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Availability of nutrients to a deep-sea benthic microbial community: results from a ship-board experiment

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Abstract

Intact sediment cores from the Vøring-Plateau (Norwegian Sea) were incubated under *in situ* temperature on board ship with and without the addition of natural detritus to follow the reaction of deep-sea benthic microbial communities to nutrient enrichment. Concentration and enzymatic decomposition of organic material, total microbial number, biomass and production were followed in time-course experiments. The addition of decomposable organic material caused an immediate stimulation of microbial metabolic processes: following the induction of enzymatic activity, microbial biomass production increased.

During the initial period of incubation metabolic processes were also stimulated in the untreated "control" sediments. This "incubation effect" competed with the "feeding effect" caused by the enrichment with organic material.

Introduction

Distribution and activity of microbial communities in deep-sea sediments are governed by the supply with organic material (TURLEY and LOCHTE 1989) which only episodically enters the seafloor. As described by several authors (e.g. HELDER 1989, MURRAY and KUIVILA 1990), most of the sedimented organic material is oxidized by oxygen and secondary oxidants at the sediment surface. Very little, however, is known about the degradation of organic material in deep-sea sediments as well as about the organisms involved. Therefore a laboratory experiment was carried out, in which the reaction of deep-sea benthic microbial communities to an input of natural detritus was investigated.

Material and methods

Sampling. Sediment cores from the Norwegian Sea (Vøring-Plateau; water depth 1240 m; 67°44'1"N, 05°55'0"E) were incubated on board ship under *in situ* temperature (- 0.5 °C). After 4 days of "acclimatization", one set of the cores was enriched with detrital material, the remaining untreated cores served as controls. The response of microbial communities to the enrichment was followed by analysing various chemical and microbiological parameters in the sediment profiles. For the analyses, sediments were dissected in 0.5 and 1.0 cm intervals,

respectively, down to a depth of 8 cm. Generally, sediments were diluted 1:5 with filter sterilized bottom water.

Food supply. Natural planktonic and detrital material (sampled from a water depth of 140 m) was concentrated through a 20 µm plankton net. The material was suspended in surface water, boiled, homogenized and washed by centrifugation. Aliquots of the natural organic material (equivalent to 200 mg of dry weight) were pipetted into the water overlying the sediment cores (surface area 78.5 cm^2). As a relative measure of nutrient enrichment, concentrations of protein were analysed from dried sediments according to the Folin method as recommended by RICE (1982).

Hydrolytic enzymes. The hydrolysis of the model substrate fluoresceindiacetate (FDA) by natural esterases was used as a measure of the decomposition of organic material (KÖSTER et al. 1991, SCHNÜRER and ROSSWALL 1982). For the enzymatic analysis, 10 µl FDA (4.8 mM in acetone) was added to 500 µl aliquots of dissected and diluted sediment. Assays were run in time-course experiments at *in situ* temperature (-0.5 °C). The release of the fluorescent dye was read in a spectrofluorometer against a standard solution of fluorescein (1 µM). Enzymatic hydrolysis rates were extrapolated from the slope of the activity curve by linear regression (for details cf. KÖSTER et al. 1991).

Incorporation of ^{3}H-leucine. As a relative measure of bacterial biomass production, the incorporation of ^{3}H-leucine in macromolecular cell material (KIRCHMAN et al. 1985) was determined. 500 µl aliquots of the dissected and diluted sediments were incubated with 10 µl of ^{3}H-leucine (0.5 µCi; specific activity 950 mCi per mg) in time-course experiments at *in situ* temperature. The sample treatment was a modification of the methods of THORN and VENTULLO (1988) and WICKS and ROBARTS (1988) (for details cf. MEYER-REIL and KÖSTER 1991). Bacterial incorporation rates of ^{3}H-leucine were extrapolated from the slope of the activity curve calculated by linear regression. For estimating bacterial biomass production, rates of leucine incorporation were divided by the proportion of leucine in protein (8.8 %), the amount of protein in bacterial cells (50 %), and the carbon content of the cells (50 %).

Bacterial number and biomass. Total bacterial number and biomass were determined by epifluorescence microscopy as described by MEYER-REIL (1983). For biomass analysis, the size of random chosen cells was measured with an eyepiece graticule (New Porton G12; Graticules Ltd, Kent). Using a conversion factor of 0.11 g C cm^{-3} biovolume was converted into bacterial carbon.

Results and discussion

As it could be expected the enrichment with organic material led to a stimulation of benthic microbial activities. From the experiment further information could be obtained about the time scale and sequences of microbial decomposition processes as well as about the influence of incubation.

During the initial phase of incubation, concentrations of protein (a relative measure of nutrient enrichment) slowly increased. Compared to the relatively homogeneous distribution of protein in the original sediment cores, the incubation caused a stratification of protein in the sediment profiles. With prolonged incubation, concentrations of protein decreased and revealed an almost homogeneous distribution pattern comparable with that of the original sediment (Fig. 1).

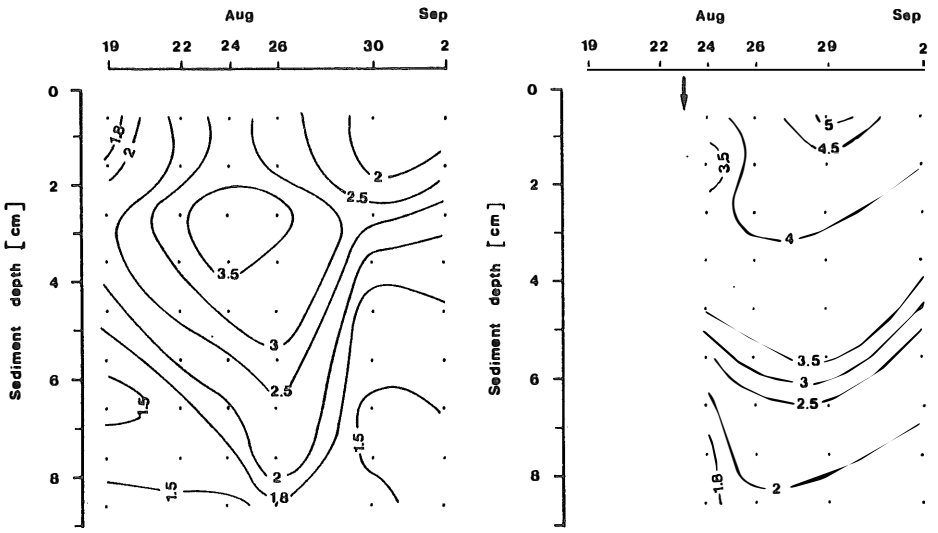

Fig. 1. Isopleth diagram of concentrations of protein equivalents (x 100 µg of bovine serum albumin per cm^3 of sediment) in untreated sediments (left) and sediments supplied with organic material (right). Arrow indicates the date of nutrient addition.

In the treated sediments, the supply with organic material (110 µg C per cm^3 of sediment) led to an increase in concentrations of protein with a maximum approximately 6 days after nutrient addition. The isopleth diagram revealed that the enrichment originated from the sediment surface and spread out relatively quickly into deeper sediment horizons (Fig. 1).

Compared to concentrations, the enzymatic decomposition of organic material (as measured by the activity of hydrolytic enzymes) showed a similar variation pattern. Upon incubation, enzymatic activities increased in the untreated sediments. After 5 days of incubation enzymatic activity decreased and remained on a relatively constant level comparable to the enzymatic activity measured in the original sediment (Fig. 2).

In sediments enriched with organic material, enzymatic decomposition processes were further stimulated by the availability of decomposable organic material. 3 days after nutrient addition maximum decomposition rates were recorded (Fig. 2).

Prior to nutrient addition, microbial incorporation of tritiated leucine (a measure of microbial biomass production) increased in the untreated sediments for a short period of time (Fig. 3). The external nutrient supply led to a second stimulation of microbial biomass production in the treated sediments. Maximum incorporation rates were reached approximately 6 days after feeding (Fig. 3). This demonstrates the existence of a time lag between maxima in concentration and enzymatic degradation of organic material and the subsequent incorporation of the hydrolysis products into microbial biomass (compare KIRCHMAN et al. 1986).

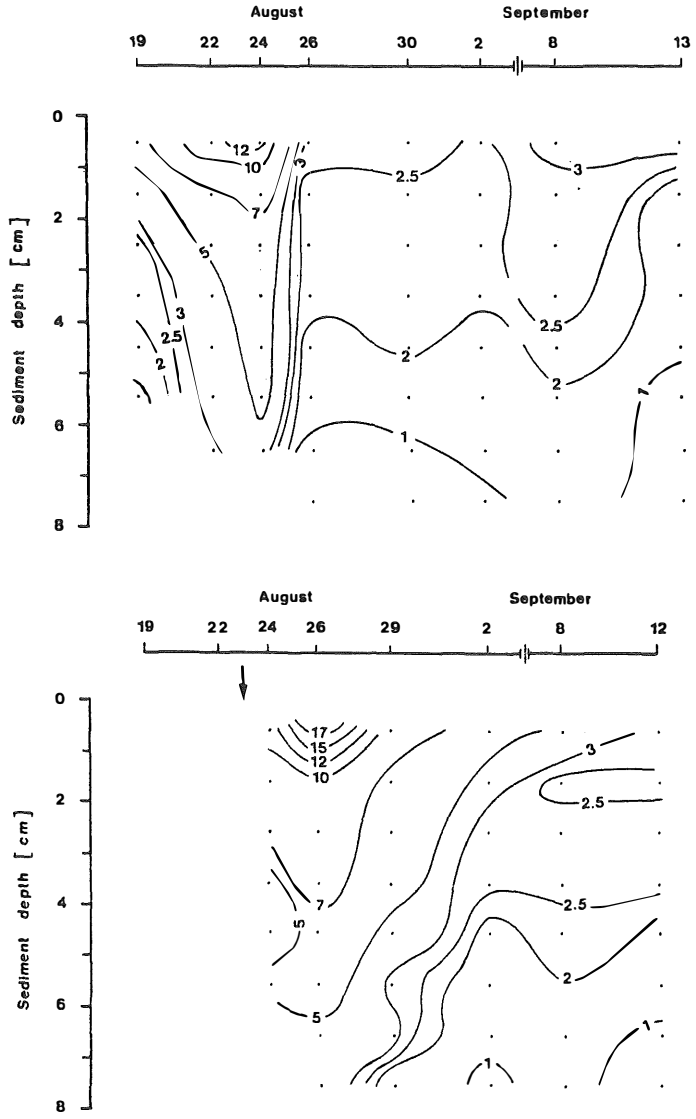

Fig. 2. Isopleth diagram of enzymatic activity as measured by the hydrolysis of fluoresceindiacetate (µmol of fluorescein per cm^3 of sediment per h) in untreated sediments (above) and sediments supplied with organic material (below). Arrow indicates the date of nutrient addition.

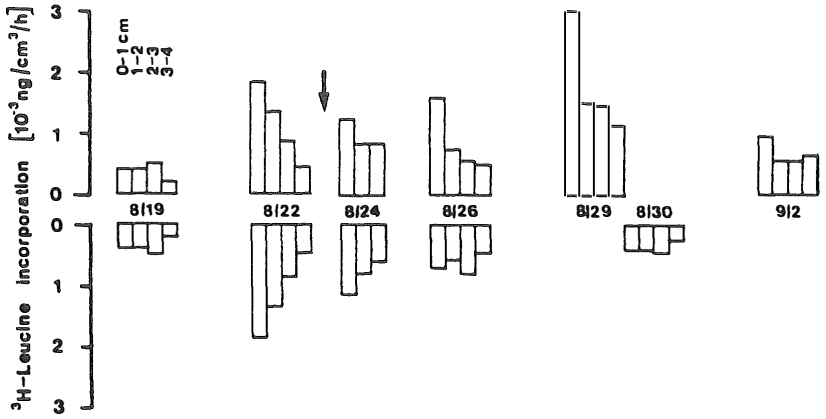

Fig. 3. Incorporation rates of tritiated leucine into microbial biomass in different horizons of untreated sediments (below) and sediments supplied with organic material (above). Arrow indicates the date of nutrient addition.

Calculations of carbon production rates based on the incorporation of ^{3}H-leucine showed that only 0.1 % of the carbon supplied was incorporated in microbial biomass. The unexpectedly low incorporation rates led to the hypotheses that during the initial breakdown only a minor part of the organic material was available for microbial decomposition processes or that a high proportion of the organic material was respired. The latter hypothesis is supported by results from epifluorescence microscopy which gave no indication of a significant increase in either microbial number or biomass after nutrient addition (Fig. 4).

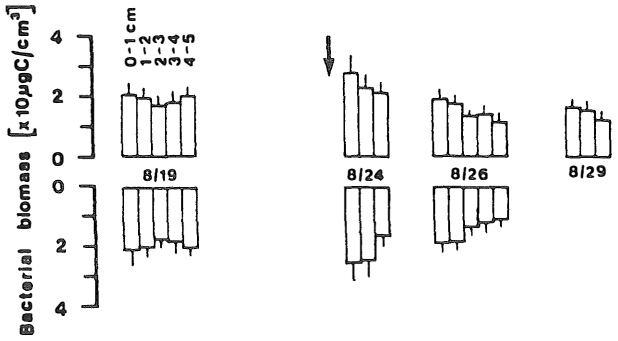

Fig. 4. Microbial biomass in different horizons of untreated sediments (below) and sediments supplied with organic material (above). Arrow indicates the date of nutrient addition.

From the analyses of concentration, enzymatic decomposition of organic material and uptake of hydrolysis products two different effects became obvious. Prior to nutrient addition, organic material accumulated in the untreated sedi-

ments. This effect will be referred to as "incubation effect". As possible explanations lysis of cells as well as redistribution or activation of nutrients caused by incubation have to be considered. The addition of "external" organic material to the sediments caused a second enrichment of organic material and a further stimulation of microbial activity referred to as "feeding effect". Organic matter was supplied after an "acclimatization" period of 4 days. At this time, the stimulation of microbial activity caused by the incubation reached its plateau, and then it slowly decreased. In the treated sediments, however, the decrease of the activity was compensated by a further stimulation of activity caused by the additional supply with organic material. This means that at the date of nutrient addition, the incubation effect competed with the feeding effect.

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References

HELDER, W., 1989. Early diagenesis and sediment-water exchange in the Savu Basin (Eastern Indonesia). Netherlands J. Sea Res. 24, 555-572.

KIRCHMAN, D., R.E. MURRAY and R.E. HODSON, 1986. Rates of DNA and protein synthesis by heterotrophic bacteria in aquatic environments: a comparison between the thymidine and leucine approaches. In: F. MEGUSAR and M. GANTAR (eds.), Perspectives in Microbial Ecology, Slovene Society for Microbiology, Ljubljana, 631-637.

KIRCHMAN, D., E. K'NEES and R. HODSON, 1985. Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. Appl. Environ. Microbiol. 49, 599-607.

KÖSTER, M., P. JENSEN and L.-A. MEYER-REIL, 1991. Hydrolytic activity associated with organisms and biogenic structures in deep-sea sediments from the Norwegian-Greenland Sea. In: R.J. CHROST (ed.), Microbial Enzymes in Aquatic Environments. Springer-Verlag, Berlin.

MEYER-REIL, L.-A., 1983. Benthic response to sedimentation events during autumn to spring at a shallow water station in the Western Kiel Bight. II. Analysis of benthic bacterial populations. Mar. Biol. 77, 247-256.

MEYER-REIL, L.-A., 1991. Ecological aspects of enzymatic activity in marine sediments. In: R.J. CHROST (ed.), Microbial Enzymes in Aquatic Environments. Springer-Verlag, Berlin.

MEYER-REIL, L.-A. and M. KÖSTER, 1991. Fine-scale distribution of hydrolytic activity associated with foraminifera and bacteria in deep-sea sediments of the Norwegian-Greenland Sea. In: Proc. 4th Europ. Mar. Microbiol. Symp., Kieler Meeresforsch., Sonderh. 8, 121-126.

MURRAY, J.W. and K.M. KUIVILA, 1990. Organic matter diagenesis in the northeast Pacific: transition from aerobic red clay to suboxic hemipelagic sediments. Geochim. Cosmochim. Acta 37, 59-80.

RICE, D.L., 1982. The detritus nitrogen problem: new observations and perspectives from organic geochemistry. Mar. Ecol. Progr. Ser. 9, 153-162.

SCHNÜRER, J. and T. ROSSWALL, 1982. Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. Appl. Environ. Microbiol. 43, 1256-1261.

THORN, P.M. and R.M. VENTULLO, 1988. Measurement of bacterial growth rates in subsurface sediments using the incorporation of tritiated thymidine into DNA. Microb. Ecol. 16, 3-16.

TURLEY, C.M. and K. LOCHTE, 1989. Microbial response to the input of fresh detritus to the deep-sea bed. Palaeogeogr., Palaeoclimatol., Palaeoecol. (Global Planet. Change Sect.), 89, 3-23.

WICKS, R.J. and R.D. ROBARTS, 1988. Ethanol extraction requirement for purification of protein labeled with ^{3}H-leucine in aquatic bacterial production studies. Appl. Environ. Microbiol. 54, 3191-3193.