthic organisms (with respect to size, reaction time and duration of the response). 3. the participation and role of different groups of benthic organisms in benthic metabolism.

2. EXPERIMENTAL SET-UP

The complete experimental set-up consisted of 93 round polypropylene boxes (35 cm high; 30 cm diameter) placed side by side in the two North Sea mesocosm basins (Fig. 1). Boxes were filled up to 25 cm with sieved sand from the Zeegat van Texel and filled to the rim with aged sea water. Each box was equipped with a sea water supply ($10 \text{ ml} \cdot \text{min}^{-1}$) and an airflow. The incubation temperature was 12°C . After a preincubation period boxes were seeded with micro/meiobenthos. Subsequently 46 boxes were faunated with macrozoobenthos (6 *Echinocardium cordatum*, 6 *Tellina fabula* and 6 *Nephtys hombergii* per box). Two organic matter supply regimes were applied: a single pulse of $24 \text{ gC} \cdot \text{m}^{-2}$ and weekly supplies of $8 \text{ gC} \cdot \text{m}^{-2}$. The single pulse of organic matter was administered to study the rate and size of the biological and chemical response and its duration in the presence and absence of macrozoobenthos. The regime with weekly supplies was applied to study the growth of macrozoobenthos and the contribution of macrozoobenthos to benthic metabolism and sediment water exchange of phosphate. The organic matter consisted predominantly of *Phaeocystis*



Fig. 1. The North Sea boxcosm experimental set-up. Oxygen measurements are taken on the rear righthand side.

spec., which was collected during the spring bloom. Parallel, a series of control boxes (no supplies) was installed. The boxes were divided between the three research topics carried out: nutrient chemistry (P and N), microbial ecology (bacteria, protozoa) and macrozoobenthos (bioturbation and growth). One single box did not contain sufficient sediment to allow combined sediment sampling from it for all three topics. So for sediment sampling each topic had its own series of boxcosms. Similar sediment sampling schemes were maintained, where relevant. Benthic oxygen consumption and oxygen penetration depth were determined in all series.

With these measurements the development of duplicate boxes (same treatment, same incubation time) was controlled. For the methods applied in the different topics I refer to the respective papers in the appendix.

3. RESULTS

For the results of the different contributions I refer to the appendix. In brief the addition of organic matter caused a direct response of the nutrient chemistry and the benthos. A rapid release of phosphate was observed in response to the algal additions. Macrozoobenthos immediately started to sequester

the settled material and reworked the sediment to a depth of 5-6 cm within one month. The redistribution of organic matter enhanced mineralization rates. Bacterial biomass and production as well as nanoflagellate abundance and biomass increased. Also, the aerobic respiration increased, but this increase was not related to the presence of macrofauna. Presence of macrofauna in organic matter supplied boxes seemed to stimulate the anaerobic respiration in particular.

The budgets for the two different algal supply regimes are given in Table 1. The organic matter input was corrected for loss (6%) due to outflow over the rim. In this table the stock variables just before algal additions were subtracted from the stock variables as determined at the end of the experiments. The stock changes are given in the budget. Fluxes refer to the extra respiration observed due to the algal additions. Fluxes were integrated over the course of the experiment. Bacterial respiration (50% growth yield assumed) generally exceeded aerobic respiration as measured in benthic chambers implying that a considerable part of the benthic respiration occurred anaerobically. Only in the experiment with an input of 145 gC.m^{-2} in the absence of macrofauna was the bacterial respiration less (20 gC.m^{-2}) than the aerobic respiration (33.7 gC.m^{-2}). This may be ascribed to bacterial respiration in the algal mat which was not measured. The algal mat on top of the sediment surface was not systematically

included in the sediment analyses. This may also have resulted in the low recovery of TOC in the boxcosms, particularly in the ones without macrozoobenthos. The total recovery in the treatments with macrofauna was significantly higher (>80%) than the recovery in the treatments without macrofauna (23-49%). Burial of organic matter by macrozoobenthos increases the sediment organic matter content and reduces the loss of settled organic matter (due to resuspension) to the watercolumn. Burial stimulates benthic microbial mineralization. In the presence of macrofauna (bioturbation) far more effective use is made of algal sedimentation by the microbenthos (mineralization) than in the absence of macrofauna. This may imply that the risks of occurrence of suboxia/anoxia after a sedimentation event increase with increasing densities of burrowing organisms such as *Echinocardium cordatum*.

4. CONCLUSIONS

- The benthic response of microorganisms (production and biomass of bacteria, heterotrophic nanoflagellate abundance, oxygen consumption) to the single *Phaeocystis*-supply was initiated within days, reached its highest value in 3-18 days after the organic matter supply and lasted for up to 1 month. The phosphate release was mainly determined by processes taking place in the algal mat on top of the sediment surface and was back

TABLE 1

TOTAL INPUT	22.5 gC.m ⁻²	22.5 gC.m ⁻²	145 gC.m ⁻²	145 gC.m ⁻²
supply regime	at once	at once	c. 8 gC.m ⁻² .wk ⁻¹	c. 8 gC.m ⁻² .wk ⁻¹
duration experiment after first algal supply	39d	39d	130d	130d
	without macro	with macro	without macro	with macro
STOCK INCREASE				
detrital organic carbon (0-63 mm)	4.0 (18%)	6.9 (31%)	-1.3 (-0.09%)	34.2 (24%)
bacterial carbon (0-63 mm)	0 (0%)	0.2 (1%)	1.7 (1.1%)	2.5 (1.7%)
nanoflagellate carbon (0-63 mm)	0.06 (0.3%)	0.08 (0.3%)	0.23 (0.2%)	0.10 (0.1%)
macrozoobenthos carbon	-	0.5 (2.2%)	-	3 (2.1%)
FLUXES				
oxygen uptake (expressed in C-equivalents)	3.2	2.2	33.7	33.9
macrozoobenthos respiration	-	0.5 (2.4%)	-	2.3 (1.6%)
bacterial respiration (0-63 mm)	7 (31%)	11 (49%)	20 (13%)	85 (59%)
TOTAL RECOVERY OF INPUT	49.3%	84.9%	13.4% +9.4%	88.5%

to initial values 10-14 days after the introduction of the single supply.

- The presence of macrofauna had no clear effect on the sediment-water exchange of phosphate but appeared to stimulate the phosphate binding in the sediment.
- Bioturbation in the boxcosms supplied with organic matter resulted in a reworking of the sediment to 6 cm depth and a larger net increase in organic matter in the sediment compared to boxcosms without macrozoobenthos.
- The oxygen penetration depth in the sediment decreased in the 10 days after the algal input and subsequently stabilized at a thinner depth in the presence of macrofauna as compared to the penetration depth in the absence of macrofauna.
- The development in oxygen consumption in the presence and absence of macrofauna was comparable irrespective of the treatment except for the respiratory demands of the macrofauna. The burrowing activities of macrofauna apparently did not enhance the benthic oxygen consumption.
- In the presence of macrofauna, bacterial biomass and production increased in the anoxic layers of the sediment after algal additions, indicating an apparent stimulation of anaerobic mineralization.
- *Echinocardium cordatum* demonstrated growth in length after *Phaeocystis* supplies. Average growth rates of $0.75 \text{ mm.month}^{-1}$ were reached. This growth rate is 2-fold less than the maximum rate reported in field studies ($1.6 \text{ mm.month}^{-1}$).

GENERAL CONCLUSIONS

- In the boxcosm experiments bacteria are the major profiteers of increased eutrophication.
- The burrowing activities of macrozoobenthos may enhance the risks of occurrence of suboxic/anoxic conditions in the sediment surface and overlying water (resulting in mass mortalities) by their indirect stimulation of anaerobic mineralization.

5. FUTURE RESEARCH

The aim of our future boxcosm experiments is to enlarge our understanding of the effects of increased inputs of organic matter on benthic metabolism. Emphasis will be placed on determining the proportion of aerobic and anaerobic processes in benthic metabolism with increasing organic matter loads in the presence and absence of bioturbation. The development of anoxia in the overlying water and its effect on benthic organisms will receive further attention.

Assessment and analysis of the effects of increasing organic matter loads on the benthic metabolism will remain the central theme in the next series of meso/boxcosm experiments. The benthic metabolic response to increasing loads of organic matter in boxcosms for one particular regime still has to be done. Is the benthic response in proportion to the size of the organic matter load irrespective of the organic matter content of the sediment? For determination of the proportion of aerobic and anaerobic metabolism in benthic metabolism sulphate reduction in the sediment will be studied. The research will be conducted in cooperation with NIOO-CEMO in Yerseke.

In gastight boxcosms the development of anoxia will be investigated under different oxygen supply regimes by monitoring the O_2 consumption and TCO_2 production in a flow-through system. Dimethylsulphide and dimethylsulphonioacetate will be measured in the water column of these systems in the framework of a NOP project in cooperation with the State University of Groningen and TNO.

For all experiments the following conditions will be varied: 1. the amount and quality of the organic matter input, 2. the sediment type, and 3. bioturbation.

Results of meso/boxcosm experiments support the further development of the benthic module of the North Sea ecosystem model (ERSEM).

THE EFFECT OF DEPOSITION OF ORGANIC MATTER ON PHOSPHORUS DYNAMICS IN EXPERIMENTAL MARINE SEDIMENT SYSTEMS*

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ABSTRACT

The effect of deposition of organic matter on phosphorus dynamics in sandy marine sediments was evaluated using an experimental system (boxcosms) and three different strategies: (1) no supply (2) one single addition (3) weekly additions of a suspension of algal cells (*Phaeocystis spec.*). Macrofauna (3 species, 6 individuals of each) were added to half of the boxes. Both in the case of the single and weekly additions a clear effect of increased organic matter loading on phosphorus dynamics was found. Following the organic matter addition, porewater phosphate concentrations in the upper sediment layer increased, phosphate release rates from the sediment increased by a factor 3-5 and in the boxes to which a single addition was applied NaOH-extractable phosphorus increased substantially. The increase in phosphate release rates from the sediment was attributed to mineralization of the added material and to direct release from the algal cells. No clear effect of the presence of macrofauna on sediment-water exchange of phosphate could be discovered. The macrofauna were very effective at reworking the sediment, however, as illustrated by the organic carbon profiles. It is hypothesized that the sediment-water exchange rates of phosphate were regulated by the layer of algal material which was present on the sediment surface in the fed boxes. In the boxes to which the single addition was applied porewater phosphate concentrations were lower and NaOH-extractable phosphorus was higher in the presence of macrofauna, suggesting that macrofauna can stimulate phosphate binding in the sediment.

1. INTRODUCTION

Benthic phosphorus regeneration may strongly influence water column chemistry in shallow marine systems (e.g. BALZER, 1984; CALLENDER & HAMMOND, 1982; FISHER *et al.*, 1982; HOPKINSON, 1987; KLUMP & MARTENS, 1981, 1987; RUTGERS VAN DER LOEFF, 1980). Therefore, the role of sediments in phosphorus recycling and eutrophication of these systems (e.g. the North Sea, BROCKMAN *et al.*, 1988, 1990) is of major importance, even though phosphorus generally does not limit primary

production (PEETERS & PEPERZAK, 1990; RIEGMAN *et al.*, 1990).

Phosphorus cycling has mostly been studied in organic-rich, high porosity, fine-grained sediments (e.g. FROELICH *et al.*, 1988; KLUMP & MARTENS, 1981, 1987; KROM & BERNER, 1980, 1981; MARTENS *et al.*, 1978). Much less information is available on organic-poor, low porosity, sandy sediments (e.g. HOPKINSON, 1987; RUTGERS VAN DER LOEFF, 1980; VAN RAAPHORST *et al.*, 1990) which can be found in a major part of the North Sea (EISMA, 1990). In view of the general concern about increased eutrophication

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of the North Sea (POSTMA, 1985), presumably resulting in increased algal blooms (CADÉE, 1990) and oxygen deficiency in certain areas (WESTERNHAGEN *et al.*, 1986), it is important to obtain more quantitative information on the processes controlling phosphorus dynamics in sandy sediments.

Early diagenesis in marine sediments largely depends on the supply of organic carbon (BERNER, 1980; BILLEN *et al.*, 1990; KLUMP & MARTENS, 1987). Although a significant correlation between the amount of fine particles and of organic matter in sediments can often be found (CREUTZBERG *et al.*, 1984; BILLEN *et al.*, 1990) deposition of organic matter is not limited to fine-grained sediments. JENNESS & DUINEVELD (1985) have shown that considerable amounts of phytoplanktonic material can be - at least temporarily - buried in sandy sediments down to a depth of 5 cm following deposition in periods of slack tidal current.

Binding of phosphorus in the sediment may cause a time lag between organic matter mineralization in the sediment and actual regeneration of phosphorus to the water column. Sorption to iron and aluminum oxides and precipitation processes (LIJKLEMA, 1977; MARTENS *et al.*, 1978; FROELICH, 1988; FROELICH *et al.*, 1982) may substantially reduce regeneration to the overlying water. Furthermore, uptake of phosphorus by microorganisms, not only from the organic substrate but also from the porewater, may play an important role. This latter process obviously depends on the quality (*e.g.* C:P ratio) of the available organic matter (BILLEN *et al.*, 1990; GÄCHTER *et al.*, 1988, 1992). Under anoxic conditions chemically bound phosphorus may be released due to reduction of iron oxides (MORTIMER, 1941). According to GÄCHTER *et al.* (1988) polyphosphates which have accumulated in bacterial cells during oxic conditions may then be released as well.

The presence of macrofauna can stimulate mineralization of organic matter and uptake of phosphorus by microorganisms through reworking of the sediment. Furthermore, sediment-water exchange rates of phosphorus can be enhanced, mostly due to bioirrigation activity (*e.g.* ALLER, 1982; HÜTTEL, 1990; HYLLEBERG & HENDRIKSEN, 1982; YINGST & RHOADS, 1980).

In this study the effect of deposition of organic matter on phosphorus dynamics in a sandy marine sediment is evaluated. Furthermore, the role of macrofauna is discussed. The system was a modification of the boxcosms described by VAN RAAPHORST *et al.* (1992). This research was part of a

larger study on North Sea sediment eutrophication of which further results are presented in the other chapters of this report.

2. MATERIALS AND METHODS

2.1. BOXCOSMS

Sediment with a median grain size of 125-160 μm and a content of particles $<50\mu\text{m}$ of ca. 2-5% was obtained from a station in the southern North Sea (Zeegat van Texel: 52°53'N, 4°34'E; depth: 17m). The sediment was stored in large covered containers at outdoor (winter) temperatures for ca. 4 months. Before use, the sediment was sieved (<0.5 cm) and homogenized in a cement mill. The boxcosm experiments were performed in 26 cylindrical polypropylene boxes with an inner diameter and height of 30 and 35 cm, respectively. The boxes were filled with sediment up to 10 cm from the rim, resulting in a sediment depth of 25 cm in each box. Incorporation of air bubbles while filling the boxes was avoided as much as possible by adding seawater simultaneously. The thin layer (ca. 5 mm) of fine particles which subsequently developed on top of the sediment was carefully removed.

The boxes were distributed over 2 separate basins, in order to be able to maintain the "starved" and "fed" boxcosms spatially apart thus avoiding mutual contamination. No communication existed between the boxes. To each box ca. 10 cm of overlying water was added, which was continuously replaced by filtered (over sand beds, grain size 1-1.4 mm), aged (for several weeks in 2 large containers) North Sea water of constant salinity (29‰), an average dissolved organic carbon (DOC) content of 2.1 ± 0.4 mg.l^{-1} and the following average ($n = 16$) nutrient concentrations: $\text{PO}_4 = 2.7 \pm 0.9$ $\mu\text{mol.l}^{-1}$; $\text{NO}_3 + \text{NO}_2 = 54.3 \pm 7.9$ $\mu\text{mol.l}^{-1}$; $\text{Si} = 17.2 \pm 3.3$ $\mu\text{mol.l}^{-1}$. The NH_4 concentration in the inflow was ca. 1.0 $\mu\text{mol.l}^{-1}$ at the beginning, increased to ca. 7 $\mu\text{mol.l}^{-1}$ at day 14 and subsequently decreased to values < 0.8 $\mu\text{mol.l}^{-1}$ remaining at this level from day 24 onwards. The DOC present in the inflow water probably consisted of refractory components (LAANE, 1980). The inflow rate of 10.4 ml.min^{-1} resulted in a residence time of the overlying water of ca. 11 hours. Outflow took place by overflow over the rim of the boxes into the basin. Constant bubbling of air was performed to keep the water column in the boxes well-mixed and saturated with respect to

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oxygen. The boxcosms were kept in the dark at a temperature of 11.8 ± 0.5 °C.

One week after installation each box was supplied with micro-and meiofauna through a 250 ml sediment sample consisting of the 2.5 cm surface layer of freshly collected boxcores. Two weeks later three species of macrofauna (*Tellina fabula*, *Nephtys hombergii*, *Echinocardium cordatum*; 6 of each, resulting in a total density of 255 ind. per m²) were added to 13 of the boxes. Dead individuals visible at the sediment surface were replaced on a weekly basis.

Three different strategies were used to study organic matter deposition: (1) no supply ("starved"; 8 boxes), (2) one single addition ("fed"; 10 boxes), (3) weekly additions ("fed"; 8 boxes). The organic matter consisted of a suspension of *Phaeocystis spec.*, a common alga in coastal areas of the North Sea (e.g. LANCELOT *et al.*, 1987; CADÉE, 1990), which was collected in the Schulpengat south-west of Texel with 50 µm plankton nets during the 1990 spring bloom. The material was homogenized by stirring with a paddle in large containers, divided into equal portions and subsequently stored at -20°C until use (ca. 4 weeks for the first addition). 16 days after the introduction of the macrofauna and 37 days after the installation of the boxes the first portion of (thawed) organic matter was added (day 0). The organic matter supply to the boxcosms resulted in loadings of ca. 8 g C.m⁻² and 6.3 mmol P.m⁻² for the weekly additions (during 19 weeks, resulting in a total of 152 g C.m⁻² and 120 mmol P.m⁻²), and 24 g C.m⁻² and 19 mmol P.m⁻² for the single additions. The amount of carbon supplied with the single addition is approximately equivalent to the annual metabolic loss of sandy North Sea sediments as estimated by DE WILDE *et al.* (1984) and CRAMER (1991). Although the water circulation was stopped for 24 h following each addition not all of the algal material settled on the sediment surface within this period, resulting in a loss of organic matter due to outflow from the boxes. This especially was a problem in the boxcosms to which the single addition of organic matter was applied. Therefore, the actual carbon loading in these boxes was somewhat lower than 24 g C.m⁻². At each sampling event either intact boxes were used for the measurements (sediment-water exchange rates, oxygen respiration rates and penetration depth) or boxes were "sacrificed" (porewater, sediment

composition).

2.2. SEDIMENT-WATER EXCHANGE RATES

Sediment-water exchange rates of phosphate were measured in single boxes which were temporarily disconnected from the water supply. 500 ml of the overlying water was carefully removed and stored in a jar. At fixed time intervals 25 ml of sample was taken both from the overlying water and from the jar. The samples were filtered (0.45 µm cellulose acetate) and analyzed for phosphate. At the end of each experiment - which never took more than 8 h - the water supply was reconnected.

The fluxes were calculated from the concentration change in time in the overlying water of the boxcosm corrected for the consumption or production of phosphate in the jar and the decreasing depth of the overlying water due to sampling:

$$dC_o/dt = J \cdot h^{-1} - R \quad (1)$$

where:

C = concentration of the overlying water (mol.m⁻³)

t = time (s)

J = sediment-water exchange rate (mol.m⁻².s⁻¹)

h = the depth of the overlying water which decreases in time due to sampling (m)

R = change of the phosphate concentration in the overlying water (mol.m⁻³.s⁻¹) due to production/consumption in the water column (jars).

2.3. OXYGEN RESPIRATION AND PENETRATION

Benthic oxygen consumption was measured using the method described by CRAMER (1990). The boxcosms were covered with a plexiglass lid in which a stirring device, O₂ electrodes (YSI 5739) and a temperature electrode were fitted. Respiration was calculated from the change in the O₂ concentration in the chamber during incubation. Oxygen concentrations in the pore water were measured with an O₂ micro-electrode (HELDER & BAKKER, 1985) at 0.2 mm depth intervals using a micromanipulator. The oxygen penetration depth is defined as the depth at which zero oxygen concentrations or constant and low readings were obtained.

2.4. POREWATER

The boxcosms were sampled with acrylic liners (i.d. 5.2 cm, length 30 cm) which were sliced into 5, 10 and 20 mm segments (depending on sediment depth). Interstitial water was obtained by squeezing under N_2 pressure using Reeburgh-type squeezers (REEBURGH, 1967) fitted with 0.45 μ m cellulose-acetate filters. In all cases segments of three cores were pooled.

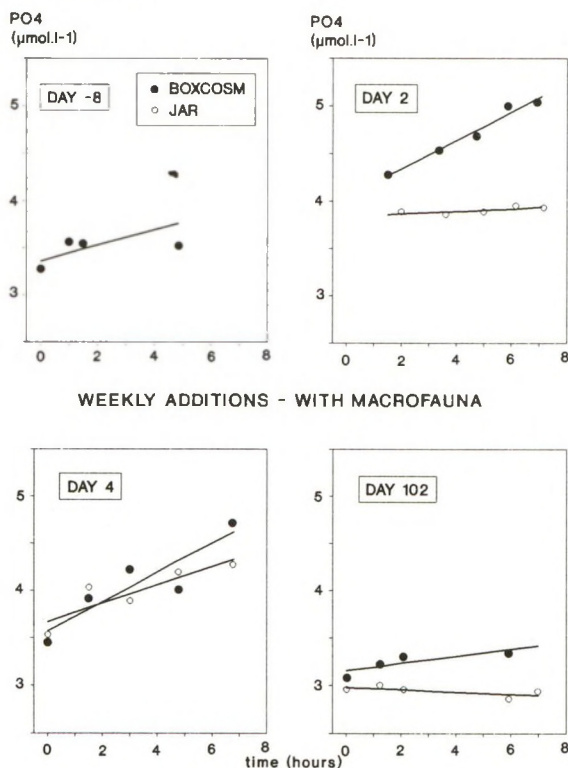


Fig. 1. Change in the phosphate concentration ($\mu\text{mol.l}^{-1}$) with time in the overlying water of weekly fed boxcosms with macrofauna and the matching jars during flux experiments on day -8, 2, 4 and 102. Solid lines were obtained through linear regression.

2.5. SEDIMENT COMPOSITION

The boxcosms were sampled with PVC tubes (i.d. 4.5 cm) and sliced into segments of 5, 10, 20 and 40 mm (depending on sediment depth). Three cores were pooled each time. Organic-C contents were measured on a Carlo Erba NA 1500-2 elemental analyzer following the procedure of VERARDO *et al.* (1990).

The phosphorus speciation was determined using the sequential extraction scheme described by HIJLTJES & LIJKLEMA (1980). 50 mg of wet sediment was extracted sequentially with 2 \times 50 ml of 1 M NH_4Cl , pH = 7 (2 \times 2 hours), 50 ml of 0.1 M NaOH (17 h) and 50 ml of 0.5 M HCl (24 h). These fractions presumably represent the loosely bound and exchangeable fraction, the fraction bound by iron and aluminum oxides and the calcium bound fraction, respectively. A shaking table was used for continuous agitation of the suspensions.

After each extraction step the suspensions were filtered (0.45 μ m cellulose-acetate), the filtrate was stored at -20°C until analysis and the filter with the sediment was added to the next extraction solution in the sequential procedure. The organic carbon content and phosphorus speciation were only determined for the sediments of the starved boxes and those that were fed once.

Easily exchangeable Fe and Mn was determined through an extraction with 0.1 M HCl (suprapur). It was assumed that most of the reactive iron and manganese oxides were released by this method. 0.1 gr of dried (60°C) and homogenized (through grinding in a agate mortar) sediment was leached with 50 ml of HCl for 18 h, followed by filtration over a pre-acid cleaned 0.45 μ m cellulose nitrate filter (Duinker *et al.*, 1974).

2.6. ANALYTICAL PROCEDURES

Phosphate concentrations (analytical precision $\pm 0.03 \mu\text{mol.l}^{-1}$ at a concentration of $1 \mu\text{mol.l}^{-1}$) were determined on a Technicon AA II autoanalyzer (fluxes, porewater) and on a Shimadzu Double beam Spectrophotometer UV-150-02 (sediment phosphorus) following the method of STRICKLAND & PARSONS (1972). The Fe and Mn content of the HCl-leachate was determined with a Perkin Elmer 5100 PC Atomic Absorption Spectrophotometer using the standard addition method for calibration (analytical precision for Fe and Mn: $\pm 1 \mu\text{mol.l}^{-1}$ and $\pm 0.5 \mu\text{mol.l}^{-1}$ at a concentration of $18 \mu\text{mol.l}^{-1}$).

3. RESULTS

3.1. SEDIMENT-WATER EXCHANGE RATES

Fig. 1 shows the concentration change with time in the overlying water of the weekly fed boxcosms with macrofauna and in the jars on day -8 (no jar measurement), 2, 4 and 102. Calculated phosphate release rates from such data (assuming a linear

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relationship between the phosphate concentration and time) are given in Fig. 2. Error bars indicate the standard error of the calculated flux. Deviations from a straight line, as found for example on day 4 (Fig. 1), resulted in large standard errors for the estimated fluxes due to the small number of samples ($n = 4-7$). Phosphate release rates were generally low in the starved boxcosms with the exception of the high initial release rates in the boxes with macrofauna. Following deposition of organic matter (day 0) an increase in phosphate release rates from the sediment was found within 4 days in the case of the single additions, followed by a period of very low phosphate release from day 10 (with macrofauna) or 15 (without macrofauna) onwards. The interpretation of the results for the weekly fed boxes is hampered by the limited amount of measurements and the large errors in the estimated fluxes. From Fig. 2 it can be observed, however, that phosphate release rates increased within 2 days after the first organic matter addition in the boxes with macrofauna and within 4 days after the second addition in the boxes without macrofauna. The maximum phosphate release rates were 2-3 times higher in the boxes fed only once compared to the weekly fed ones. Apart from a slightly higher maximum phosphate release rate in the presence of macrofauna in the boxes which were fed once, no clear effect of the presence of macrofauna on the phosphate fluxes was observed.

The phosphate concentration in the overlying water was generally higher than in the inflow water, particularly in the fed boxcosms. In the case of the single addition the phosphate concentration in the overlying water increased from ca. 3 to ca. 10 $\mu\text{mol.l}^{-1}$ immediately following the food supply. This was followed by a decrease to 2-3 $\mu\text{mol.l}^{-1}$ within 2 days. The same pattern was observed in the case of the weekly additions, corresponding values being ca. 3, 6 and 3-4 $\mu\text{mol.l}^{-1}$, respectively.

3.2. POREWATER PROFILES

In the starved boxcosms the porewater concentration of phosphate (Fig. 3) slowly decreased in time, to a concentration of less than 5 $\mu\text{mol.l}^{-1}$ in the upper sediment layer both with and without macrofauna. When the boxcosms were fed only once an immediate increase of the porewater phosphate concentration was found ($>20 \mu\text{mol.l}^{-1}$) in the upper 30-40 mm of the sediment, followed by a rapid decrease, especially in the presence of macrofauna (Fig. 3b). In the case of weekly

additions of organic matter only a minor increase (Fig. 3b, with macrofauna) or even a decrease (Fig. 3a, without macrofauna) of the phosphate concentration in the porewater of the upper sediment layer could be detected following the addition of organic matter. Both in the presence and absence of macrofauna the phosphate concentration subsequently decreased rapidly, even though organic matter additions continued. In all boxes the porewater phosphate concentrations measured in the upper 5 mm were higher than those of the overlying water.

The porewater phosphate concentration declined in all of the boxes during the course of the experiments, apart from the initial increase due to the organic matter additions found in the fed boxcosms. The phosphate concentrations found at the start of the measurements, however, were very high: 15-25 $\mu\text{mol.l}^{-1}$. Presumably phosphate was released from the sediment during the period prior to the first measurements either due to mineralisation of organic matter and/or due to desorption from binding sites.

3.3. OXYGEN RESPIRATION

Deposition of organic matter caused the benthic oxygen consumption to increase substantially (Fig. 4a). After a single organic matter addition oxygen respiration increased to ca. 20 and 30 $\text{mmol O}_2\text{.m}^{-2}\text{.d}^{-1}$ in the boxes with and without macrofauna, respectively. Oxygen respiration rates subsequently decreased to ca. 10 $\text{mmol O}_2\text{.m}^{-2}\text{.d}^{-1}$ within 30 days, slightly higher than the original rates (ca. 8 $\text{mmol O}_2\text{.m}^{-2}\text{.d}^{-1}$). In the case of weekly supply, oxygen respiration rates increased from approximately 10 to a maximum of 40 $\text{mmol O}_2\text{.m}^{-2}\text{.d}^{-1}$. After an initial almost linear increase the oxygen respiration rates seemed to stabilize around 30-35 $\text{mmol O}_2\text{.m}^{-2}\text{.d}^{-1}$. No clear effect of the presence of macrofauna on oxygen respiration could be discovered in the fed boxcosms.

3.4. OXYGEN PENETRATION

The addition of organic matter generally caused the oxygen penetration depth to decrease (Fig. 4b). Especially the single addition had a very clear effect on the oxygen penetration depth both in the boxes with and without macrofauna. In these boxes oxygen penetration gradually decreased to depths $< 1 \text{ mm}$ 10 days after the addition, and subsequently increased again to ca. 5-15 mm. In the weekly fed

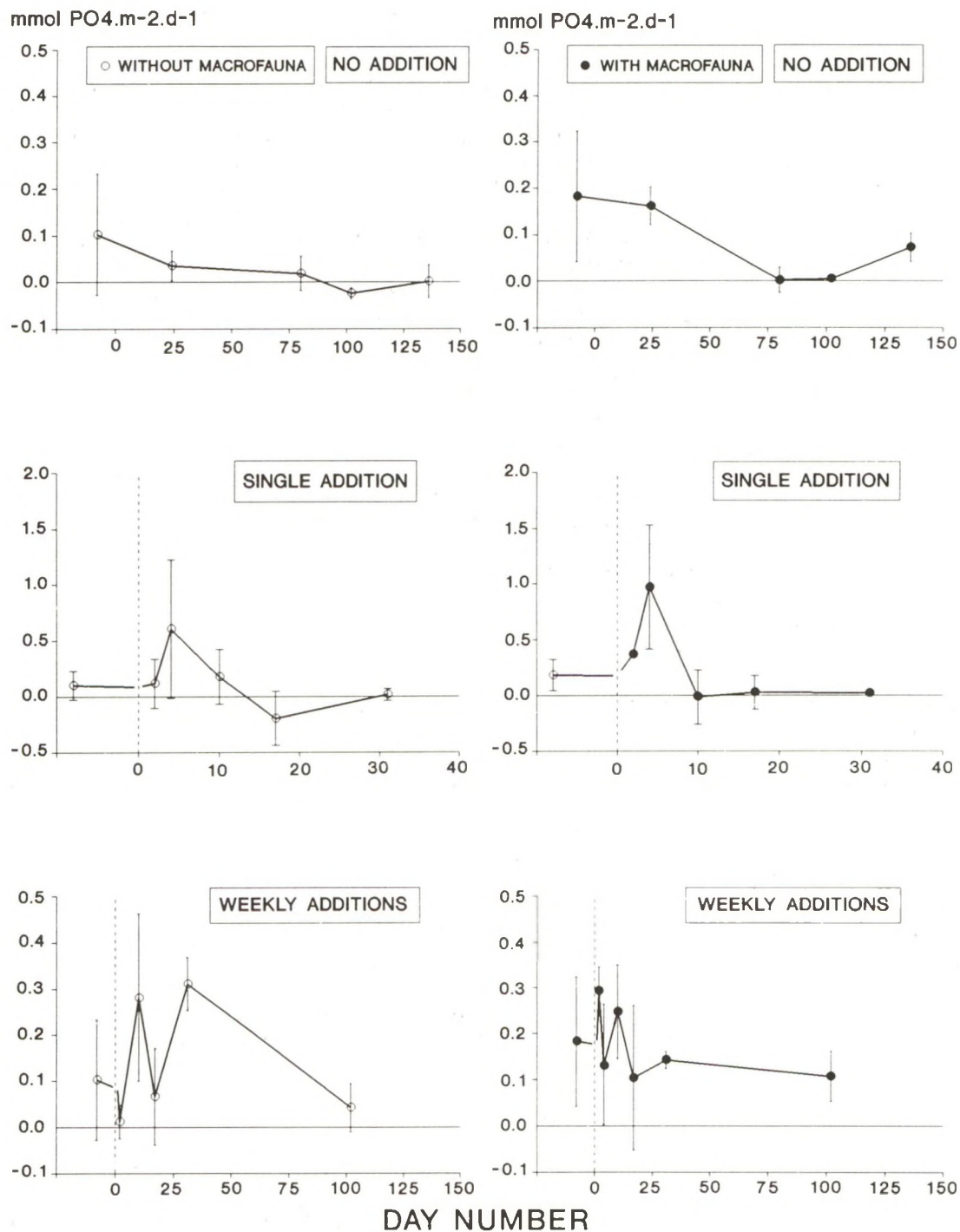


Fig. 2. Sediment-water exchange rates of PO_4 ($\text{mmol} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$) measured in the boxcosms. Error bars indicate the standard error of the estimated flux.

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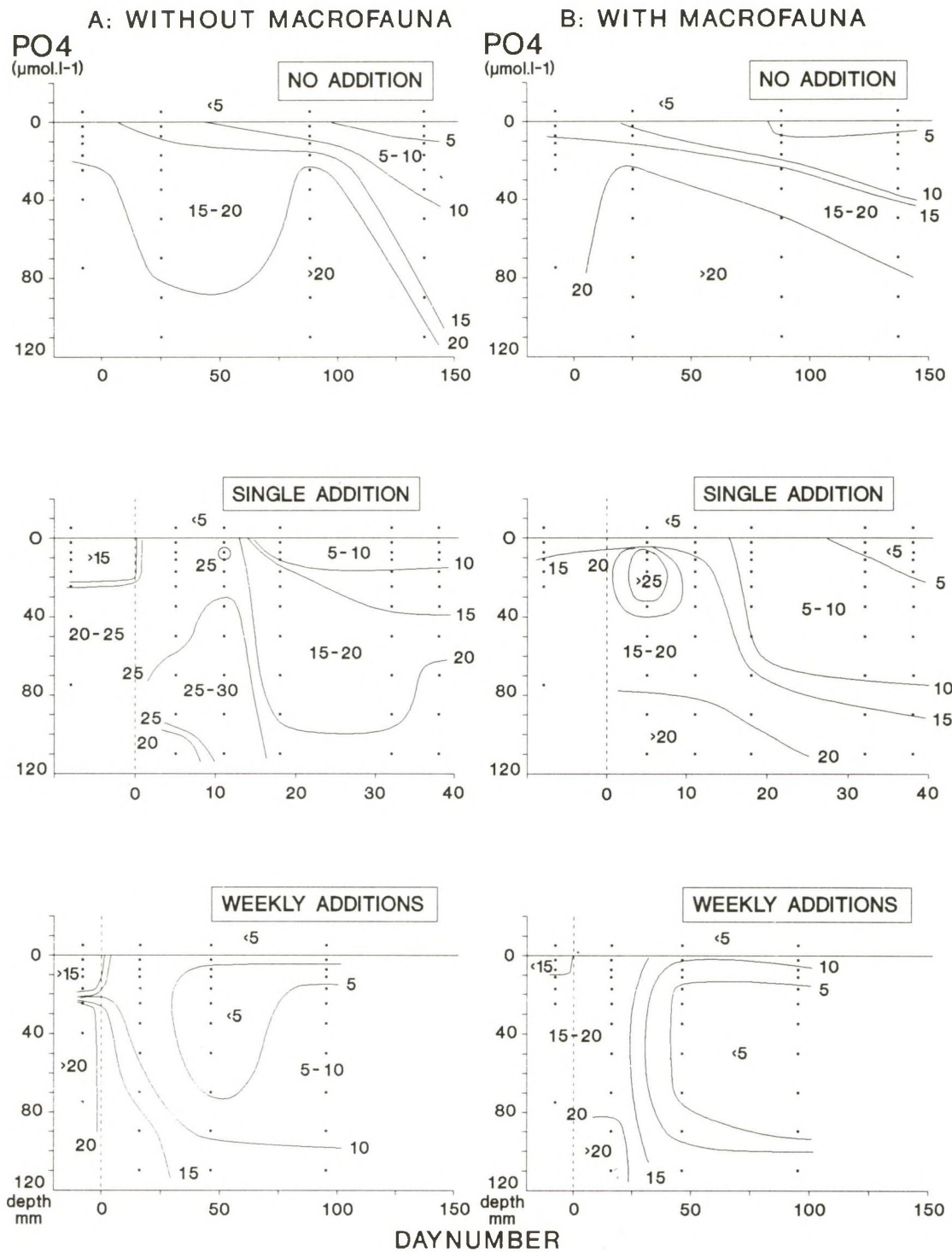
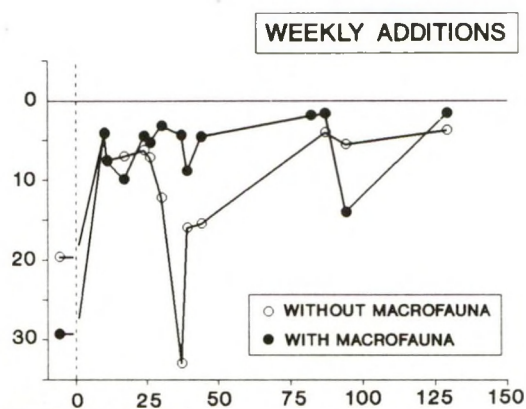
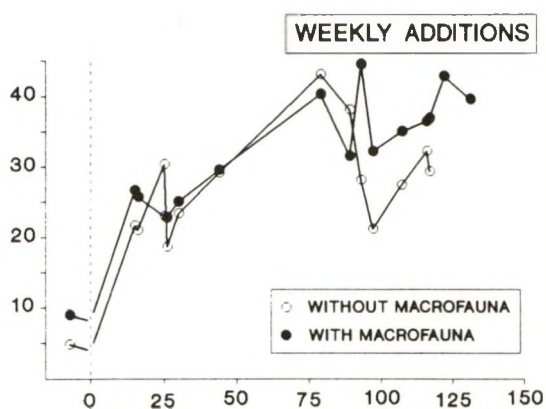
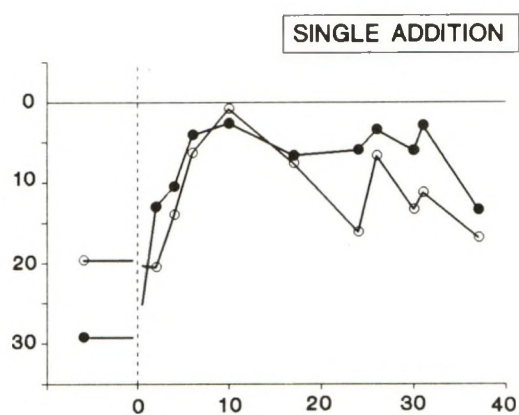
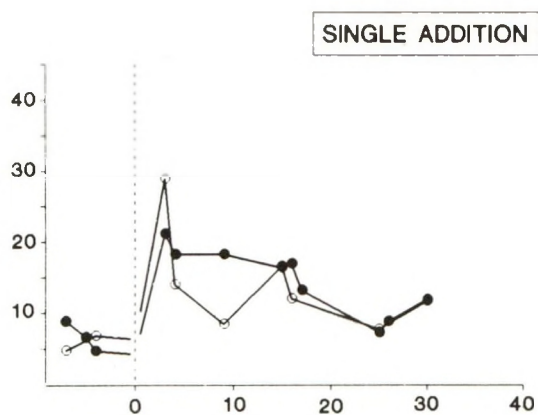
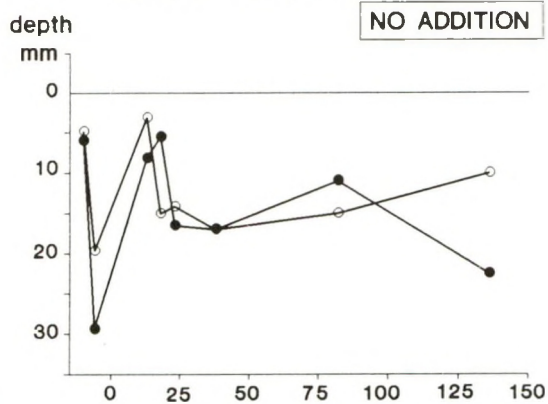
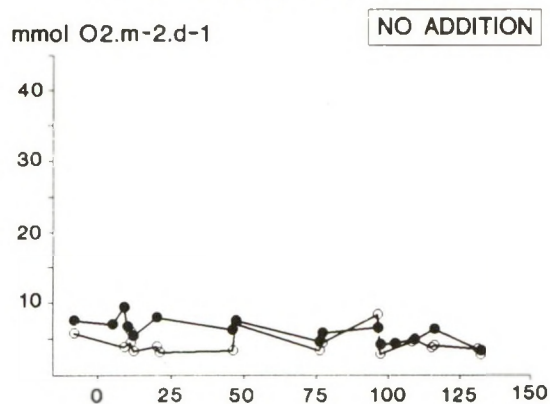


Fig. 3. PO_4 concentrations ($\mu\text{mol.l}^{-1}$) in the porewater of the boxcosm sediment. a. without macrofauna. b. with macrofauna.

A: O₂ RESPIRATION

B: O₂ PENETRATION



DAY NUMBER

Fig. 4a. Oxygen respiration rates (mmol O₂.m⁻².d⁻¹). b. Oxygen penetration depths (mm) in the boxcosms.

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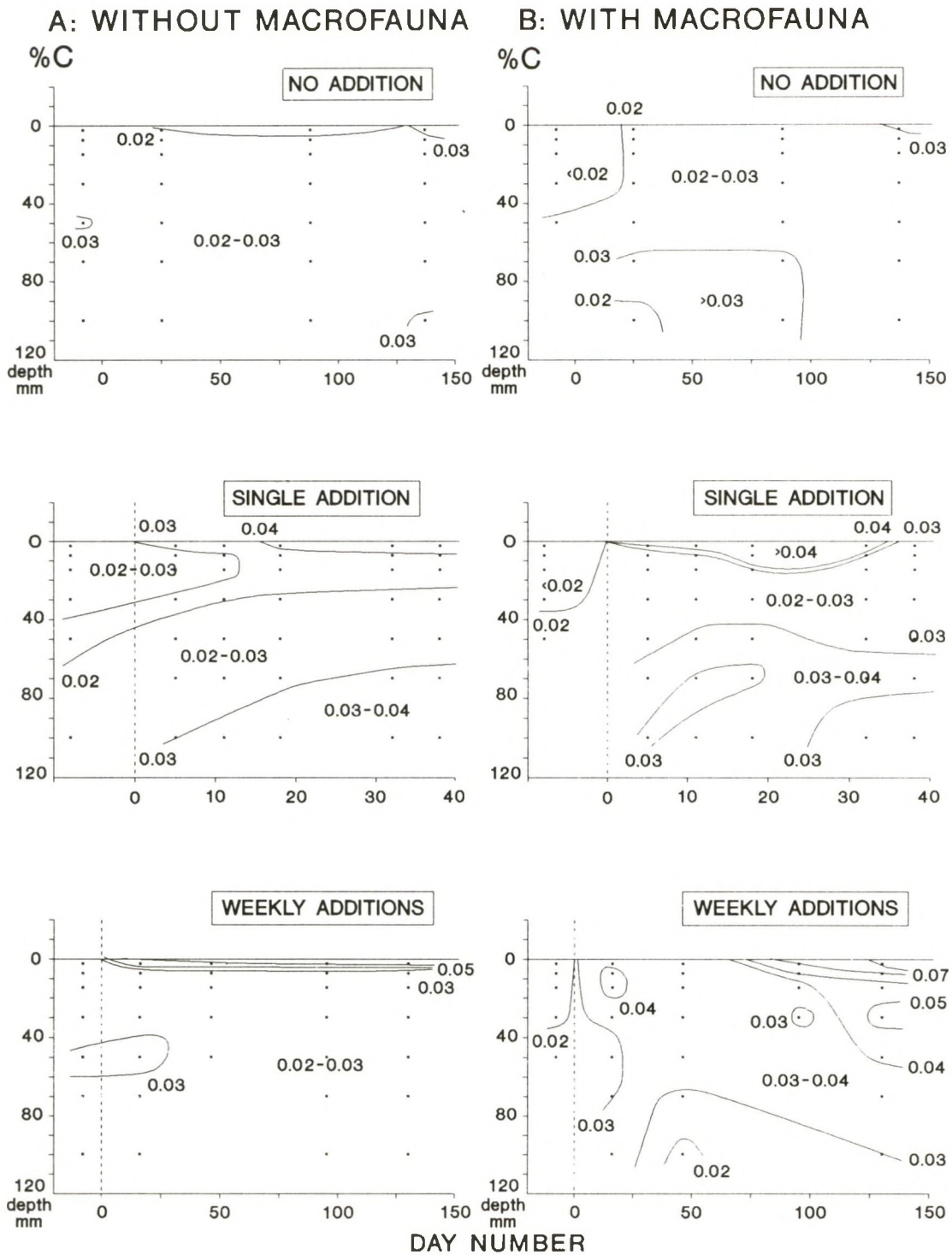


Fig. 5. Organic carbon content (%C) of the sediment in the boxcosms. a. without macrofauna b. with macrofauna.

boxcosms with macrofauna oxygen penetration depths decreased from ca. 30 to 2 mm during the experiment. In the weekly fed boxes without macrofauna large oscillations in the oxygen penetration depth were found, but overall, a decrease from 20 mm to 4 mm was observed. The oxygen penetration depth in the fed boxcosms with macrofauna was generally smaller than in the boxcosms without macrofauna (Wilcoxon's test; $n = 21$; $p < 0.05$).

3.5. SEDIMENT COMPOSITION

In the boxcosms to which no organic matter was added the carbon content remained very low, ranging from 0.01 to 0.03% (Fig. 5). After a single addition of organic matter the carbon content rapidly rose to 0.04-0.09% in the upper sediment layer, both with (Fig. 5b) and without macrofauna (Fig. 5a). When organic matter was added weekly and macrofauna were present, this increase of the carbon content was not limited to the upper sediment layer but extended down to 50 mm in the boxcosms, indicating substantial sediment mixing.

The leachable Fe- and Mn-contents of the sediment were very low: 0.03-0.04% ($5.4\text{--}7.2\ \mu\text{mol Fe.g}^{-1}$) and 0.002% ($0.4\ \mu\text{mol Mn.g}^{-1}$), respectively. NH_4Cl -, NaOH- and HCl-extractable phosphorus amounted to 0.01-0.03, 0.02-0.05 and 0.05-0.11 $\mu\text{mol P.g}^{-1}$ sediment, respectively. The phosphorus content determined with this extraction was lower than found at a comparable sandy station in the North Sea where values of 0.05, 0.25 and 2.2 $\mu\text{mol P.g}^{-1}$ were found for the NH_4Cl , NaOH and HCl fraction, respectively (unpublished results). The low values found here can probably be explained by the removal of a part of the fine sediment fraction after filling of the boxes. No clear reaction to the single addition of organic matter could be discovered in the NH_4Cl and HCl fractions. Only NaOH-extractable phosphorus showed a clear response (Fig. 6) with the largest increase occurring in the presence of macrofauna.

4. DISCUSSION

4.1. EFFECT OF ORGANIC MATTER ADDITIONS

Both in the case of the single and weekly additions a clear effect of increased organic matter loading on phosphorus dynamics was found. Following deposition of organic matter porewater phosphorus concentrations in the upper sediment layer

increased, phosphate release rates showed a 3-5 fold increase and NaOH-extractable phosphorus increased substantially in the boxes to which a single addition was applied. Furthermore, oxygen respiration rates showed an immediate response. In the case of the single additions of organic matter this was accompanied by a rapid initial decrease of the oxygen penetration depth.

Previous field research on organic matter deposition on sediments has shown a rapid response of benthic microbial activity (GRAF, 1982, 1989; MEYER-REIL, 1983; JENSEN *et al.*, 1990) following deposition of a phytoplankton spring bloom. During laboratory experiments similar to ours using intact cores from the field, GRAF (1987) found a maximum oxygen uptake 3 days after the addition of algal matter. In experimental microcosms KELLY & NIXON (1984)

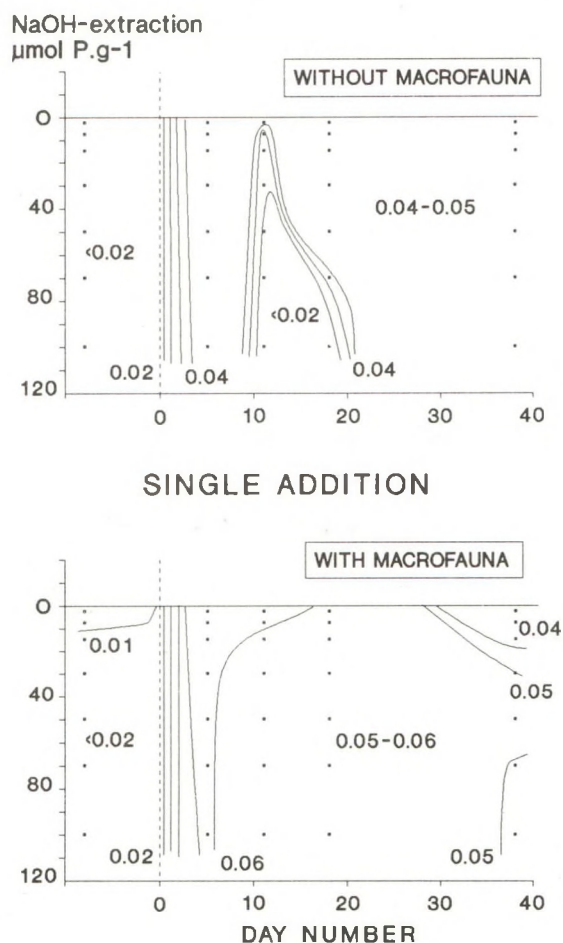


Fig. 6. NaOH-extractable phosphorus ($\mu\text{mol P.g}^{-1}$ sediment) in the sediment from the boxcosms which were fed once.

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observed a time lag of 2-20 hours between an organic matter addition and maximum ammonium release rates. ENOKSSON (1987) found maximum oxygen consumption and phosphate release rates ca. 6 days following an organic matter addition in the form of algal material. The results of our experiments are in accordance with these observations: following the single addition of organic material a large increase in oxygen respiration and phosphate release rates occurred within 2 and 4 days, respectively. Due to the relatively large intervals between sampling events it is impossible to say when the maxima occurred exactly.

When studying phosphorus dynamics it is of major importance to know what mechanism is controlling whether phosphorus is being released or bound in the sediment. The mechanisms involved can be of a chemical (adsorption/desorption and precipitation/dissolution) or biological nature (uptake or release by bacteria, excretion by macrofauna) or a combination of both (e.g. anoxic conditions mediated by bacteria resulting in release of sorbed phosphorus from iron oxides).

In the starved boxcosms a relatively large release of phosphorus, especially in the presence of macrofauna, was observed compared to the corresponding oxygen uptake. The average O_2 -uptake/P-release atomic ratio in the starved boxes was low, ca. 55, indicating that oxic mineralization was not the dominating process. Excretion by macrofauna (Nixon *et al.*, 1980) may explain part of these results. Initial porewater concentrations in the sediment were high and only gradually decreased, supporting the observed phosphorus release during almost the entire length of the experiment.

Following the addition of organic matter increased phosphate release occurred in the fed boxcosms. This can be attributed to (1) mineralization of the added organic matter, (2) release from iron oxides due to reduction and (3) direct release from algal cells due to cell lysis.

In the same set-up, VAN DUYL *et al.* (1992) measured increased bacterial numbers (from ca. 0.5 to $1.5 \cdot 10^9$ bacteria.cm⁻³) and bacterial production rates (from ca. 7 to 140 mg C.m⁻².d⁻¹; methyl-³H-thymidine incorporation method) on day 5 compared to day -4 in the 0-3 mm sediment layer of the boxes which were fed once. In combination with the higher oxygen respiration rates from day 2 onwards, this indicates the potential importance of oxic mineralization for the phosphate fluxes.

If reduction of iron oxides controls the phosphate release, a decrease in the oxygen penetration depth

would be expected during this period. Figure 4b shows that oxygen penetration decreased to depths < 1 mm on day 10 in the case of the single additions. Although sulfate reduction may have taken place in locally reduced spots, diagenesis probably did not proceed beyond nitrate reduction as nitrate was generally still present in the porewater (not shown). Furthermore, only a relatively small amount of phosphorus was present in the NaOH-extractable phosphorus fraction at the start of the experiment and this fraction was found to increase from ca. 0.02 to 0.05 $\mu\text{mol P.g}^{-1}$ during the period of maximum phosphate release. Apparently, the adsorption capacity of the sediment was not eliminated, making a chemical control of the increased phosphate release improbable.

Preferential P-release due to cell lysis is known to occur rapidly on death of algal cells (BALZER, 1984; GARBER, 1984; KROM & BERNER, 1981) and certainly may have occurred in the *Phaeocystis* suspensions.

In the boxes to which a single addition was applied the sediment-water exchange rates of phosphate were very low from day 10-15 onwards, coinciding with a gradual decrease in the oxygen consumption rate. When the organic matter was applied weekly, however, substantial phosphate release continued to occur. Furthermore, the oxygen respiration rates roughly stabilized and further depletion of the porewater did not occur. It is unlikely that a steady state situation was reached, however, as the sediment organic carbon content still continued to increase.

A tentative budget for the fate of the phosphorus added to the boxcosms through the organic matter additions is presented in Table 1. As the various input and output terms could not be quantified accurately, a great deal of assumptions were necessary. Each output/storage term in Table 1 is the highest value of the estimates with and without macrofauna. A loss of 20% of the added organic matter due to outflow over the rim was assumed for both feeding regimes. The release of phosphate from the sediment was estimated from the area under the curves in Fig. 2. The amount of added phosphorus which was bound in sediment organic matter and in the NaOH-extractable fraction of the sediment was calculated from the Figs 5 and 6 (assuming a sediment density of 2.65 g.cm⁻³, an average porosity of 0.40 , a sediment depth of 10 cm and a C:P ratio for the organic matter of 106 ; REDFIELD *et al.*, 1963). An estimate of the amount of phosphorus bound in bacteria was obtained from the increase in bacterial biomass in the weekly fed

TABLE 1

A tentative budget for the fate of the phosphorus added to the fed boxcosms (in mmol P.m⁻²), n.d. = not determined

	SINGLE ADDITION (in mmol P.m ⁻²)	WEEKLY ADDITION
Input	+ 15	+ 95
Output/storage		
- P flux	- 4	- 29
- Organic P	- 0.2	- 3
- NaOH-extr. P	- 5	n.d.
- Bacterial P	n.d.	- 4
Unaccounted for	+ 5.8 (39%)	+ 59 (62%)

boxes with macrofauna integrated over 63 mm depth during 130 days (2.4 g C.m⁻²; VAN DUYL, 1992) assuming a C:P weight ratio of 20 for the bacteria (GÄCHTER *et al.*, 1992). Ca. 40-60% of the phosphorus added through the organic matter is not accounted for in Table 1. Neither the phosphorus bound in bacteria in the case of the single additions nor the NaOH-extractable phosphorus in the sediment of the weekly fed boxes can account for this difference.

During the experiment total amounts of ca. 0.26 and 3.3 mol O₂.m⁻² (calculated from the area under the curve in Fig. 4 and corrected for the respiration in the starved boxes) were consumed in the boxes with single and weekly additions, respectively. This corresponds to a carbon respiration of ca. 18-36% of the added material for both treatments when assuming a respiration quotient of 0.85 (HARGRAVE, 1973) and a loss due to outflow of 20%. These results suggest that for both feeding regimes a major part of the added organic matter was not mineralized during the experiment. This is in accordance with the fact that a layer of algal material was present on the sediment surface in most of the fed boxes during the entire experiment.

4.2. EFFECT OF MACROFAUNA

The macrofauna added to the boxcosms consisted of sub-surface and surface deposit feeders generally found in sandy sediments in the North Sea (CREUTZBERG *et al.*, 1984). Apart from the sessile bivalve *Tellina*, of which up to 5 individuals per boxcosm had to be replaced during the experiment, the macrofauna generally had a low mortality and

were very effective at reworking the sediment, as illustrated by the organic carbon profiles. Only *Echinocardium* reached high growth rates (DUINEVELD *et al.*, 1992). In some of the sampled boxes, however, the macrofauna (*Echinocardium* in particular) were completely inactive. Further details on the macrofauna in this study will be published elsewhere.

Previous research has shown that macrofauna can directly increase sediment-water exchange rates of oxygen and nutrients due to bioirrigation activity (ALLER & YINGST, 1985; HYLLEBERG & HENDRIKSEN, 1980; KRISTENSEN & BLACKBURN, 1987) and indirectly due to the fact that macrofaunal feeding and burrowing can stimulate microbial activity in the sediment (ALLER, 1982; ALLER & YINGST, 1985; KRISTENSEN & BLACKBURN, 1987; YINGST & RHOADS, 1980).

Both in the case of the weekly and single additions of organic matter no clear effect of the presence of macrofauna on sediment-water exchange of phosphate and oxygen respiration rates could be discovered. The role of the burrowers *Echinocardium* and *Nephtys* was most likely limited to reworking of the sediment. Therefore, only indirect effects of macrofauna on solute transport would be expected.

The increase in substrate availability (higher organic carbon contents) at greater depths probably resulted in increased mineralization (VAN DUYL *et al.*, 1992) below the uppermost sediment layer explaining the smaller oxygen penetration depth in the presence of macrofauna. The increase of oxic mineralization below the upper sediment layer was apparently too small to have a substantial effect on

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the total oxygen respiration rates. The organic carbon profiles show that mixing of the food into the sediment took several weeks, thus causing a major portion to remain at the sediment surface. In any case, this holds for the most labile, freshly deposited material in the weekly fed boxcosms. Consequently, most of the organic matter mineralization probably took place in the algal layer on the sediment surface and the processes in this layer most likely determined the phosphate and oxygen fluxes. Apparently, the processes in this layer were not substantially affected by the presence of macrofauna.

In the weekly fed boxes with macrofauna higher phosphate concentrations were observed in the upper cm's of the sediment in the second half of the experiment, corresponding to the higher organic carbon contents in these boxes. In the case of the single additions this was not observed, probably because time was too short to mix a substantial amount of carbon deeper into the sediment. In these boxes porewater phosphate concentrations were lower and NaOH-extractable phosphorus was higher in the presence of macrofauna. This suggests that macrofauna can stimulate phosphate binding in the sediment.

Measurements in experimental systems of this type are generally associated with a great number of problems. Although the influence of sediment heterogeneity is limited when using boxes filled with homogenized sediment instead of intact sediment cores, large differences between similarly treated boxes can still occur. Due to the manipulation of the sediment, processes may take place in the boxes which do not occur in natural sediments. Furthermore, the number of sampling events, different treatments and variables to be measured are limited by the maximum number of boxes that can be handled. Despite the drawbacks, this study illustrates that interesting results on the effect of organic matter deposition on phosphorus dynamics in sediments can be obtained in this type of system.

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THE IMPACT OF ORGANIC MATTER AND MACROZOOBENTHOS ON BACTERIAL AND OXYGEN VARIABLES IN MARINE SEDIMENT BOXCOSMS*

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ABSTRACT

In boxcosm experiments the impact was studied of organic-matter input and the presence of macrozoobenthos on benthic bacterial abundance and production, oxygen consumption and penetration depth in manipulated marine sediments. Different regimes of organic-matter supply (a single pulse or weekly supplies of *Phaeocystis*) were applied to boxcosms with and without macrozoobenthos. Both regimes of organic-matter supply affected the top layer of the sediment: an increase in numbers and production of bacteria and consumption of oxygen and a decrease in the oxygen penetration depth (oxic zone). The impact of a single pulse appears to be restricted to a period of about 1 mo. The presence of macrozoobenthos had no clear impact on the bacterial variables in the top sediment layer in either regime of organic-matter supply. In the deeper (anoxic) layers it resulted in an enhancement of the bacterial production in both regimes. Bacterial abundance in these deeper layers also increased in the boxcosms weekly supplied with *Phaeocystis*. The enhanced bacterial production at deeper layers was not reflected by the benthic oxygen consumption. The presence of macrozoobenthos resulted in a further decrease in the oxygen penetration depth. In the presence of macrozoobenthos a net transport of organic matter from the sediment surface to deeper sediment layers (bioturbation) was assessed, resulting in an increase in total organic-matter content in the sediment and an increase in benthic metabolism.

1. INTRODUCTION

The fate of organic matter after deposition on marine sediments has been the subject of several studies. The rapid response of the benthic metabolism to organic input has received considerable attention (e.g.

GRAF *et al.*, 1982, 1983; MEYER-REIL, 1983; KELLY & NIXON, 1984; VAN DUYL *et al.*, 1992). Macrozoobenthic activities have been shown profoundly to affect the physical and chemical nature of the sediments (e.g. MAHAUT & GRAF, 1987; GERINO, 1990) and are generally perceived as stimulating decomposition (e.g. YINGST & RHOADS, 1980; BLACKBURN, 1987; KRISTENSEN & BLACKBURN, 1987). The effects of burrowing organisms on decomposition were reviewed by ANDERSEN & KRISTENSEN (1991). In the present study the decomposition of algal deposition events in the presence and absence of burrowing organisms in sandy North Sea sediments was studied.

In the Dutch and Belgian coastal waters, each year dense blooms of the alga *Phaeocystis* spec. develop. Due to eutrophication over the last 30 years (VAN DER VEER *et al.*, 1989), the intensity of blooms as well as their length has increased (CADÉE & HEGEMAN, 1986; CADÉE, 1990). WASSMANN *et al.* (1990) observed mass sedimentation of *Phaeocystis pouchetii* in the upper 100 m of Atlantic water in the central Barents Sea. Even in turbulent shallow and coastal North Sea waters this algal material sinks out during periods of slack tidal current and settles on the sediment surface (JENNESS & DUINEVELD, 1985). Part of it is subsequently worked into the sediment either by physical processes (JENNESS & DUINEVELD, 1985) or by biological processes (e.g. YINGST & RHOADS, 1980). The *Phaeocystis* spring bloom provides the benthos with the first substantial organic matter input of the season and also instantaneously enhances the activity of the microbial benthic community in sandy North Sea coastal sediments (BAK *et al.*, 1991; VAN DUYL *et al.*, 1992).

Field studies on the role of organic matter supply and of organic matter processing and burial by macrozoobenthos in the microbial distributions and activities are difficult to carry out. Meso- and boxcosm studies are tools which allow such experiments to be performed under controlled semi-natural conditions. The present boxcosm study was initiated to quantify

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the effect of organic-matter supply and of macrozoobenthos on aspects of benthic metabolism. Bacterial and oxygen variables were studied in *Phaeocystis*-supplied boxcosms to compare their development in the presence and absence of macrozoobenthos and assess to what extent the macrozoobenthos enhances the microbial processes in the benthic system and thus influences the degrading capacity of the bottom.

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2. METHODS

2.1. EXPERIMENTAL SET-UP

Sandy sediment (median grain size 125 to 160 μm) was collected in the Zeegat van Texel in the southern North Sea (52°53'N; 4°34'E) at 17 m depth and transported to the laboratory. The wet sediment was kept outdoors in large covered containers during the winter. In the spring it was sieved (mesh size 0.5 cm) to remove large particles such as shells and gravel. After homogenization in a cement mill, the sediment was placed at a depth of 25 cm in 24 round water-filled polypropylene boxes (35 cm high and 30 cm diameter) equipped with watertight removable bottom plates. The overlying water (~ 10 cm) was aerated just above the sediment surface. The air tube was fixed in the centre of the boxes and the airflow provided both water agitation and circulation. The boxes were supplied with aged filtered seawater ($S=29$) at a rate of $10.4 \pm 6.1 \text{ cm}^3 \text{ min}^{-1}$ (retention time ~ 11 h) and incubated in the dark at 11.8 ± 0.5 °C.

2.2. FAUNA INTRODUCTION

One week after installation, the boxes were inoculated with micro- and meiofauna. The inoculum consisted of the 2.5 cm surface layer of freshly collected intact sediment cores from the Zeegat van Texel. Sediment material of several separate cores was combined, mixed and divided into 250- cm^3 portions which were subsequently supplied to each box (boxcosm) in the set-up. The fresh sediment layer was approximately 3 mm thick. Compaction of the sediment in the boxcosms was allowed to proceed for 3 weeks before macrofauna was introduced. Half of the boxcosms were populated with macrozoobenthos consisting of a combination of common species occurring in sandy North Sea sediments: the spat-

goid *Echinocardium cordatum* (size class 25 to 35 mm), the bivalve *Tellina fabula* (size class 11 to 14 mm) and the predatory polychaetous worm *Nephtys hombergii* (size class 4 to 6 cm). Six individuals of each species were introduced per boxcosm. Dead specimens visible on the sediment surface were replaced on a weekly basis. The remaining half of the boxcosms provided a control where only micro- and meiofauna were present.

2.3. ALGAL DETRITUS INTRODUCTION

Two weeks after macrofauna introduction, organic matter was supplied. It was collected with 50- μm -mesh plankton nets during the April phytoplankton bloom of *Phaeocystis* on the North Sea, in the Schulpengat, SW of Texel. After each tow, the nets were vacuum cleaned, producing a suspension of algal material in the receptacle of the vacuum cleaner. This material, consisting predominantly of *Phaeocystis* colonies, was divided into equal portions of 161 g containing 0.6 g C. These portions were double wrapped in plastic bags and deep-frozen until used.

Besides the controls (no algal addition) two treatments were applied: a single pulse of 24 g C m^{-2} and a weekly supply of 8 g C m^{-2} . Before the organic matter was added, the water supply was turned off and approximately 500 cm^3 water was taken out of each boxcosm to prevent loss of material over the edge. All algal material was rinsed out of the plastic bag, using the water within each boxcosm. The water supply remained off for 24 h to allow the algal material to settle on to the sediment surface. Twice a week the algal mat was resuspended by softly stirring with a spoon to reduce risks of anoxic conditions in the mat (checked with O_2 profiles). To assess the loss of organic carbon over the edge, water samples were taken at periodic intervals. Total organic matter content (TOC) in the water samples was determined by the wet-oxidation method using $\text{K}_2\text{S}_2\text{O}_8$ on a carbon analyser (0524B Total Carbon System of Oceanography International Corp., Texas USA) according to CADDÉE (1982).

2.4. SEDIMENT SAMPLING

The boxcosms were successively sacrificed to obtain sediment samples. Intervals between sediment sampling were 30 to 60 d for starved boxcosms, 5 to 14 d for pulsed boxcosms and 8 to 50 d for boxcosms weekly supplied with organic matter. Corers of 2.5 cm diameter were inserted into the sediment and then closed on the top with rubber stoppers. If algal mats were present, they were removed by siphoning the day before sampling. The overlying water was siphoned off, after which the bottom plate was loosened and the outer wall of the boxcosm was

removed. Cores were sectioned and the 0 to 3 mm, 30 to 33 mm and 60 to 63 mm layers were used. Per depth, slices from 15 cores were combined. These depth layers have been selected in earlier studies and appeared suitable to determine the gradients of microbial variables with depth (e.g. BAK & NIEUWLAND, 1989). The sediment of different depth layers was analysed for total numbers of bacteria and average cell biovolume (biomass estimation) with epifluorescence microscopy. Bacterial production was measured by (methyl- ^3H) thymidine incorporation. Methods are described in detail by van DUYL & KOP (1990). Isotope dilution of the thymidine was assessed in the surface layer and extrapolated to the deeper layers. Meiofauna samples were taken from the 2-cm surface layer of the sediment. Samples were fixed with a heated (70°C) 4% solution of formaldehyde with rose bengal to stain the meiobenthos. Incubation times ran for 39 days in the pulse boxcosms and for 140 days in the weekly supplied and starved boxcosms.

2.5. OXYGEN CONSUMPTION AND PENETRATION DEPTH

Benthic oxygen consumption was measured in PVC bell jars with oxygen electrodes, according to CRAMER (1989). The rate of oxygen uptake was calculated over the periods with a linear rate of reduction in oxygen concentration. Measurements in boxcosms were conducted daily. At the sediment water interface the thickness of the oxic zone in the sediment was measured using oxygen microelectrodes with tip-diameters of 3 to 10 μm and an external reference electrode. The oxygen electrode was inserted into the sediment by a micromanipulator attached to a tripod placed over the boxcosm. The signals were sent to a picoammeter via a coaxial cable and recorded on paper; from this the oxygen penetration depth in the sediment was determined. In total, approximately 160 profiles were measured.

3. RESULTS

3.1. SURVIVAL OF MEIO- AND MACROZOOBENTHOS

Initial densities of nematodes in boxcosms, as estimated from the densities in the inoculum from the field, were approximately 8 to $10 \cdot 10^3 \text{ ind} \cdot \text{m}^{-2}$. During the experiment, the numbers of nematodes declined in most boxcosms. In a few of the weekly supplied boxcosms, however, meiofauna densities rose to more than $10^6 \text{ ind} \cdot \text{m}^{-2}$ after 80 to 100 d. The development of meiofauna was highly variable both in boxcosms with similar and different treatments (organic matter supply, macrozoobenthos). *Tellina fabula* demonstrated a high mortality irrespective of

organic matter supply. Up to 5 *Tellina* per boxcosm died and were replaced during the experiment. *Nephtys hombergii* and *Echinocardium cordatum* experienced little mortality. Only 3 of the 78 individuals of *Echinocardium* had to be replaced. All *Nephtys* specimens were recovered during sediment sampling.

3.2. THE EFFECT OF ORGANIC MATTER SUPPLY

In both regimes of organic matter supply (pulse and weekly supply), the algal material settled on the sediment surface within a few hours. It formed a mat of algal detritus (2 to 3 mm thick) completely covering the sediment surface. The responses to organic-matter load in the absence of macrozoobenthos will be treated first (open symbols in Figs 2 to 5).

3.2.1. BACTERIAL ABUNDANCE

In the starved boxcosms, bacterial abundance remained constant at all depths throughout the experiment (Fig. 1a). In response to organic matter supply, the bacterial abundance in the sediment surface layer (0 to 3 mm) became higher than in the starved boxcosms. In the pulsed boxcosm (Fig. 1b), bacterial numbers more than doubled in the sediment surface 5 days after the *Phaeocystis* supply of $24 \text{ g C} \cdot \text{m}^{-2}$. Bacterial numbers returned to initial values after 2 weeks. Bacterial abundance did not increase in deeper layers. In the weekly supplied boxcosms, a steady increase in bacterial abundance in the sediment surface was recorded (Fig. 1c). After 90 to 95 days, densities in weekly supplied boxcosms equalled the densities in boxcosms which had received a single pulse of organic matter. Bacterial abundance in deeper layers did not respond to the *Phaeocystis* input and gradually dropped during the experiment.

3.2.2. BACTERIAL PRODUCTION

Bacterial production remained below $12 \text{ mg C} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ ($4 \mu \text{g C} \cdot \text{cm}^{-3} \text{ wet sediment} \cdot \text{d}^{-1}$) in the sampled sediment layers of the starved boxcosms and experienced limited variations over the time of the experiment (Fig. 2a). In the *Phaeocystis*-supplied boxcosms the sediment surface layer showed enhanced bacterial production. In the boxcosms which received a single pulse of *Phaeocystis* of $24 \text{ g C} \cdot \text{m}^{-2}$ (Fig. 2b), the response of the bacterial production in the sediment surface was recorded within 5 days, demonstrating an 18 to 19-fold increase in bacterial production. For this first measurement after the *Phaeocystis* supply the algal mat was not removed as was done for all the subsequent measurements. In the deeper layers no response was noted. In the box-

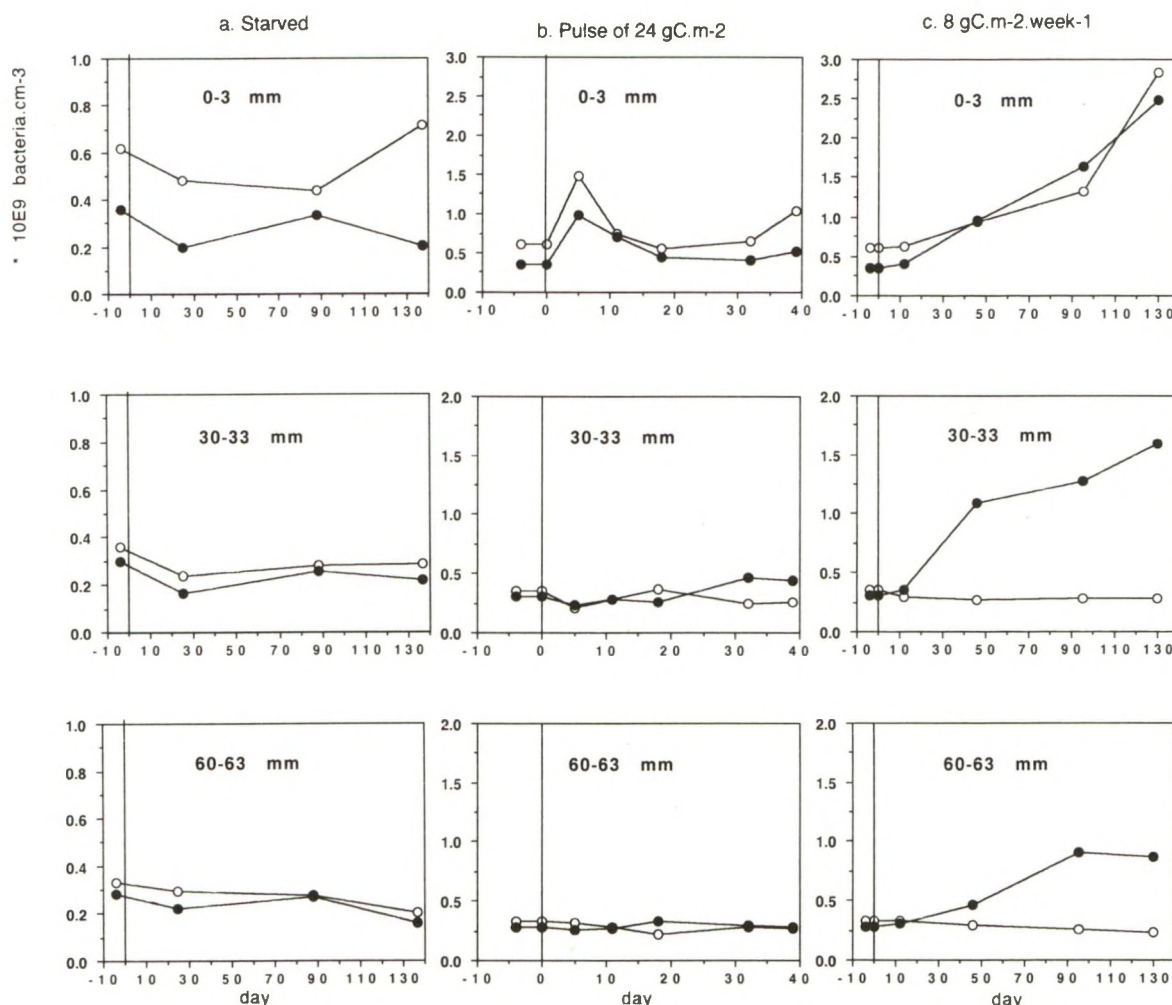


Fig. 1. Bacterial abundance in 3 depth layers of the sediment in a. starved, b. single-pulse and c. weekly *Phaeocystis*-supplied boxcosms with (●) and without (○) macrozoobenthos.

cosms weekly supplied with *Phaeocystis* (Fig. 2c), an overall increase in bacterial production was observed, particularly in the sediment surface. The *Phaeocystis* supply had no effect on the bacterial production in deeper sediment layers (30 to 33 mm, 60 to 63 mm).

3.2.3. OXYGEN CONSUMPTION

In starved boxcosms, oxygen consumption slowly declined during the experiment (Fig. 3a). The pulse of $24 \text{ g C}\cdot\text{m}^{-2}$ of *Phaeocystis* material enticed a strong reaction of the benthic oxygen consumption, increasing the consumption 4-fold within 5 days (Fig. 3b). The increase was followed by a steep drop to half the peak value with a subsequent return to initial values which were reached approximately 25 days after the introduction of the *Phaeocystis* supply. A clear

increase in oxygen consumption was observed in the weekly supplied boxcosms until day-25. By then they had received $32 \text{ gC}\cdot\text{m}^{-2}$ and the oxygen consumption equalled values reached in the pulse after 3 days. From day-25 onwards, oxygen consumption roughly stabilized around $30 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ with enhanced values on day-79 and -89 (43 and $38 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$) and reduced values on day-97 ($21 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$) (Fig. 3c). The oxygen concentration in the water above the sediment (3 to 5 cm) ranged from 230 to $370 \mu\text{mol}\cdot\text{dm}^{-3}$.

3.2.4. OXYGEN-PENETRATION DEPTH

Oxygen-penetration depths in the sediment ranged from 0 to 35 mm. Most measurements fell in the 0 to 20 mm penetration-depth range (>90% of 163 measurements, of which 60 were in boxcosms without

MARINE SEDIMENT BOXCOSMS

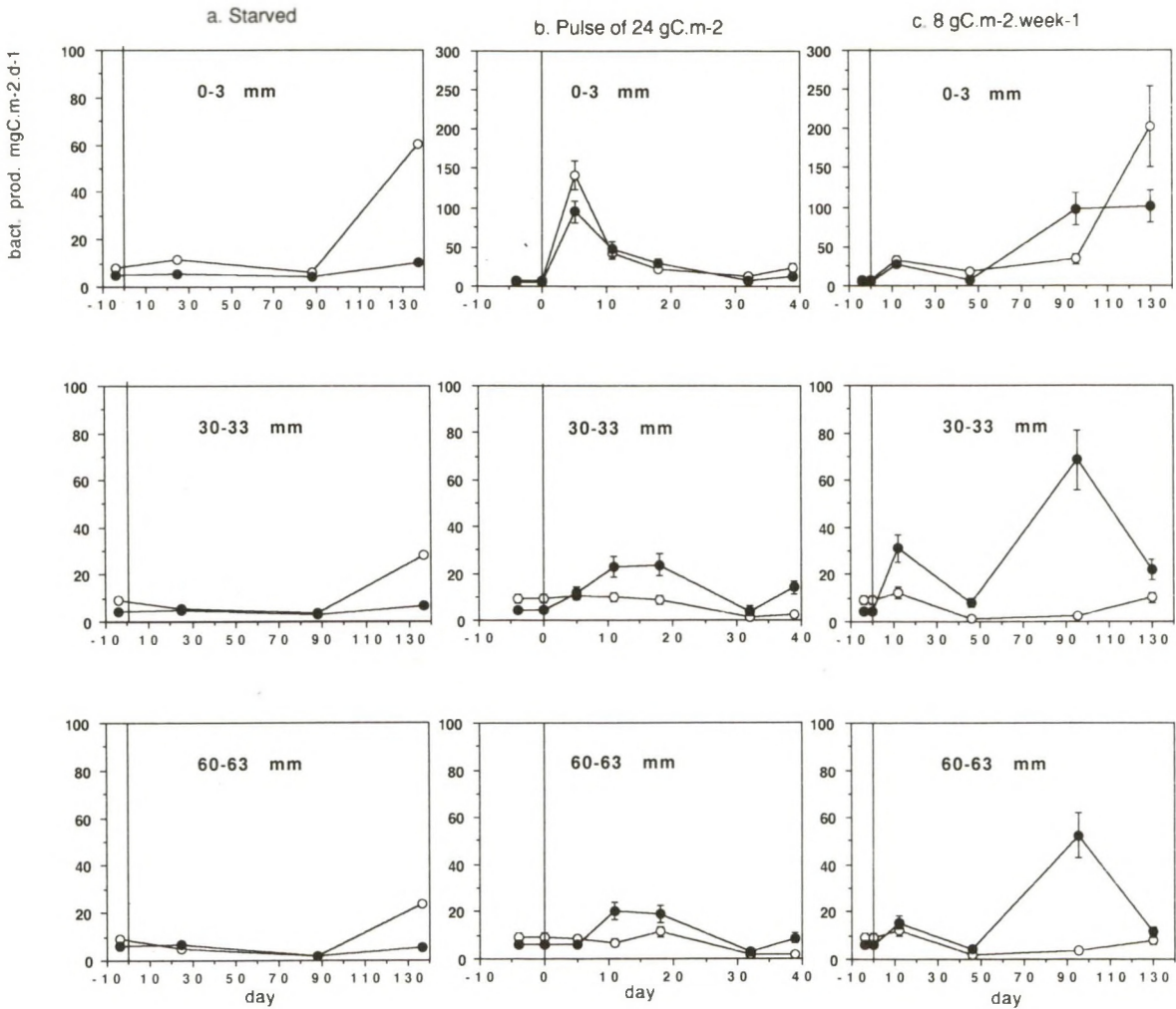


Fig. 2. Bacterial production in 3 depth layers of the sediment in a. starved, b. single-pulse and c. weekly *Phaeocystis*-supplied boxcosms with (●) and without (○) macrozoobenthos.

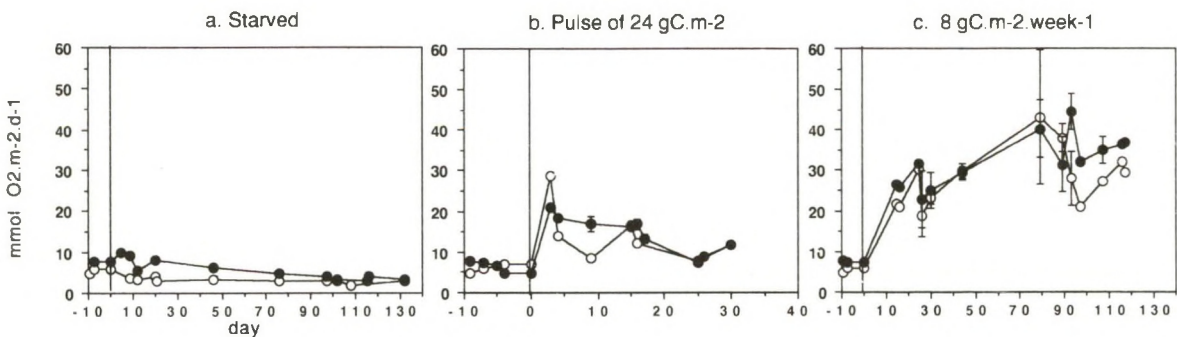


Fig. 3. Benthic oxygen consumption in a. starved, b. pulse and c. weekly *Phaeocystis*-supplied boxcosms with (●) and without (○) macrozoobenthos.

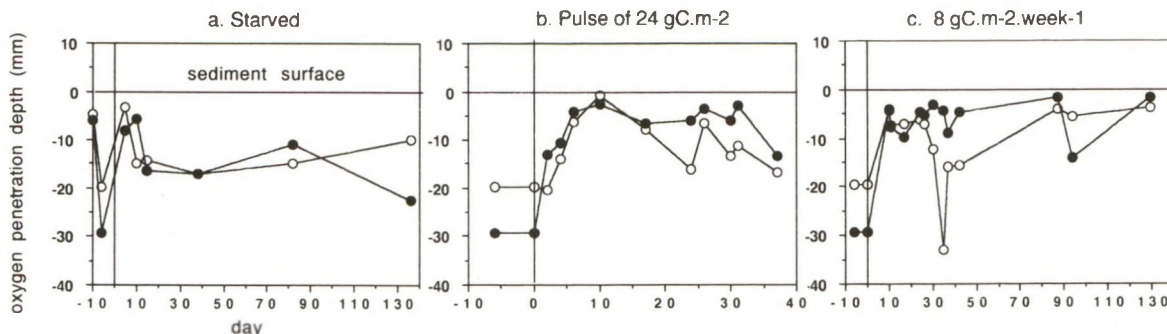


Fig. 4. Thickness of the oxic layer in a. starved, b. single-pulse and c. weekly *Phaeocystis*-supplied boxcosms with (●-) and without (○-) macrozoobenthos.

macrozoobenthos). Variations in the thickness of the oxic layer were considerable within one boxcosm and in boxcosms with similar treatment and incubation time. Despite the variation between measurements (standard deviations between 0 and 11 mm) the patterns of the average values showed differences between treatments (Fig. 4). In the starved boxcosms (Fig. 4a), the thickness of the oxic zone stabilized (after initial oscillations) at an average thickness of 14 mm around day-18. The average changes in oxygen-penetration depth after introduction of a *Phaeocystis* supply were best demonstrated in the boxcosms which received a single input (pulse) (Fig. 4b). Two days after the introduction of *Phaeocystis* a steep decrease in the oxic zone was observed, dropping from 20 mm to <1 mm in 10 days. Subsequently, a recovery occurred resulting in an average oxic layer thickness of 11 mm for the remaining period (30 d) of the incubation (Fig. 4b). In the weekly supplied boxcosms a slightly different pattern was found (Fig. 4c). An initial decrease in the oxic layer was followed by oscillations. The expected consistent decline of the thickness of the oxic zone occurred from day-40 onwards. Oscillations were related to the variable time span between the most recent *Phaeocystis* supply and the oxygen-penetration depth measurement.

3.3. THE EFFECT OF MACROZOOBENTHOS

In boxcosms with macrozoobenthos the algal-mat cover declined within days. Five days after the supply about 65% of the sediment surface was covered with an algal mat in the boxes without macrofauna. In the boxes with macrofauna this cover was reduced to about 17%. *Echinocardium cordatum* was observed to clear organic debris from the sediment by surface feeding, funnel feeding and burial.

3.3.1. BACTERIAL ABUNDANCE

On the sediment surface lower bacterial numbers were found in boxcosms with macrofauna than in boxcosms without macrofauna (Fig. 1a). Bacterial numbers in the deeper sediment layers of the starved boxcosms were not affected by the macrozoobenthos. In the pulsed boxcosm (Fig. 1b) the doubling of bacterial numbers in the sediment surface on day-5 occurred irrespective of the presence or absence of macrozoobenthos. The differences in bacterial abundance in the sediment surface in boxcosms with and without macrozoobenthos was initially maintained but disappeared during the experiment. In the 30 to 33 mm sediment layer, macrozoobenthos was found

TABLE 1

Bacterial biomass change in presence and absence of macrozoobenthos in the weekly *Phaeocystis*-supply treatment over the duration of the experiment in mg C·m⁻² (3-mm depth layers).

	without macrozoobenthos		with macrozoobenthos	
	initial	after 130-d incub.	initial	after 130-d incub.
0- 3 mm	91	143-424	42	157-242
30-33 mm	41	33	39	127
60-63 mm	38	21	35	123
0-63 mm (integrated)	1120	2778	814	3278

to enhance the bacterial abundance 20 to 30 days after the introduction of *Phaeocystis*. No such enhancement of numbers was found in the deepest sampled sediment layer (60 to 63 mm). In boxcosms supplied with $8 \text{ g C} \cdot \text{m}^{-2} \cdot \text{wk}^{-1}$ (Fig. 1c), bacterial numbers in the deeper layers only increased in the boxcosms with macrofauna. The increase in bacterial abundance was evident for all depths after about 20 days.

Average biovolume of bacteria ranged from 0.120 to $0.311 \mu\text{m}^3 \cdot \text{cell}^{-1}$ with the largest variations in the pulsed boxcosms. Five days after the carbon supply the average biovolume had increased from 0.179 to $0.311 \mu\text{m}^3 \cdot \text{cell}^{-1}$ in the boxcosms with macrofauna. Variations in biovolume were usually smaller, implying that the patterns found for bacterial abundance were comparable to the patterns of bacterial biomass (not shown). In Table 1 an overview is given of the changes in bacterial biomass during the 130-day incubation in both the presence and absence of macrozoobenthos.

3.3.2. BACTERIAL PRODUCTION

Bacterial production in the sediment surface of starved boxcosms was reduced in the presence of macrozoobenthos compared to bacterial production in the absence of macrozoobenthos (Fig. 2a). In the deeper sediment layers, the initial differences in bacterial production between boxcosms with and without macrozoobenthos disappeared. In the *Phaeocystis*-supplied boxcosms the presence of macrofauna did stimulate bacterial production in deeper layers (Fig. 2b and c). In the boxcosms which received a single pulse (Fig. 2b) the response of the bacterial production in the deeper layers was noted after 5 to 11 days with a 5-fold increase at 30 to 33 mm depth and a 3-fold increase at 60 to 63 mm depth. In the weekly supplied boxcosms (Fig. 2c) bacterial production in the sediment surface was not markedly influenced by macrozoobenthos. The production pattern appeared independent of the presence of macrozoobenthos. At the deeper sediment layers (30 to 33 mm, 60 to 63 mm), bacterial production was enhanced in the presence of macrofauna, but appeared to be subject

to variations related to variations in activity of the macrofauna. After 46 days the macrozoobenthos, in particular *Echinocardium cordatum*, appeared completely inactive and evidently had been so for several days prior to this time. In Table 2 the average bacterial production during the experiment for the 3 depth layers sampled is given for the different treatments. The effect of the macrozoobenthos on the average bacterial production was most pronounced in the deeper sediment layers of the weekly supplied boxcosms.

3.3.3. OXYGEN CONSUMPTION

The development in oxygen consumption in the presence of macrofauna under different *Phaeocystis*-supply regimes is shown in Fig. 3 (closed symbols). In starved boxcosms oxygen consumption declined during the experiment. Oxygen consumption in boxcosms with macrofauna exceeded consumption in boxcosms without macrofauna (Fig. 3a) and was on average 1.8 to $4 \text{ mmol O}_2 \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ higher than in boxcosms without macrozoobenthos. The pulse of *Phaeocystis* induced an instantaneous response in the benthic oxygen consumption, increasing the consumption 4-fold in less than 5 days (Fig. 3b). The increase passed into a 15-d period of stabilization at $\sim 18 \text{ mmol O}_2 \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ followed by a return to initial values, which were reached approximately 25 days after the introduction of the pulse. No clear differences in overall oxygen consumption between boxcosms with and without macrofauna were found at the pulse regime (Fig. 3b) or in the weekly supplied boxcosms (Fig. 3c). Only after ~ 90 days did weekly supplied boxcosms with macrozoobenthos tend to have higher oxygen consumption rates than boxcosms without macrofauna. This difference exceeded the oxygen demand of the macrozoobenthos.

3.3.4. OXYGEN-PENETRATION DEPTH

In the starved boxcosms the thickness of the oxic zone was independent of the presence of macrofauna (Fig. 4a). Only after ~ 100 days did the oxic layer become thicker in the presence of macrozoobenthos

TABLE 2

Average bacterial production ($\text{mg C} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$, 0 to 3 mm sediment layer) per depth layer in starved single-pulse and weekly *Phaeocystis*-supplied boxes with and without macrozoobenthos over the respective durations of the experiments.

	starved controls (over a 130-d period)		pulse of <i>Phaeocystis</i> (over a 39-d period)		weekly <i>Phaeocystis</i> -supplies over a 130-d period)	
	without macro	with macro	without macro	with macro	without macro	with macro
0- 3 mm	16	5	39	33	50	53
30-33 mm	8	4	39	14	5	33
60-63 mm	7	4	7	11	5	23

TABLE 3

Overview of the overall influence of macrozoobenthos on bacterial and oxygen variables. Bacterial variables were integrated over the 0 to 63 mm sediment layer.

	starved (over a 130-d period)		pulse (over a 39-d period)		weekly supplied (over a 130-d period)	
	without macro	with macro	without macro	with macro	without macro	with macro
bacterial biomass (mg C·m ⁻²)	816	703	1036	1032	1374	1948
bacterial production (mg C·m ⁻² ·d ⁻¹)	205	90	320	388	358	747
oxygen consumption (mmol O ₂ ·m ⁻² ·d ⁻¹)	3.66	7.63	11.8	13.2	29	31.6
oxygen penetration depth (mm)	13.4	14.5	11.4	6.87	10.2	5.5

than in its absence. After the introduction of *Phaeocystis* the oxygen-penetration depth decreased (Fig. 4b and c). The pattern of oxygen-penetration depth was comparable in pulsed boxcosms with and without macrozoobenthos. The same accounted roughly for the weekly supplied boxcosms. Oxygen-penetration depth in *Phaeocystis*-supplied boxcosms with macrofauna was significantly smaller than in the boxcosms without macrofauna for both *Phaeocystis* supply regimes (Student t-test; $p < 0.05$). In pulsed and weekly supplied boxcosms with macrozoobenthos, the oxic layer was on average 4.5 and 4.7 mm, respectively, thinner than in the absence of macrozoobenthos.

In Table 3 an overview is given of the average values of the different variables measured for the treatments to present a quantification of the effect of *Phaeocystis* supplies and the presence of macrozoobenthos on the total benthic metabolism.

3.4. CARBON BUDGET

In Table 4 the carbon budget is given for the different experimental treatments. The *Phaeocystis* input was corrected for 6% loss in both *Phaeocystis*-supply treatments (single and weekly supply). TOC analyses

in the waterphase of carbon-supplied boxcosms showed that organic material in the waterphase was lost over the edge of the boxcosm for 2 days after the water flow was resumed. From the boxcosms which received 8 gC·m⁻²·wk⁻¹, a loss of 6% was determined. For the boxcosms receiving a single pulse of 24 gC·m⁻² a similar loss was assumed. The benthic oxygen consumption was expressed in C-equivalents according to HARGRAVE (1973). The bacterial respiration was derived from the production values assuming a growth efficiency of 50% (e.g. MORIARTY *et al.*, 1985). Budgets are relative to the starved boxcosms. The benthic respiration accounted for about 30 to 60% of the C-input. Macrozoobenthos stimulated the C-respiration by 18% in the pulse and by 46% in the weekly supplied boxes. This resulted in an increase in the benthic metabolism by a factor of 4.

4. DISCUSSION

4.1. EXPERIMENTAL CONDITIONS

In boxcosms field conditions are best simulated with intact cores. However, this introduces variability between the boxcores and the composition and quantity of macrobenthos is not known at the start of the

TABLE 4

Total benthic oxygen consumption and bacterial production expressed in g C·m⁻² respired in the respective *Phaeocystis*-supply regimes and the impact of macrozoobenthos on the benthic metabolism. The bacterial C-respiration refers to the respiration by bacteria in the sediment from 0 to 63 mm depth comprising aerobic and anaerobic respiration. The C-respiration derived from oxygen consumption measurements mainly refers to respiratory processes in the oxic layer.

	input	oxygen consumption (expressed in C-equivalents)		bact. C-respiration (0-63 mm)		increased respiration due to macrozoobenthos
		without	with	without	with	
pulse (24 g C·m ⁻²) (duration 39 d)	22.5	3.2	2.2	7 (31%)	11 (49%)	18%
weekly supplies (154 g C·m ⁻²) (duration 130 d)	145	33.7	33.9	20 (13%)	85 (59%)	46%

experiment. Therefore, all cores were filled with sieved sediment and stocked with known types and numbers of benthos. Such a sediment manipulation results in an equal starting situation for all boxcosms with respect to structure, grain-size composition, degree of compaction and organic matter content. This is crucial when interpretation of different effects is based on the comparison of individual boxcosms that were sacrificed at different time intervals.

A disadvantage of sediment manipulation is a deviation from the natural situation by distorting or inhibiting expected developments or processes and by disturbing the geochemistry of the sediment. During instalment of the sediment in the water-filled boxcosm, gas bubbles were locked into the sediment, which might have obstructed the living space of benthic organisms. Their numbers decreased during the experiment but could still be traced at the end. The artificial habitat, the pre-incubation period or the size of the inoculum of fresh sediment slurry was inadequate or insufficient to initiate a consistent nematode development in boxcosms. To what extent this may have influenced the results is unclear.

Echinocardium cordatum and *Nephtys hombergii* survived and appeared to behave naturally under the experimental conditions. *Echinocardium* actively took up the *Phaeocystis* detritus, showed a high activity level (bioturbation) and a low mortality. It fed both at the surface and in funnels. Comparable feeding habits were observed by CRAMER *et al.* (1991). It moved about at the surface of the sediment and burrowed into it. Given the high mortality of the bivalve *Tellina fabula* in the boxcosms, the effects of macrozoobenthos on the benthic metabolism should be ascribed to the activities of *Echinocardium* and *Nephtys*.

4.2. IMPACT OF ORGANIC MATTER INPUT

The metabolic response to the single *Phaeocystis* supply ($24 \text{ gC} \cdot \text{m}^{-2}$) was instantaneous and short. In approximately 30 days the effect disappeared. Comparable results for benthic metabolism have been reported earlier (e.g. GRAF *et al.*, 1982, 1983; MEYER-REIL, 1983; KELLY & NIXON, 1984; VAN DUYL *et al.*, 1992; VAN RAAPHORST *et al.*, 1992). The patterns of bacterial variables and oxygen consumption after the *Phaeocystis* pulse were roughly comparable and characterized by an increase just after the introduction of the organic matter supply. With a growth yield of 50%, the carbon demand of bacteria roughly equalled the C-respiration (O_2 consumption recalculated to C-equivalents based on HARGRAVE (1973)) during the bacterial production maximum. This implies that the bacterial oxygen demand in the surface layer was responsible for the benthic oxygen consumption directly after the *Phaeocystis* input. In the

boxcosms weekly supplied with *Phaeocystis*, the relationship between bacterial abundance and production and oxygen consumption did not align as well as for the pulse. The bacterial abundance showed a consistent increase not reflected by the other variables such as bacterial production and oxygen consumption. The bacterial production rates in the sediment surface layer in the weekly supplied boxcosms were considerably lower than in the pulse-supplied boxcosms 15 to 25 days after a comparable amount of *Phaeocystis* had been given (24 to $32 \text{ g C} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ accumulated). The explanation may be that during the first measurement after the pulse supply the algal mat was not removed as was done in subsequent measurements, and therefore the bacterial production may have comprised the activity in the mat as well as in the upper regions of the sediment. If only the sediment had been sampled, much lower values for bacterial variables are likely to have been found, comparable to the values in the weekly supplied boxcosms after 10 to 25 days. Apparently most of the bacterial activity took place in the *Phaeocystis* detritus on top of the sediment. This may also have influenced the oxygen consumption pattern at the beginning of both *Phaeocystis* supply regimes. After day-25 the increase in the oxygen consumption in the boxcosms weekly supplied with *Phaeocystis* dropped and stabilized roughly. This suggests that throughout the experiment, the share of anaerobic metabolism (nitrate reduction, sulphate reduction) versus aerobic metabolism increased. Simultaneously the average thickness of the oxic layer decreased. After a steep initial decline it remained on average thicker than 3 mm. This would mean that during the experiment the oxygen consumption of the sediment under the algal mat gradually increased and that the consumption by the algal mat on top of the sediment surface declined.

The *Phaeocystis* input did not generate a response of bacterial variables in the deeper sediment layers. Either the rapid uptake of solubilized organic matter and/or the mere absence of meiofauna slowed down the distribution of organic solutes deeper into the sediment. From these experiments, it is clear that sedimentation events in the absence of macrozoobenthos only affect the surface and a thin sediment layer.

4.3. IMPACT OF MACROZOOBENTHOS BEFORE THE ORGANIC MATTER SUPPLY

In the 12 days preceding the introduction of organic matter, macrozoobenthos might have caused the reduction of both abundance and production of bacteria, particularly in the sediment surface layer. Deposit feeding of macrozoobenthos consuming sediment bacteria at a rate not exceeded by bacterial

production might have been partly responsible for this. This implies that the bacterial values of boxes with and without macrozoobenthos were different at the start of the *Phaeocystis*-supply regimes, with generally lower values in the boxes with macrozoobenthos. This difference was maintained for bacterial abundance in starved boxes with macrozoobenthos throughout the experiment. The benthic oxygen consumption was enhanced after macrozoobenthos introduction in starved boxcosms. The enhancement agreed with the estimated oxygen demand of the macrozoobenthos (Duineveld, pers. comm.).

4.4. IMPACT OF MACROZOOBENTHOS AFTER THE ORGANIC MATTER SUPPLY

4.4.1. BACTERIAL ABUNDANCE AND PRODUCTION

Bacterial variables were not significantly affected in the sediment surface by the presence of macrozoobenthos in boxcosms supplied with *Phaeocystis*. The decline in the cover of the algal mat caused by the macrozoobenthos did not result in any change of bacterial variables in the sediment surface layer (0 to 3 mm). The material transported by the macrozoobenthos was probably not the most labile part. In deeper sediment layers, where the fresh detritus was transferred to, a stimulation of bacterial production was observed in both *Phaeocystis* regimes within five days at 30 to 33 mm depth and within 5 to 10 days at 60 to 63 mm depth. The enhancement decreased with depth. Bacteria in the sediment were obviously limited in their growth by carbon, as bacterial production in starved boxcosms did not respond to the presence of macrozoobenthos. Thus, the stimulation was either triggered by the organic matter *per se* transferred to deeper layers by macrozoobenthos or by the excretion products (faeces) of macrozoobenthos which were assumed to increase after introduction of *Phaeocystis* supplies. In the same experimental set-up, Van Noort (pers. comm.) ob-

served by means of luminophores that bioturbation affected the sediment to a depth of 5 to 6.5 cm. Therefore stimulation of bacterial production is not expected to have occurred much deeper in the sediment. Despite enhanced bacterial production, bacterial abundance patterns in deeper layers were not influenced by the single pulse of *Phaeocystis* supply. Grazing by macrozoobenthos probably prevented the extension of the bacterial standing stock into the deeper sediment layers. Abundance patterns in weekly supplied boxcosms increased significantly at all depth layers and were apparently not controlled by deposit-feeding macrozoobenthos. In the same experimental set-up, SLOMP *et al.* (1992) determined the carbon content of the sediment in the presence and absence of macrozoobenthos after *Phaeocystis* supplies. In Fig. 5, the data of SLOMP *et al.* (1992) are reproduced as average carbon content in the 0 to 6 cm sediment layer during the experiment. It shows the immediate influence of macrozoobenthos on the carbon distribution and the increase in net accumulation of carbon in the sediment. In the presence of macrozoobenthos more organic matter was worked into the sediment than in its absence.

4.4.2. BENTHIC METABOLISM

The enhancement of bacterial production in deeper sediment layers and thus the enhancement of the total benthic metabolism in the presence of macrofauna was not reflected by the benthic oxygen consumption. This implies that the biological oxygen uptake in the presence of macrofauna explained only part of the estimated total C-demand of the benthic bacteria. Electron acceptors other than O_2 were presumably utilized by bacteria. Relatively high concentrations of nitrate (SLOMP *et al.*, 1992) suggest that denitrifying bacteria contributed to the enhanced bacterial production in deeper sediment layers in the presence of macrozoobenthos. In accordance KRISTENSEN (1985) found stimulation of NO_3^-

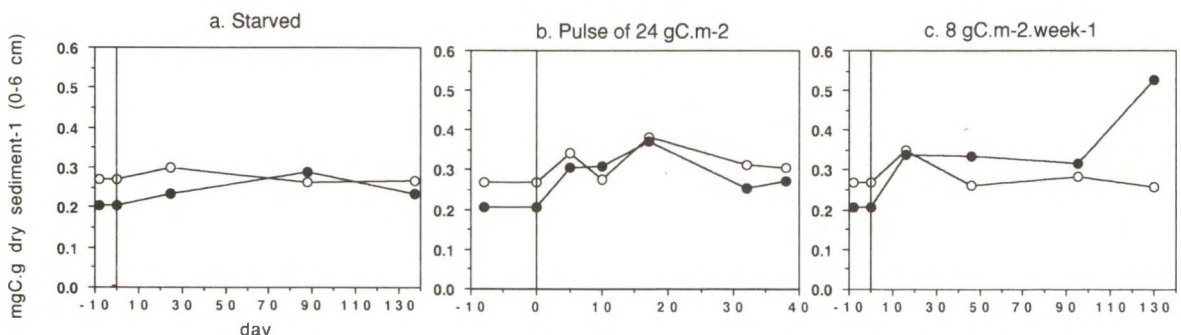


Fig. 5. Sediment organic carbon content integrated over the upper 6 cm of the sediment in a. starved, b. single-pulse and c. weekly *Phaeocystis*-supplied boxcosms with (●) and without (○) macrozoobenthos.

respiration in the presence of macrozoobenthos. From day-90 onwards blackening of the sediment and H_2S production suggest that sulphate reduction was increasing, further reducing the sediment. It has been demonstrated in mesocosms as well as in the field that with organic matter enrichment the share of sulphate reduction in benthic metabolism increases (JØRGENSEN, 1982; SAMPOU & OVIATT, 1991).

After day-90, oxygen consumption in the presence of macrozoobenthos exceeded oxygen consumption in boxes without macrozoobenthos. From that moment onwards some chemical oxidation possibly occurred. Reduced compounds may have been transported to the sediment surface by bioturbation and subsequently oxidized. The decreasing bacterial production may also be an indication that sulphate reducing bacteria and the associated sulphate reduction were increasing. Sulphate-reducing bacteria generally do not incorporate thymidine in their DNA (MORIARTY, 1986; GILMOUR *et al.*, 1990; PIKER & REICHARDT, 1991), implying that their production cannot be measured by the thymidine method.

4.4.3. OXYGEN-PENETRATION DEPTH

After the introduction of *Phaeocystis* the changes in the thickness of the oxic layer were initiated faster in the presence than in the absence of macrozoobenthos. This was to be expected, considering their role in the sediment-water exchange which enhances the effective diffusion of solutes through bioturbation and bioirrigation (e.g. ALLER & YINGST, 1985; KRISTENSEN, 1984, 1985; HÜTTEL, 1988), and suggests that in the presence of macrozoobenthos, the thickness of the oxic layer would increase. However, the opposite happened: after the initial decrease in thickness in response to the *Phaeocystis* supply, the oxic zone stabilized at a shallower depth in the presence of macrozoobenthos than in its absence. The explanation may be that the expected enhanced oxygen diffusion due to bioturbation was counteracted by enhanced decomposition of organic matter in the anoxic part of the sediment. This assumption is supported by the enhanced bacterial production, particularly deeper in the sediment. However, this would also require an increased oxygen consumption in the presence of macrozoobenthos. As already indicated, such an increase was not found. At present no satisfying explanation can be given other than that the sediment was further reduced during the experiment and was not in equilibrium with the benthic oxygen consumption.

4.4.4. CARBON BUDGET

Data presented in Table 4 clearly show that macrozoobenthos enhanced the anaerobic metabolism

and had no effect on the aerobic benthic metabolism. The single *Phaeocystis* input elicited less stimulation than the weekly supplies. The percentage of the input which was worked into the sediment must therefore have been lower in the pulsed than in the weekly supplied boxcosms. Irrespective of the *Phaeocystis*-supply regime, it was evident that the presence of macrozoobenthos stimulated the bacterial production and abundance in deeper sediment layers. Evidence was found that the bioturbation activities of *Echinocardium cordatum* were particularly important in the incorporation and storage of *Phaeocystis* material into the sediment. This stresses the point that the activities of macrozoobenthos (such as deposit feeding and bioturbation) are important in the burial of organic matter in the sediment and strongly influence the benthic metabolism.

4.5. INPUT OF ORGANIC MATTER IN NORTH SEA SEDIMENTS: CONCLUSIONS BASED ON THE BOXCOSM EXPERIMENT

The present boxcosm experiments have confirmed that:

- 1. Input of organic matter to the sediment is enhanced by the burrowing of macrozoobenthos, in accordance with the findings of e.g. ANDERSEN & KRISTENSEN (1991). In the absence of burrowing organisms more organic matter will be lost to the water column than in their presence. *Echinocardium cordatum* appears to burrow organic matter effectively. It redistributes organic matter by transferring deeper into the sediment the organic matter that settles on the sediment surface. Considering its common distribution in the southern North Sea with adult densities of tens of $\text{ind}\cdot\text{m}^{-2}$ and maxima of up to hundreds of $\text{ind}\cdot\text{m}^{-2}$ (CREUTZBERG *et al.*, 1984; BEUKEMA, 1985; DUINEVELD *et al.*, 1990, 1991), its contribution to the burial of organic matter in North Sea sediments may be of importance in comparison with other processes.
- 2. The benthic metabolism is enhanced by macrozoobenthos. The presence of 80 *Echinocardium cordatum* per m^2 enhanced the benthic metabolism by 400% in boxcosms weekly supplied with organic matter compared to boxcosms without macrozoobenthos.
- 3. After enrichment with organic matter, the presence of macrobenthos resulted in less thick oxic zones than in boxcosms without macrozoobenthos. This process is more likely to occur in sandy sediments where shafts and burrows of organisms cannot be maintained. After the organic-matter input, bioturbation may have induced increased use of

electron acceptors deeper in the sediment which appeared not to be replenished at the same rate with oxygen by diffusion and bioirrigation.

Organic enrichment of the sediment due to eutrophication was demonstrated to increase the macrofaunal biomass in the Skagerrak-Kattegat (JOSEFSON, 1990) and in the Wadden Sea (BEUKEMA & CADÉE, 1986; BEUKEMA, 1989, 1991). If this coincides with an increase in bioturbation, burrowing organisms may deteriorate their own environment by enhancing the microbial activity, which may ultimately lead to anoxia. Hypoxia and anoxia of North Sea sediments reducing the macrobenthic community have been observed in the German Bight and the Kattegat (VON WESTERNHAGEN *et al.*, 1986; ROSENBERG & LOO, 1988; BADEN *et al.*, 1990). Additional research is needed to investigate to what extent bioturbation contributes to the risks of anoxia in different sediment types, which can ultimately lead to mass mortality of macrozoobenthos.

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RESPONSES OF BENTHIC HETEROTROPHIC NANOFLAGELLATES TO SETTLEMENT OF ORGANIC MATTER UNDER EXPERIMENTAL CONDITIONS

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1. INTRODUCTION

Sedimentation of organic material on marine sediments draws responses from the benthos, such as change in respiration rate per bottom surface area, and it is clear that there are significant reactions of the microbial communities in the field (e.g. GRAF *et al.*, 1982, 1983; MEYER-REIL, 1983). The role of the different components of benthic microbial communities is, however, far from obvious.

A potentially important group in the flow of material through sediments are the heterotrophic nanoflagellates. They are little studied, because of the difficulties involved in quantitatively extracting such fragile small organisms from their heterogeneous sediments. We showed that nanoflagellates can accurately be sampled (BAK & NIEUWLAND, 1989). Our results showed that densities can be high and that there are significant seasonal changes. In sandy North Sea sediments numbers varied from 50 to $300 \times 10^3 \text{ cm}^{-3}$ through the year. High densities occur in spring and summer.

The observed variation in the field suggests that high densities are linked to phytoplankton blooms and subsequent sedimentation. This assumption was tested in a mesocosm experiment (BAK *et al.*, 1991). We exposed undisturbed North Sea sandy sediments to algal sedimentation and studied the response of the heterotrophic nanoflagellates and their potential prey, the benthic bacteria. The results showed that there was a significant reaction in terms of bacterial productivity and flagellate density to the input of organic matter. Also, flagellates could have a considerable impact on bacterial production, at least in sandy North Sea sediments.

To study these processes more closely, having data points at smaller time intervals, and the

experiment performed in the framework of a controlled biotic environment, such as known numbers and biomass of macrobenthos, we adjusted the environmental setting and repeated the experiment. Our questions were; 1. how do heterotrophic nanoflagellates densities vary under different organic input regimes, 2. what are the relevant time scales, 3. are there density fluctuations related to the characteristics of the biotic environment, i.e. bacteria, macrofauna? These questions are posed to give information on the basic problem: what is the fate of the pelagic carbon input in benthic systems. Does it fuel bacterial production mainly or does an appreciable part of the energy supply support the macrofauna and stimulate the growth of these organisms? Also, if bacterial production is significantly stimulated will this be followed by a response in the nanoflagellate communities, indicating a link between bacterial production and their potential microbial predators in marine sediment?

2. MATERIAL AND METHODS

To closely follow the immediate response of flagellates to For experimental set-up, including treatment of sediment, stocking with organisms, origin and nature of carbon supply, as well as sampling methods see VAN DUYL *et al.* (1992). All boxcosms (round, diameter 30 cm, height 35 cm) were seeded with micro-organisms but, to study the effects of macro-fauna, only half of the boxcosms were stocked with macro-fauna (*Echinocardium cordatum*, *Tellina fabula*, *Nephtys hombergii*, $n=6$ each boxcosm). Two regimes of carbon addition to boxcosms were established: a weekly supply of 8 g C m^{-2} and a single pulse of 24 g C m^{-2} . Controls

were starved throughout the experiment. For time intervals between sampling points during the experiment see abscissa of Figs.

the carbon pulse (24 g) we subsampled the sediments during the 10 days directly after addition of the organic material. In these subsamples no complete cores were obtained but only the sediment surface was sampled. For time intervals see Fig. 2.

Heterotrophic nanoflagellates were extracted from sediment cores ($n=5$ at each sampling point, inner diameter of core 26 mm).

Each core was sampled at three depth, 0-3, 30-33 and 60-63 mm into the sediment. Briefly, each sample was fixed, washed, stained and counted using epifluorescence microscopy (Hobbie *et al.*, 1977). Numbers of protists were grouped in size classes >2 , 2-5, 5-10 and 10-20 μm . Conversion to carbon values was done using geometrical shapes and a carbon content of $200 \text{ fg C } \mu\text{m}^{-3}$. This is a mean value of Fenchel (1982) and Børseim & Bratbak (1987). For details of methods see Bak & Nieuwland (1987), Bak *et al.* (1991).

3. RESULTS

Densities of flagellates were low in the surface layer of the controls and they remained so throughout the experiment (Fig. 1). At lower densities, this constancy in numbers was reflected in the deeper sediment layers. Numbers were lower in the control with macrofauna compared with controls without macrofauna.

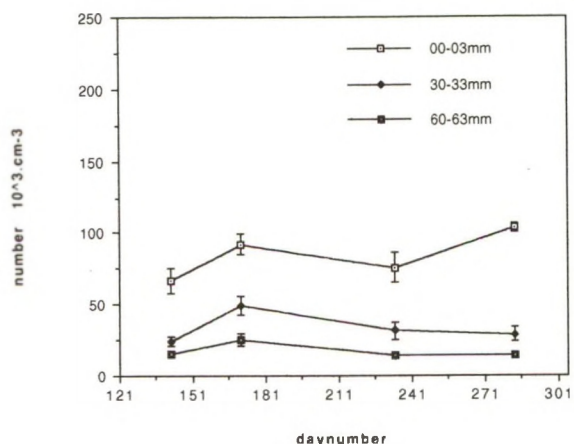


Fig. 1. Densities of heterotrophic nanoflagellates at three depths in the sediment of starved mesocosms.

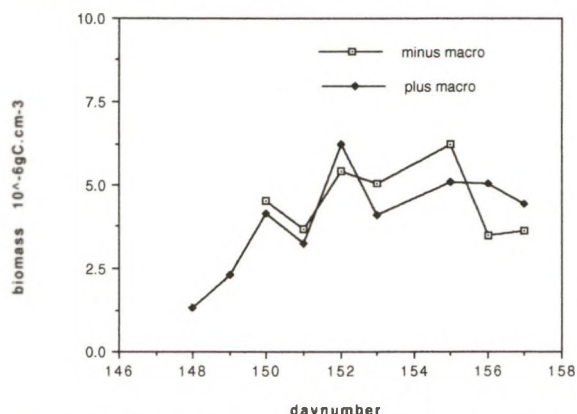


Fig. 2. Biomass of heterotrophic nanoflagellates the first 9 days after the carbon pulse in mesocosms with and without macrofauna.

The addition of the single carbon pulse resulted in a rapid response of the protist community. Densities increased from $80 \times 10^3 \text{ cm}^{-3}$ to $400 \times 10^3 \text{ cm}^{-3}$ in a week. Because cell size increased during this period the increase in protist biomass was even more outspoken (Fig. 2). Densities increased to $500 \times 10^3 \text{ cm}^{-3}$ two weeks after the carbon pulse but were lower at the subsequent data points. When macrofauna was present in the sediments highest densities were not maintained for the same length of time (Fig. 3). A second obvious difference caused by the presence of macrofauna was the response deeper in the sediment. In the absence of macrofauna flagellate abundances only increased in

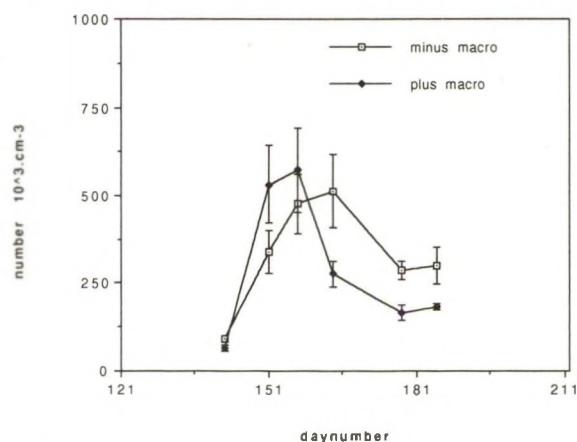


Fig. 3. Densities of heterotrophic nanoflagellates after carbon pulse in bottoms with and without macrozoobenthos (closed and open dots respectively). Depth in sediment 0-3 mm.

BENTHIC HETEROTROPHIC NANOFLAGELLATES

the surface layer of the sediment, but when macrofauna was present flagellate densities also increased significantly at depths of 3 and 6 cm in the bottom.

In bottoms which received a weekly supply of carbon flagellate densities showed high numbers at the sediment surface throughout the experiment. However, densities were much higher in the absence of macrofauna, 750 compared to $250 \times 10^3 \text{ cm}^{-3}$ respectively. Increased densities at 3 and 6 cm depth in the sediment occurred only in the presence of macrofauna.

4. DISCUSSION

Abundances of the heterotrophic nanoflagellates in our experiment are wholly comparable with those of shallow temperate seas such as the Wadden Sea, the sandy North Sea and others (BAK & NIEUWLAND, 1989; BAK *et al.*, 1991; BAK & NIEUWLAND, in press). Also, flagellates in the size class $< 5 \mu\text{m}$ are, as usually, most numerous.

All experiments clearly show the influence of carbon supply to the surface layer of the sediment. Increased carbon not only resulted in increase of flagellate abundance but there was a similar response by their prey. The high flagellate densities were paralleled by an increase in bacterial variables such as bacterial production (VAN DUYL *et al.*, 1992). There appeared to be a direct relation between these groups, e.g. in case of the single pulse experiment the increased values and activity lasted as long, a month, in bacteria and flagellates.

The effect of the presence of macrofauna was obvious in various ways. Firstly, in comparable situations, e.g. in the starved controls, flagellate densities were lower in the presence of macrofauna. A similar observation was made for the bacterial densities. VAN DUYL *et al.* (1992) suggest deposit feeding by macrofauna, most noticeable in the surface layer of the sediment, to be responsible for lower densities here. The same effect of the macrofauna showed in the bottoms receiving weekly carbon supplies: flagellate densities were lower in the presence of macrofauna. We assume that this indicates that part of the energy added was not available for use by the flagellate communities but channelled into macrofauna growth and maintenance (DUINEVELD *et al.*, 1992). The same phenomenon, competition for available energy between flagellates and macrofauna, appears in the pulse experiment where flagellate densities drop relatively soon in the presence of macrofauna (Fig.

3). An alternative explanation, that macrofauna would directly interfere with microbial densities through predation, is unlikely (see KEMP 1987; ALONGI 1988).

Secondly, there is the clear influence of macrofauna demonstrated deeper in the sediments. Enhanced flagellate densities at any depth below the surface only occur in the presence of macrofauna. Again there is a clear relation with the bacteria, the most likely source of food for the flagellates. Bacterial productivity deeper in the sediment was significantly higher in bottoms with macrozoobenthos (VAN DUYL *et al.*, 1992). Bioturbation is apparently of overwhelming importance in transporting organic matter into the sediments.

We conclude, firstly, that under our experimental conditions the response of heterotrophic nanoflagellates in marine sediments to sedimentation of organic matter was clearly demonstrated, and secondly, that there is interaction between bacterial production, protists densities and the presence/absence of macrofauna.

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MEASUREMENT OF BIOTURBATION IN A MESOCOSM EXPERIMENT USING A NEW METHOD

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1. INTRODUCTION

Bioturbation is a process of sediment movement or mixing by burrowing and feeding macrofauna. Glass beads or sand grains marked with fluorescent paint are often used to trace this sediment movement in studies of horizontal and vertical sediment transport, as well as in determining the role of bioturbation (TEUCHER, 1991; MAHAUT & GRAF, 1987; NACHTIGALL, 1964; GERINO, 1990; RIJKEN, 1979). MAHAUT & GRAF (1987) AND GERINO (1990) found the painted sand grains back till a depth of 5 cm reworking by macrofauna in three or four weeks. The painted sand grains are often placed on the surface of the sediment and at periodic intervals samples of the sediment are examined to ascertain the movement of these stained particles through the sediment. Formerly, in order to follow the fate of the marked grains, it was necessary to count them visually. Nachtigall (1964) indicated that separating and visual counting of these grains can be a very time-consuming process. To alleviate this problem, a number of methods have been developed over the years in an attempt to find a way to automatically count the colored grains (Nachtigall, 1964; Striggow, 1966; Teucher, 1991).

Rather than even attempting to count the painted sand grains, we took a completely different approach for solving this problem. By first redissolving the paint from sediment samples, it was possible to measure the resulting solution fluorometrically. Thus, the concentration of paint in a sample becomes an index of the number of painted sand grains.

2. MATERIAL AND METHODS.

Sand grains (grainsize 125-160 μm) were prepared using a modification of the method as given by WILKENS (1969) and NIEUWENHUIZE & SIPS (1977).

After some trial and error, the following procedures were adopted. 50 kg of sand were washed in a 5 % solution of hydrofluoric acid for 15 minutes, and then rinsed for one hour with tap water. The wet sand was drained over a 50 μm sieve and shaken to remove as much water as possible. The resulting moist, etched sand was mixed for 2 hours in a concrete mixer with 1 kg fluorescent paint (Visprox Fluorescent Flame Red) and 120 ml ethyl alcohol. After mixing, the sand was placed in an oven at 50 $^{\circ}\text{C}$ until dry. The dried sand was sieved over a 500 μm sieve to separate any grains that stuck together. To remove any potentially poisonous solvents, the painted sand was washed for a period of two weeks in flowing sea water. The painted sand was then tested to be certain that all poisonous materials had been removed. This was done by first filling two 10 liter Erlenmeyer flasks with seawater, one with painted sand covering the bottom and the other with unpainted sand. Ten shrimps (*Crangon crangon*) were placed into each flask. Both flasks were aerated and kept at 12 $^{\circ}\text{C}$, but the sea water was not refreshed. This was to ensure that any poisonous material that might be released by the painted sands would not be rinsed away.

The following test was done in order to determine how much time was necessary to redissolve all of the paint from treated sand particles. Ten samples of painted sand, varying in dry weight from 1-12 mg, were prepared. To each sample was added 5 ml of the extraction solution, composed of 4 ml acetone and 1 ml dimethylsulfoxide. The concentration of paint in each sample was measured after 1, 3, 27 and 46 hours, using a Hitachi F2000 fluorospectrophotometer (EX 418; EM 600).

2.1. EXPERIMENTAL SET-UP

Three cylinders, each 35 cm high with a diameter of 30 cm, were filled up to 25 cm with North Sea sand

(125-160 μm grainsize) and to the rim with sea water. Cylinders were kept at a constant temperature of 12 °C and overflowed by water and aerated. After an acclimatization period of 7 days, test animals were placed into two of the three cylinders. The third cylinder was used as a control and had no animals in it. The animals used consisted of 5 individuals each of the heart urchin (*Echinocardium cordatum*), a bivalve (*Tellina fabula*) and a polychaete (*Nephtys hombergii*). Within 24 hours, all animals had buried themselves. One week after the animals had been placed in the containers, a layer of approximately 3/4 cm thick fluorescent sand was placed on the sediment surface in all three cylinders. One of the cylinders containing animals was also supplied with fresh-frozen *Phaeocystis* (8 g C. m^{-2}) as a food source. Three sediment samples per container were taken after a month and thereafter every 2 weeks, using a coring tube supplied with a plunger. The tube had an outside diameter of 6 mm and an inner diameter of 3 mm.

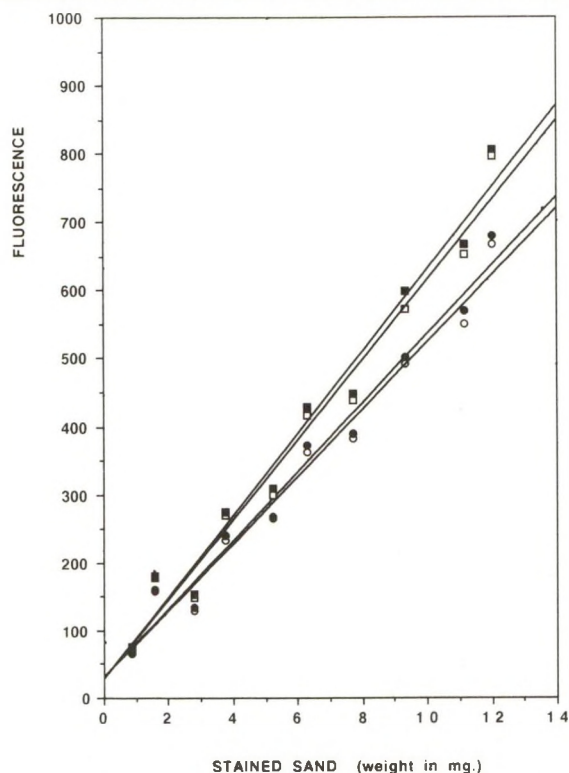


Fig. 1. Linear regression lines showing the relation between the amount of extracted sediment (stained) and fluorescence at increasing extraction time. \circ 1 hour, \bullet 3 hours, \square 27 hours, \blacksquare 46 hours.

As the coring tube was pressed into the sediment, the face of the plunger remained on the sediment surface. Holding the plunger in place while removing the tube from the sediment maintained the integrity of the sample core. Cores were divided into 1 cm lengths to a total depth of 10 cm. Each 1-cm section was placed into 5 ml. of the extraction solution (4 ml acetone; 1 ml dimethylsulfoxide). After 24 hours of extraction, the fluorescence of each sample was measured.

3. RESULTS AND DISCUSSION

3.1. METHODOLOGY

The linear relation between fluorescence and the weight of the sample (a measurement of the number of sand grains) is shown in Fig. 1. The correlation between weight and fluorescence for the different extraction times remained significant irrespective of the extraction times. The regression coefficient, however, increased at longer extraction times. It took apparently more time to resolve most of the paint in the heavier samples than in the lighter samples. An extraction time of 24 h or more is required for the best results (Fig. 2). After the two weeks incubation of the shrimps with the painted sand, they were still alive, and behaved like the shrimps in the vial with the untreated sand. This indicates that the painted sand seems to be not fatal and that the paint might be free of poisonous components. We concluded that the painted grains are suitable for use in bioturbation studies. The

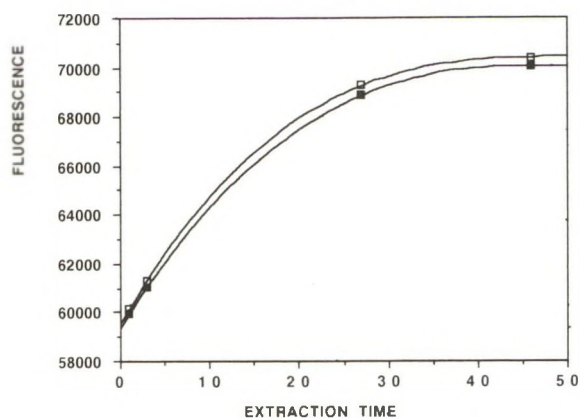


Fig. 2. Fluorescence (per gram sediment) as a function of extraction time (h) (in duplo).

BIOTURBATION

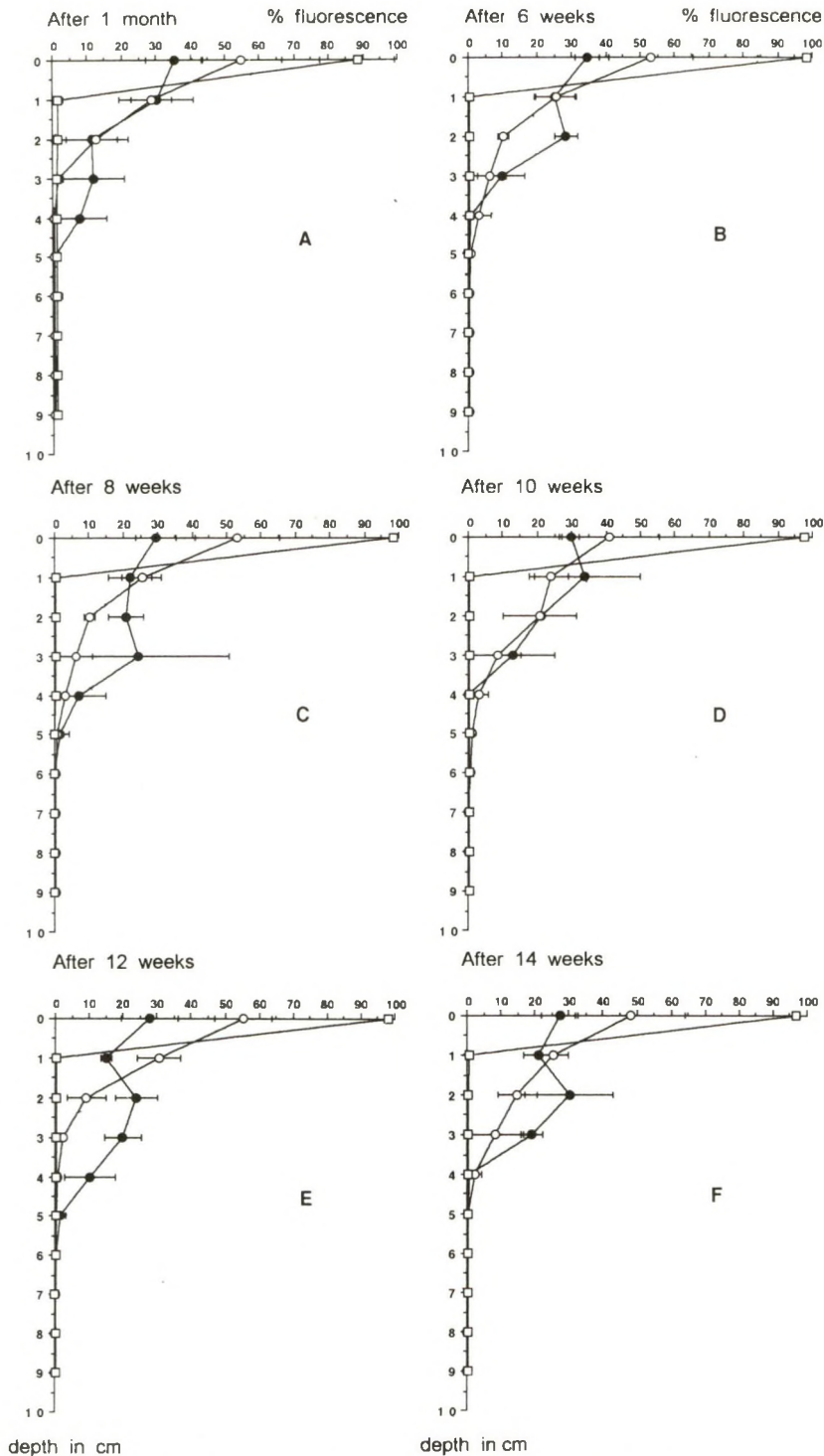


Fig. 3. Vertical distribution of percent fluorescence in bioturbation experiment, (A) one month after the start and (B-F) bi-weekly intervals until the end of the experiment. The three conditions tested include: fed macrofauna (●), unfed macrofauna (○), and a control (□) without animals.

method presents a good alternative for the methods with luminophore presently in use to measure bioturbation (e.g. MAHAUT & GRAF, 1987). With respect to the other methods it is time-saving. The amount of paint as measured with simple fluorescence in the diluted pore water after extraction replaces the more time-consuming and/or complicated measurement of the amount of painted sand grains in the sample (by either microscopy or automatic counting).

3.2. BIOTURBATION

The results of the bioturbation experiment obtained in the North Sea bottom sediments are given in Fig. 3. The three treatments - fed with macrozoobenthos (●), unfed with macrozoobenthos (○) and unfed without macrozoobenthos (□) - are indicated in all figures. The series of A to F represent the development of the differently treated containers in the course of the experiment (14 weeks).

The first thing to notice is the difference between the cylinders with animals (●,○) and the control without animals (□). In the latter, the total fluorescence remains concentrated in the top (1 cm) sediment layer over the entire duration of the experiment (14 weeks). In the two containers with animals (●,○), fluorescent sand appeared after one month at a depth of 4-5 cm, although it was much more homogenized in the cylinder containing fed animals (●). For the remaining 10 weeks of the experiment, hardly any change was observed in the fluorescent profile of the unfed animals (●,○). At the close of the experiment, all of the unfed animals were removed and found to be still alive. The results of their bioturbation activity is recognizable after the first month, although it remained confined to the upper layers of the sediment. Because the gradient hardly changed with time, this is an indication that there was hardly any vertical movement and thus little bioturbation took place in the lower levels.

The fluorescent profile in the container with fed animals (●) changed continuously throughout the course of the experiment. There is a gradual development from the initial profile towards a more homogenized sediment mixture. These mixing activities are most apparent in the top 4-5 cm of the sediment.

From these observations, it would appear that fed animals were able to homogenize sand to a greater depth by bioturbation than unfed animals. The greatest portion of the bioturbation observed in this experiment is almost certainly due to the five *Echinocardium*s, as their ventilation funnels were regularly observed to change position over the course of the experiment. The *Tellinas*, on the other hand, remained virtually in the same place throughout the entire experiment. Something similar has been reported by DE RIDDER & JANGOUX (1985); they found that unfed individuals of *Echinocardium* ingested more of the upper sediment than did those which were fed. This undoubtedly came out because unfed animals, as we have found, remain nearer to the sediment surface.

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REPORT ON MACROFAUNAL GROWTH IN A MESOCOSM EXPERIMENT WITH SINGLE DOSAGE AND CONTINUOUS FEEDING

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1. INTRODUCTION

One of the questions related to eutrophication of coastal areas concerns the effect of the increased algal biomass on the benthic macrofauna and more specifically in the ability of macrobenthic animals to store excess carbon as biomass or transfer it to higher trophic levels. In the Wadden Sea, BEUKEMA (1991) observed a doubling of macrobenthic biomass and production over the past 20 yrs in connection with eutrophication. Although it is recognized that in many subtidal sediments a major part of the carbon-input is mineralized by the microbial system, simultaneous measurements on the metabolic activity of macrofauna and microfauna have not frequently been conducted.

In an earlier series of mesocosm experiments where the effect of a single dosage of food on a natural community was investigated, most of the carbon appeared to be rapidly recycled within the bacterial system (VAN DUYL, 1991). The use of a natural community in this case, however, did not allow an accurate assessment of the macrofauna. In order to gain better insight in the role of macrofauna, an experiment was designed whereby a macrobenthic community was assembled of three species with known weights and respiratory activity. This set-up also gave opportunity to study the interaction between macrofauna and microbial metabolism by manipulating macrofauna density. One aspect of this interaction, i.e. the bioturbation activity of the macrofauna, was studied in detail by means of fluorescent tracer particles (VAN NOORT & KRAAY, 1992) and by means of pigment analysis of the sediments. The present report provides the data on the measurements of macrobenthic respiration and production.

2. MATERIAL AND METHODS

For a detailed description of the experimental set-up we refer to VAN DUYL *et al.* (1992). This section only contains features of the experiment which are relevant with regard to the introduction of the macrofauna. Three species were selected for the experiment, viz. the urchin *Echinocardium cordatum*, the bivalve *Tellina fabula* and the polychaete *Nephtys hombergii*. Animals were collected from a shallow subtidal site near the coast of Texel. The experimental containers (0.076 m²) were stocked with 6 equally sized specimens of *Tellina* and *Echinocardium*. Total macrobenthic biomass in a container was approximately 14.5 g Ashfree Dry Weight.m⁻². *Echinocardium* and *Tellina* were measured to the nearest 0.5 mm before they were introduced. For both species, a length-ashfree dry weight (AFDW) relationship was calculated on basis of spare material in order to have an estimate of the initial weights of the experimental animals. For *Echinocardium* the length-weight relationship was as follows (W=AFDW in g; L=length in mm):

$$W=1.358E-05 * L^{2.862}$$

and for *Tellina*:

$$W=1.778E-05 * L^{3.45}$$

Specimens that were retrieved during the course of the experiment were measured and their individual ashfree dry weight was determined. Because the actively crawling *Nephtys* could not be measured accurately, the total wet weight of the 6 specimens was recorded prior to their release in the containers.

Two types of experiments were conducted simultaneously. In the first experiment, containers were regularly fed during a period of 153 days.

Food (*Phaeocystis*) was administered in pre-weighted portions containing 0.61 g Carbon. At periodic intervals, a set of 2 containers were sieved while in 2 containers feeding was stopped. Sieving took place in week 4, 9, 19 and 22 after the start of the experiment. At these dates the containers had received respectively 1.2, 4.3, 8.5 and 11.6 g C. Animals that died during the experiment were replaced by similarly sized ones. The latter were marked and not taken into account in the calculation of growth. In the second experiment only one dosage of food (1.8 g C) was added to the containers at the start of the experiment. At intervals, 2 containers were sieved and the animals weighed and measured. Because there were not enough *Echinocardium* of the same size available, the animals in the second experiment had a lower initial size (21.5 mm) than those taking part in the first experiment (25.5 mm).

The respiratory demand by the macrobenthos in the containers was estimated by means of allometric weight-respiration relationships for the three species in question (unpublished data). Total community respiration was measured by sealing the containers with a lid holding a stirrer and a YS oxygen electrode. Data from the electrodes were recorded on an IBM PC. The electrode readings were calibrated with the Winkler titration. Respiration data

(in $\mu\text{mol O}_2$) were converted into carbon using an RQ of 0.85.

Daily production by each macrobenthos species in a given interval was estimated by taking the difference in total weight of the stock at the end and the beginning of an interval and dividing this amount by the relevant number of days. Production in g ashfree-dry weight was converted to carbon by multiplication with factor 0.4.

3. RESULTS AND DISCUSSION

3.1. CONTINUOUS FEEDING EXPERIMENT

Table 1 shows the changes in body weight of three species during the continuous feeding experiment. *Echinocardium* showed a clear increase in individual weight and, moreover, suffered little mortality. The majority of the *Tellina* specimens decreased in weight during the course of the experiment. Especially during the later phases, increasing numbers of *Tellina* died and had to be replaced. The weights (wet weight) of the polychaete *Nephtys* did not show a clear trend, i.e. in one of the two replicate containers a positive increment was found whereas in the other a negative value were found. On average, however, the *Nephtys* stock in the two containers seems to have lost weight in the course of the experiment. Part of the erratic variation in the *Nephtys* weight is caused by the fact that we only could use wet weight which is a rather crude measure.

TABLE 1

Body weights (plus standard deviation) of *Echinocardium* and *Tellina* in the continuous feeding experiment. Last column shows the weight increments of the *Nephtys*-stock (6 individuals specimens) in each container during successive intervals.

CONTINUOUS FEEDING				
Time after start of experiment in days (daynr)	Total food received mg C	<i>Echinocardium</i> ind. weight mg AFDW(s.d)	<i>Tellina</i> ind. weight mg AFDW (s.d)	<i>Nephtys</i> WWt - WWt-1 mg Wet weight ^a
0 (146)	0	143 (26)	13.7 (5.1)	
12 (158)	1220	194 (24)	13.8 (3.7)	-586/+183
46 (192)	4270	226 (32)	10.8 (2.8)	-79/+110
100 (246)	8540	252 (38)	6.1 (0.6)	-375/+70
132 (278)	11590	238 (57)	7.1 (1.6)	-263/+278

^a Total weight increment of 6 individuals in each of the two containers

MACROFAUNAL GROWTH

TABLE 2

Continuous food experiment: Carbon input and respiratory demands by the community and by the macrobenthos separately. Last column shows the macrobenthic production in different time intervals; negative production (=weight loss) is not included in the production figures.

SINGLE FOOD PULSE

Time after start of experiment in days (daynr)	Total food received mg C/box	Community respiration mg C/d/box	Macrobenthic respiration mg C/d/box [estimated]	Macrobenthic production mg C/d/box
12 (158)	1220	10.6	2.7	10.2
62 (192)	4270	21.9	2.9	2.2
100 (246)	8540	22.5	3.2	1.2
132 (278)	11590	28.5	2.9	0.0

Table 2 shows the results from the measurements of community respiration and the calculations of macrobenthic respiration and production. The respiration by the macrobenthos, calculated from literature data, is very close to the measured difference between community respiration in containers with and without macrofauna, viz. 1.8-4 mmol O₂.m⁻².d⁻¹ (VAN DUyl *et al.*, 1992). The macrobenthic production in Table 2 consists entirely of growth by the sea-urchin *Echinocardium*. Negative production (=decrease in weight) by the other species was not taken into account in the production figures, because this organic material was lost from another source than the food that was introduced.

Remarkable is the decrease in production of *Echinocardium* during the experiment in spite of a nearly linear increase in size (Fig. 1a). An explanation for this discrepancy could be that an increasing proportion of the *Echinocardium* stock spawned during the experiment. This is not unlikely as the first months of the experiment (May-June) cover the main reproductive period of *Echinocardium* in the Southern Bight of the North Sea (DUINEVELD, pers. obs.). Further evidence comes from a comparison between the measured weights (Fig. 1b) and the weights predicted by the length-weight relation established at the start of the experiment. The measured weights appear to be substantially higher, with maximum deviations at daynr 192 (35 % higher). This 'extra weight', viz. 0.06 g AFDW for a 27 mm long animal, is comparable to the increase of the gonadal weight of *Echinocardium* that LOOSE (1991) observed over a

two-month period (June-August 1991) in a parallel feeding experiment in the mesocosm. In this period, the gonads of *Echinocardium* with a length of approximately 27 mm increased from ca. 0.2 to 0.5 g Wet Weight, which is very close to our observed 'extra weight' if one assumes an AFDW : Wet Weight ratio between 10 and 20 %. So it seems as if the animals put on additional weight, probably in the form of gonadal tissue, which they gradually lost by the end of the experiment.

The length increments of the experimental *Echinocardium* seem to be lower than the ones Beukema (1985) found in a subtidal population near Texel. In the field population, animals of 24 mm grew in one year to 35 mm. Assuming a growing season of 7 months, leads to a monthly length increment of 1.6 mm for the field situation. In the mesocosm, we found a monthly increment of roughly 0.7 mm for the duration of the experiment. The *Echinocardium* density in the experiments was, however, twice that in the field population (40 ind./m²) and the experimental temperature 12°C was lower than maximum temperatures in the field.

Fig. 1 also shows the final lengths and weights of *Echinocardium* from the containers where feeding was stopped after a certain time but which were kept intact until the end of the experiment. Comparing these animals with the corresponding ones that were weighed on the day that feeding stopped (broken lines in Fig. 1), shows that animals which starved from the beginning (daynr. 146) and from daynr. 158 onwards, lost a significant amount of weight and, moreover, did not grow any further in length (Mann-Whitney U-Test; p < 0.05).

The final weights of these animals were invariably lower than predicted by the length-weight relationship.

Animals that were not fed from daynr. 192 onwards, neither grew any further in length. Their average weight decreased slightly but not significantly. Animals that were stopped being fed on daynr. 246, seem, on the other hand, to have increased in length whereas their weights remained constant (Fig. 1). The length increments, however,

turned out to be insignificant when tested. The final weights of these two batches of starved *Echinocardium* were always higher than the weights predicted by their final length. The difference in final and expected weight was, however, highly variable (6-30 %) both within and between treatments. Thus it seems as if the organic material that had accumulated in the sediment by daynr. 192 (see VAN DUYL, *et al.*, this volume) was sufficient to meet the basic metabolic demands *Echinocardium*, but not enough to produce any significant growth in length and weight.

3.2. SINGLE DOSAGE EXPERIMENT

Just as in the continuous feeding experiment, only *Echinocardium* showed a noticeable increase in length and, more important, in weight after a single supply of *Phaeocystis* material. The weights of both other species declined after the introduction of the food (Table 3). Table 4 shows the results from the measurements of community respiration and the calculations of macrobenthic respiration and production.

Significant growth of *Echinocardium* was measured in the interval (daynr. 157-178) that community respiration as well as bacterial production in the top 3mm layer had dropped from maximum values elicited by the introduction of the food to a much lower level (VAN DUYL *et al.*, 1992). This decreased activity in the surface layer points at a diminished amount of degradable carbon in the oxic top layer. However, in the deeper sediment layers, VAN DUYL *et al.* (ibid) found an increased bacterial production in this period, probably fuelled by buried organic material. Total organic content of the sediment (0-6 cm depth) reached its maximum value in this interval (see VAN DUYL *et al.*, ibid). There is no definite answer to what the food source for *Echinocardium* has been in this period. It may have been the less degradable remainder of organic material in the top layer, the buried organic material or the bacterial production. Notable, in this respect, is that during the final short interval (daynr. 178-185), *Echinocardium* losted a significant part of its weight. Both bacterial production and sediment organic content in this interval also appear to be lower than in the preceding interval.

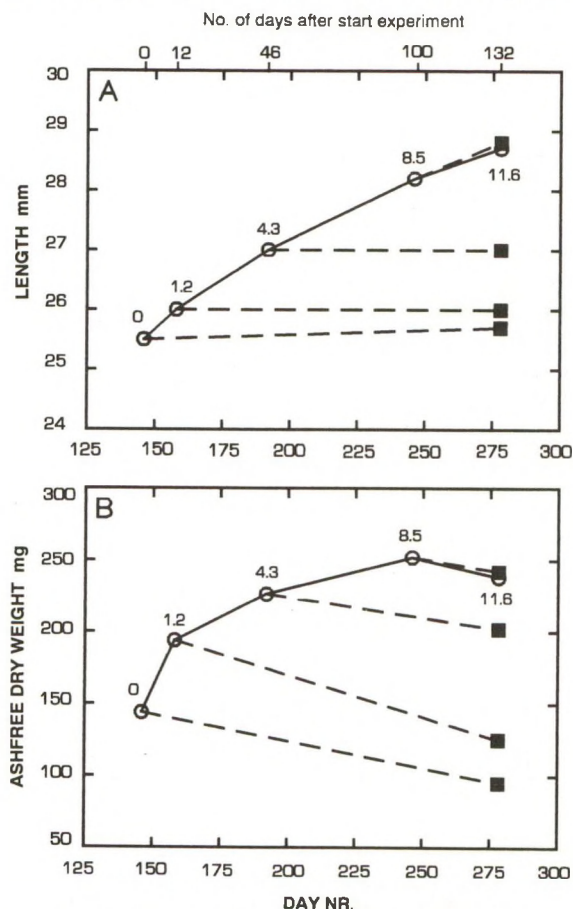


Fig. 1. Circles: average length (A) and ashfree dry weight (B) of *Echinocardium* recovered at different times from the continuous feeding experiment. Squares: final average length (A) and weight (B) of *Echinocardium* from containers where feeding was stopped after a certain time (and amount of food, see values in graph in g Carbon)

MACROFAUNAL GROWTH

TABLE 3

Body weights (plus standard deviation) of *Echinocardium* and *Tellina* in the single food pulse experiment. Last column shows the weight increments of the *Nephtys*-stock in each container during successive intervals.

SINGLE FOOD PULSE

Time after start of experiment in days (daynr)	Total food received mg C	<i>Echinocardium</i> ind. weight mg AFDW(s.d)	<i>Tellina</i> ind. weight mg AFDW (s.d)	<i>Nephtys</i> WW _t - WW _{t-1} mg Wet weight ^a
0 (146)	0	82 (14)	15.5 (4.0)	
11 (157)	1800	96 (16)	15.7 (3.9)	-251/-200
32 (178)	1800	120 (18)	12.8 (3.7)	-417/-227
39 (185)	1800	102 (17)	10.0 (3.1)	-475/-189

^a Total weight increment of 6 individuals in each of the two containers

TABLE 4

Single food pulse experiment: Carbon input and respiratory demands by the community and by the macrobenthos separately. Last column shows the macrobenthic production in different time intervals; negative production (=weight loss) is not included in the production figures.

SINGLE FOOD PULSE

Time after start of experiment in days (daynr)	Total food received mg C/box	Community respiration mg C/d/box	Macrobenthic respiration mg C/d/box [estimated]	Macrobenthic production mg C/d/box
11 (157)	1800	12.3	2.0	2.9
32 (178)	1800	6.4	2.2	2.8
39 (185)	1800		1.9	0.0

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