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Diffusion scale dependent change in anaerobic carbon and nitrogen mineralization: True effect or experimental artifact?

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ABSTRACT

Burrowing macrofauna is known to influence the diffusion properties and solute transport within sediments as well as promoting aerobic microbial processes. It has recently been argued that changes in diffusion scales also affect anaerobic microbial processes in sediments. We tested this contention on coastal sandy sediment using a widely employed sediment plug flux-incubation technique. Our results confirm that volume specific microbial carbon and nitrogen mineralization apparently are enhanced (6–15 and 3–9 times, respectively) in thin (1 mm) compared with thick (10 cm) sediment plugs incubated in anoxic seawater. However, differential accumulation of inhibiting substances among plug sizes cannot explain this phenomenon, as the same pattern was observed for plugs incubated in anoxic porewater. Instead, we found that microbial activity in the overlying water and biofilms along the walls of incubation chambers affects the results significantly. A low, but constant production of 3–7 (TCO_2) and 0.5 (NH_4^+) nmol cm^{-3} d^{-1} in the water phase and 51–140 (TCO_2) and 4 –16 (NH_4^+) nmol cm^{-2} d^{-1} in biofilms irrespective of plug thickness can explain a large part of the apparent diffusion scale dependent change in volume specific solute production in the sediment. Model considerations show that sediment independent water phase and biofilm reactions occurring in the incubation chambers result in highly overestimated volume specific rates for thin plugs. The overestimate is negligible for plugs thicker than 1 cm because the total reactions occurring within these larger sediment plugs exceed those in the water and biofilms considerably. We therefore conclude that most of the apparent inverse relationship between diffusion scales and anaerobic microbial processes in sandy sediment found by the use of flux-incubation chambers is an experimental artifact rather than a true diffusion scale dependent effect.

1. Introduction

In recent years, extensive studies have elucidated that sediment processes are greatly influenced by bioturbating macrofauna (Aller, 1982; Kristensen, 2000; 2001; Aller *et al.,* 2001). Infaunal burrows and other biogenic structures within the sediment may change the otherwise unidirectional vertical redox succession to a mosaic of oxic and anoxic microniches at a mm spatial scale (Jørgensen, 1977; Fenchel, 1996). Benthic metabolism is typically enhanced several fold more than explained by the infauna itself, suggesting a

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stimulation of microbial activity (Kristensen, 1985; Kristensen *et al.,* 1992; Banta *et al.,* 1999).

Macrofaunal burrows affect the diffusional properties of sediments, by increasing the area of the sediment-water interfaces (up to 400%), and by changing metabolite exchange from molecular diffusion to irrigation-driven active transport (Fenchel, 1996; Aller and Aller, 1998; Furukawa *et al.,* 2001). As a consequence, microbial activity benefits from the exposure to frequent redox oscillations causing increased supply of electron acceptors and effective removal of inhibitory products (Aller, 1994; Hulthe *et al.,* 1998; Banta *et al.,* 1999; Kristensen and Hansen, 1999).

Only few studies have examined the effects of changing diffusion scales associated with infauna on anaerobic processes. Aller and Aller (1998) argued that volume specific anaerobic mineralization can be stimulated by decreased diffusion scales. From experiments with anaerobic sediment plugs of different thickness, they convincingly showed that net production of NH_4^+, TCO_2, Mn^{2+} and I^- as well as bacterial numbers were inversely related to the diffusion scales in the sediment. Other studies have also found indications of increased mineralization in anoxic sediment adjacent to the bioturbated zone (Aller, 1978; Aller and Yingst, 1985; Sun *et al.,* 1999).

Aller and Aller (1998) hypothesized three possible explanations for the diffusion scale effects. (1) Accumulation of inhibitory metabolites is prevented in the thin plugs due to increased diffusive contact between sediment and water. (2) The rate of organic matter decay (e.g. nutrient release) is determined by a first-order rate constant, k, while incorporation of nutrients into new biomass is described by Monod kinetics. Lower concentration of nutrients in diffusively open sediment, therefore, results in a lower uptake and thus a higher net release. (3) Concentration dependent abiogenic precipitation decreases with diffusion scale and thus results in higher net release from thin plugs.

The purpose of the present study was to evaluate diffusion scale dependent effects on anaerobic mineralization and to determine possible underlying mechanisms. Four independent experiments were conducted using the sediment plug flux-incubation technique of Aller and Mackin (1989). Anaerobic carbon and nitrogen mineralization was used to validate the effects observed by Aller and Aller (1998). Assays were also designed to test for experimental errors. The hypothesis is that even small concentration changes in the overlying water driven by processes other than those in the sediment will affect the calculated volume specific net production rates to the highest extent in thin plugs. Among possible processes, we examined the impact of bacterial activity in the water phase and in biofilms along the walls of incubation chambers.

2. Materials and methods

a. Study site and sediment handling

The sediment was collected at the shallow lagoon, Kærby Fed, in Odense Fjord, Denmark. The study site is a typical estuarine environment with large fluctuations in

salinity caused by nearby freshwater inputs and general tidal water movements (for more information consult Kristensen *et al.* (2003)). Sandy sediment was collected from 1 to 20 cm depth on several occasions and sieved through a 1 mm mesh to remove any macrofauna. The sediment was subsequently homogenized by hand.

b. Sediment characteristics

Wet density (g cm^{-3}) of the homogenized sediment was determined as the weight of 5 cm^3 sediment contained in a cut-off syringe. Water content was determined as weight loss after drying for 12 hours at 105°C. Organic content was determined as weight loss of dried sediment after combustion for 5 hours at 520°C.

The dimensionless linear adsorption coefficient for NH_4^+, K_{NH}, was determined by the enrichment method described by Holmboe and Kristensen (2002). Subsamples of sediment were incubated in slurries with different NH_4^+ concentrations (0, 0.75, 1.5 and 3 mM) followed by KCl extraction (Mackin and Aller, 1984). The exchangeable NH_4^+, K_{NH}^*, was determined as the slope of a plot of extracted NH_4^+ (μmol g^{-1} dry weight sediment) vs. porewater NH_4^+ concentration (μmol cm^{-3}). The dimensionless adsorption coefficient, K_{NH}, was obtained from: $K_{NH} = ((1 - \Phi)/\Phi) \rho_s K_{NH}^*$, where Φ is the porosity and ρ_s is the density of the sediment (Krom and Berner, 1980).

c. Experimental set up and sampling procedures

i. Plug incubations. All plug incubations were done in a Plexiglas "test-tank" embedded in a larger glass aquarium (Fig. 1A). The "test-tank" contained 11.4‰ artificial seawater and the glass aquarium was sealed gas-tight from the atmosphere with a glass lid. Anoxia in the aquarium was achieved by a constant flow of N$_2$ gas combined with continuous recycling of the gas head space through a Na$^+$-ascorbate solution ("O$_2$-trap"). Tests using 5 mg L^{-1} resazurin (Tratnyek *et al.,* 2001) proved that complete anoxia was achieved after 4–6 hours.

Sediment layers of variable thickness ("plugs") providing different diffusion scales were placed in circular Plexiglas chambers with a fixed bottom (i.d. 5.2 cm) (Fig. 1B). The height of the incubation chambers varied according to plug thickness to assure that the height of the overlying water was similar in all chambers (7.5 cm) regardless of plug thickness. The chambers were sealed with a magnetic stirring motor during flux-incubations, preventing contact between chamber and surrounding water. Chamber water was allowed to exchange with the surrounding water before and between incubations by inserting a perforated Plexiglas collar between the stirrer motor and the incubation chamber (Fig. 1C). Stirring was continuous throughout all experiments at a level well below the resuspension level.

Plugs were prepared by weighing the desired amount of homogenized sediment. The sediment was packed gently into an even layer in incubation chambers, avoiding gas spaces within the sediment. Subsequently, the chambers were carefully filled with artificial seawater and submerged into the "test tank" before the glass aquarium was sealed and
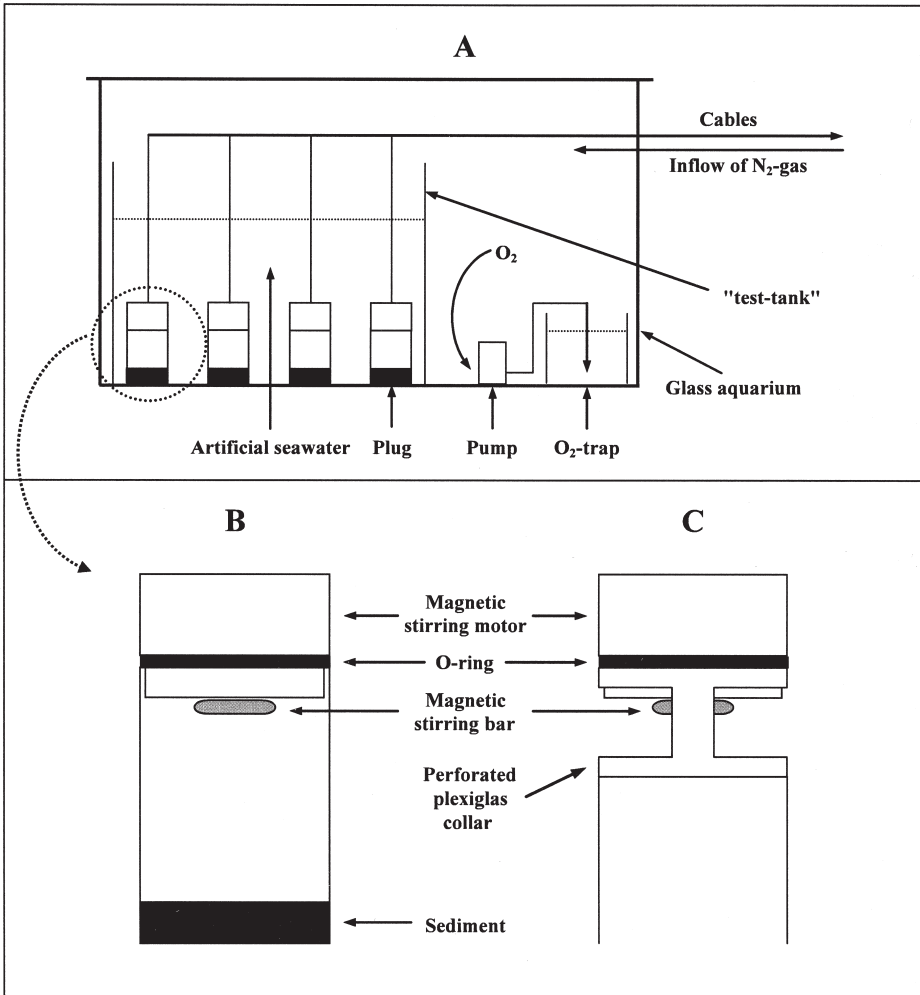
Figure 1. Experimental set up for plug experiments. (A) Incubation chambers are submerged in artificial seawater inside "test-tank," which is enclosed inside a larger glass aquarium. (B) Incubation chamber consisting of a closed bottom cylindrical plexiglass container sealed with O-ring capped magnetic stirring motor. (C) Insertion of a perforated plexiglas collar assures continuous contact between sediment in incubation chamber and surrounding water during pre-incubation/acclimatization.

flushed with N_2. The plugs were left in the dark at 15°C under anoxia for a period of days to weeks, depending on plug thickness and purpose, before the start of incubations.

 Flux-incubations were initiated by removing the lid of the glass aquarium briefly, while start water samples were taken from the overlying water in each incubation chamber. The samples were taken through the perforated collar. The collars were then removed, and the

incubation chambers sealed with stirring motors. The aquarium was closed again and flushed with N_2. Incubations were terminated by removing each chamber quickly from the "test-tank" and lifting the magnetic stirring motor briefly while end samples were taken. The chambers were subsequently returned to the "test-tank" where the collars were mounted. The entire sampling procedure for each chamber lasted between 30 and 60 s, resulting in a 10–12 min period, during which the aquarium was open to the atmosphere. Oxygen contamination was not considered a problem since the large 70 L reservoir of N_2 purged artificial seawater in the "test tank" acted as an O_2 buffer. Furthermore, each incubation chamber was only in direct contact with the atmosphere for a brief period of time (30–45 s).

ii. Jar incubations. In some experiments, the plug incubations were supplemented with a series of closed anoxic sediment incubations ("jar incubations") to establish closed system reaction rates. The jar incubations were performed by a method similar to that used by Kristensen and Hansen (1995). A number of jars consisting of 20 mL glass vials were carefully filled with homogenized sediment. Jars were closed with a screw cap, taped, and incubated head-down in anoxic sediment in the dark at 15°C.

Three jars were sacrificed at the time with 3 to 5 days interval by replacing the screw cap with a perforated cap containing a Whatman GF/F filter. Jars were placed head-down in centrifuge tubes, and porewater was extracted by centrifugation at 1500 rpm for 10 min. Porewater samples were stored for later analysis as described below.

d. The experiments

i. Experiment 1. A series of plug and jar incubations was conducted to validate the results observed by Aller and Aller (1998). Sediment was collected at Kærby Fed in late spring, and prepared as described above. Plugs with a sediment thickness of 0.1, 0.2, 0.5, 1, 5, and 10 cm were prepared to represent a wide range of diffusion distances. Three replicas were made of each thickness. The plugs were allowed to pre-incubate for 14 days before the start of the first flux-incubation. The 0.1, 0.2 and 0.5 cm plugs were incubated for 4 days, whereas the 1, 5 and 10 cm plugs were incubated for only 2 days. Incubations were conducted two times using the same plugs interrupted by a 2-day acclimation interval. The measured accumulation or removal of TCO_2 (H_2CO_3, HCO_3^-, CO_3^{2-}), NH_4^+ and DOC in the water phase were converted to apparent net volume specific sediment reaction rates (R_{app}, μmol cm^{-3} day^{-1}) according to:

$$R_{app} = \frac{(C_{pe} - C_{ps})V_w}{V_s t} \tag{1}$$

where, C_{pe} and C_{ps} are the final and initial concentration in the water phase, V_w is the volume of water in chambers (fixed at 159 cm^3) and V_s is the volume of sediment in plugs and t is the incubation time. It should be noted that R_{app}, in addition to sediment processes, also includes any reactions occurring in the water phase and biofilms along chamber walls.

The experiment was terminated after the second incubation, and the 5 and 10 cm plugs were sectioned for determination of vertical porewater profiles. Plugs were sliced in 0.5 cm intervals to 1 cm depth and 1 cm intervals below. Sediment was transferred to double centrifuge tubes and porewater was extracted by centrifugation at 1500 rpm for 10 min. The porewater was filtered through Whatman GF/F filters and samples were taken for TCO_2, NH_4^+ and DOC analysis. The contribution of solute changes in the porewater of plugs during incubations proved to be insignificant compared with changes in the overlying water (see "Results" and "Discussion" for more details) and is therefore ignored in Eq. (1) to avoid unnecessary complexity. Additional sediment subsamples were taken from each depth interval for enumeration of bacterial densities in the sediment.

Sediment used in the associated jar incubation series was identical to that of the plug incubations. However, the porewater SO_4^{2-} concentration was initially increased by 10 mM to avoid depletion during incubation. A total of 15 jars were prepared, and they were terminated as described above. The extracted porewater was analyzed for the same parameters as in the plug incubation. The closed system volume specific reaction rates (R_{sj}, μmol cm^{-3} day^{-1}) were calculated using the slope ($\Delta C/\Delta t$) from a plot of porewater solute concentration in jars vs. time:

$$R_{sj} = \Phi(1 + K)\,\frac{\Delta C}{\Delta t} \tag{2}$$

where, Φ is the porosity of the sediment and K is the dimensionless adsorption coefficient of the solute (Kristensen *et al.,* 1999).

ii. Experiment 2. Only plug incubations were conducted to investigate possible effects of inhibiting substances in the porewater on anaerobic microbial activity. An excessive amount of Kærby Fed subsurface sediment (3 to 10 cm depth) was collected in summer and centrifuged until a total volume of 2 L porewater was obtained. The extracted porewater was filtered through 0.2 μm filters, to remove fine particulate matter and frozen at $-20°$C until later use.

A total of 6 plugs were prepared for determination of R_{app}; 3 of 0.1 cm and 3 of 1 cm thickness. In addition, 3 incubation chambers were established without sediment for determination of solute changes caused by bacterial activity in both the water phase and in biofilms along walls ($R_w + R_b$, see below). The filtered porewater was thawed and purged with N_2 for 10 minutes before it was carefully transferred as the water phase to the 9 chambers, avoiding resuspension of sediment particles. The chambers were incubated for TCO_2, NH_4^+, DOC and DON fluxes in the "test-tank," as previously described. Incubations were terminated by the final sampling after 5 days. Chambers were quickly refilled with anoxic porewater and left for 2 days in the aquarium before a second flux-incubation identical to the first was conducted. Subsequently, a third flux-incubation was performed with porewater enriched by 7 mM DOC (Na-acetate). Final water samples were taken after each incubation for enumeration of bacterial abundance in the water phase.

iii. Experiment 3. Microbial activity along chamber walls (biofilm effect) and in the water phase was examined separately to differentiate possible effects on the apparent volume specific plug rates (R_{app}). The aim of this experiment was therefore to evaluate the three potential contributors to R_{app}: Sediment (R_{sp}), water (R_w) and biofilm (R_b).

Sediment was collected at Kærby Fed in winter and treated as previously described. Triplicate plugs were made with the thickness 0.1, 0.2, and 1 cm, whereas 9 plugs of 0.5 cm were made. The 5 cm and 10 cm plugs were excluded because Experiment 1 showed that values of R_{app} in these are comparable to R_{sj} obtained from closed jar incubations. All plugs were transferred to the "test-tank" where they were submerged in 70 L of artificial seawater (11.4‰) and allowed to pre-incubate for 3 days under anoxia.

Three identical 4-day flux-incubations, interrupted by 2-day acclimation intervals, were conducted on the same plugs to obtain R_{app} for TCO_2, NH_4^+, DOC and DON. At the end of each incubation, a separate water sample from each chamber was transferred to 10 mL pre-combusted bottles and incubated anaerobically for 4 days to determine water phase activity. The volume specific reactions in the water phase (R_w, $\mu mol\ cm^{-3}\ day^{-1}$) were calculated as:

$$R_w = \frac{(C_{we} - C_{ws})}{t} \qquad (3)$$

where C_{we} and C_{wa} are the final and initial concentration in the water phase. We assume that biofilm effects were negligible in the bottle incubations because of the short duration and lack of sediment contact.

After each plug incubation, three chambers containing 0.5 cm plugs were cleaned of all sediment by suction with a small silicone tube (removing sediment without resuspension and disturbance of possible biofilms). The cleaned incubation chambers were then incubated for 4 days as before to determine biofilm activity. The area specific biofilm activity along the chamber walls (R_b, $\mu mol\ cm^{-2}\ day^{-1}$) was calculated as:

$$R_b = \frac{(C_{be} - C_{bs})V_w}{tA_c} - \frac{R_w V_w}{A_c} \qquad (4)$$

where C_{be} and C_{bs} are the final and initial concentration in the water phase, and A_c is the total inner surface area of the empty incubation chambers (fixed at 165 cm^2, including both top and bottom).

A single series of jar incubations with homogenized sediment enriched by 10 mM SO_4^{2-} was conducted as described above to determine the closed system activity (R_{sj}).

iv. Experiment 4. The vertical distribution of bacteria along chamber walls near the sediment-water interface was determined to justify the presence of biofilms. Visual observations have previously indicated a distinct biofilm on chamber walls immediately above the sediment-water interface.

Plexiglas slides (length 8 cm; width 2 cm) were inserted into 8 plugs (four 0.5 cm plugs and four 3 cm plugs). Two slides were carefully inserted vertically into each of the plugs leaving at least 5 cm above the sediment-water interface. The plugs were submerged in the anoxic 70 L water reservoir of the "test-tank," and left undisturbed for 4 days. The water phase of the plug chambers was in contact with the shared water reservoir.

The slide incubations were terminated over a period of 4 days. The slides were removed from the sediment and gently cleaned on one side with paper tissue moistened with ethanol. They were left to dry in the dark for 10 min with the uncleaned side facing upward. The bacteria on the uncleaned side of the slides were stained with a DAPI (4,6-diamidino-2-phenylindole) solution (1 μg mL^{-1}), and left in the dark for 10 min. Subsequently, slides were gently washed in 0.2 μm filtered water and left to dry in the dark for about 30 min.

Bacteria on the slides were counted by epifluorescence microscopy. The counting effort was focused on the region near the sediment-water interface. The slides from the 0.5 cm plugs were counted vertically at -3, -1, 0, 1, 3, 5, 10 and 20 mm depth (0 represents the sediment-water interface, and $-$ indicates down into the sediment), whereas the slides from the 3 cm plugs were examined at -20, -10, -5, -3, -1, 0, 1, 3, 5, 10 and 20 mm depth. Bacterial densities were calculated as cells cm^{-2}.

e. Sample storage and analysis

TCO_2 samples from the plug experiments were stored at 5°C in 1.5 mL glass vials until analysis within 24 h by the flow-injection/diffusion-cell technique of Hall and Aller (1992). TCO_2 samples from the jar experiments were preserved with saturated $HgCl_2$ (9 : 1 by volume). All precipitate of HgS was removed by ultra-centrifugation (10,000 rpm) for 10 min and the supernatant was transferred to 1.5 mL glass vials and stored at 5°C until analysis within 2–3 days.

NH_4^+ samples were stored at -20°C in 10 mL plastic vials and analyzed by the salicylate-hypochlorite method as described by Bower and Holm-Hansen (1980). Samples for DOC and DON were stored at -20°C in 1.5 mL glass vials that were pre-combusted for 5 hours at 520°C. DOC of acidified (2 M HCl) samples was analysed on a Shimadzu TOC-5000 Total Organic Carbon analyzer. DON was determined by subtracting NH_4^+ concentrations from total dissolved nitrogen (TDN) analyzed on a Dohrmann DN-1900.

Samples for enumeration of bacterial populations were preserved by transferring about 40 mg of sediment to 1.5 mL Eppendorf tubes containing 1 mL of 5 vol% glutaraldehyde in 10‰ seawater. Samples were stored at 5°C and enumerated by the direct count approach on DAPI stained subsamples (Porter and Feigh, 1980). Briefly, sediment samples were diluted with 0.2 μm filtered water to a final volume of 1.5 mL and bacteria were detached from particles by sonication (Andresen and Kristensen, 2002). The sample was stained with DAPI (1 μg mL^{-1}), filtered onto a 0.2 μm black polycarbonate filter ($d = 25$ mm) and counted by epifluorescence microscopy. Bacterial densities were calculated as cells cm^{-3}, assuming that all bacteria were detached from the sediment during sonication.
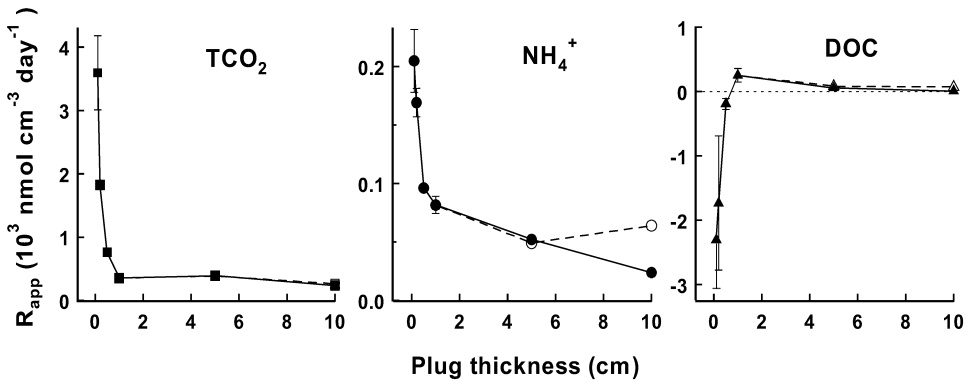
Figure 2. Experiment 1. Apparent volume specific reaction rates (R_{app}) of TCO_2, NH_4^+ and DOC as a function of plug thickness. Positive values represent solute production. The broken line indicates rates where porewater accumulation is included (see text for explanation). Error bars indicate SE ($n = 6$).

f. Statistical methods

Results from the plug experiments were tested statistically, first by ANOVA. If the H_0 hypothesis was rejected, the data were grouped with Tukeys Test. The level of significance α is 0.05 for all tests. Statistical methods are described in Zar (1999).

3. Results

a. Sediment characteristics

The sediment at the sampling site was rich in polychaete (*Nereis diversicolor*) and crustacean (*Corophium volutator*) burrows in the upper 5–10 cm. The upper oxidized zone was 0.3–0.5 cm thick, while burrows were surrounded by 0.2–0.3 cm oxidized sediment. The sediment between burrows was greyish black, and solid black with a distinct sulfide odor beneath the bioturbated zone. The sediment turned similarly black from top to bottom in all experimental plugs during incubations with a distinct sulfide odor at the end.

The organic poor (1% loss on ignition) sediment used in all experiments consisted of sand with a median grain size of 160 μm and a silt + clay content of 4%. The water content was 20% and the porosity was 0.38. The dimensionless adsorption coefficient for NH_4^+, K_{NH}, was 0.71 in the homogenized sediment.

b. Experiment 1—Effect of plug thickness on reaction rates

The apparent volume specific sediment reaction rates (R_{app}) involving TCO_2, NH_4^+ and DOC showed a strong inverse relationship with plug thickness and thus diffusion distance (Fig. 2). TCO_2 and NH_4^+ were generated, whereas DOC generally was consumed by the sediment. $R_{app}(TCO_2)$ was increased 15 times (from $2.4 \cdot 10^2$ to $3.6 \cdot 10^3$ nmol cm^{-3} day^{-1}), whereas $R_{app}(NH_4^+)$ increased 9 times (from 23 to 205 nmol cm^{-3} day^{-1}), when the
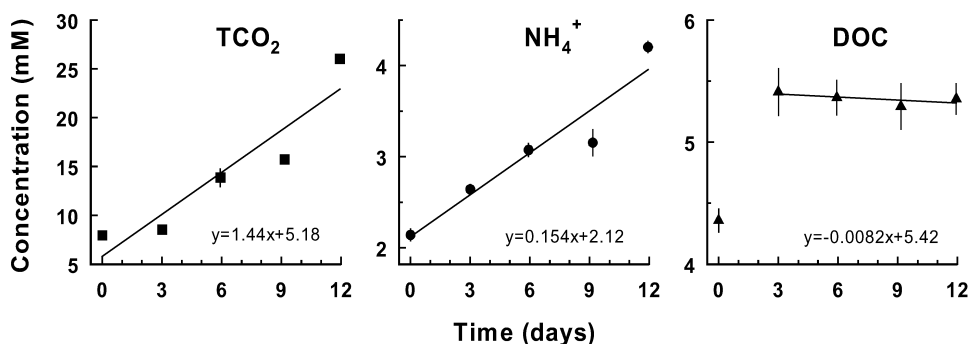
Figure 3. Experiment 1. Time dependent linear changes in TCO_2, NH_4^+ and DOC concentration in the closed anoxic jar incubations. Equations represent least squares linear regressions. Error bars indicate SE ($n = 3$).

maximum diffusion distance was lowered from 10 to 0.1 cm. The increase in TCO_2 and NH_4^+ reaction rates was most conspicuous for plugs thinner than 1 cm, and statistically significant only for 0.1 and 0.2 cm plugs. R_{app} was related by a power function to plug thickness ($r^2 = 0.85$–0.96) with comparable exponents (-0.54 for TCO_2 and -0.43 for NH_4^+), indicating that similar mechanisms may control the relationships for both solutes. R_{app}(DOC) in the 0.1 and 0.2 cm plugs was also significantly larger than the reaction rates in the thicker plugs.

The closed jar incubations exhibited linear time dependent change in TCO_2, NH_4^+ and DOC (Fig. 3). R_{sj} for TCO_2, NH_4^+ and DOC were 547, 58.5 and -3.1 10^{-3} nmol cm^{-3} day^{-1}, respectively, and were for TCO_2 and NH_4^+ about twice the R_{app} estimated for 10 cm plugs.

Porewater profiles of TCO_2 increased with depth until -2.5 cm (to about 5 mM in 5 cm plugs and 10 mM in 10 cm plugs) followed by a gradual decrease below (Fig. 4). Porewater NH_4^+ on the other hand increased steadily from the sediment surface and downward, reaching about 1.5 mM in the bottom of the 5 cm plugs and 3 mM in the 10 cm plugs. Porewater DOC increased more or less linearly from top to bottom in all plugs, reaching about 8 mM and 14 mM in 5 and 10 cm plugs, respectively. A maximum estimate of porewater contribution to the volume specific reaction rates can only be provided by assuming constant build up (not unrealistic) of porewater solutes during the 20-day period from plug establishment to the final slicing. Using the initial porewater concentrations of TCO_2 (4.10 mM), NH_4^+ (1.26 mM) and DOC (4.4 mM), the depth integrated accumulation of solutes within the 5 and 10 cm plugs corresponds to maximum rates of -3.9 and 24.8 nmol cm^{-3} day^{-1} for TCO_2, -3.1 and 40.1 nmol cm^{-3} day^{-1} for NH_4^+, and 28.2 and 61.8 nmol cm^{-3} day^{-1} for DOC. Thus, porewater accumulation potentially contributed to reaction rates only in 10 cm plugs, and significantly only for NH_4^+ (Fig. 2). Porewater contribution was negligible in the thinner plugs since steady state was achieved within a few days (see the Discussion).
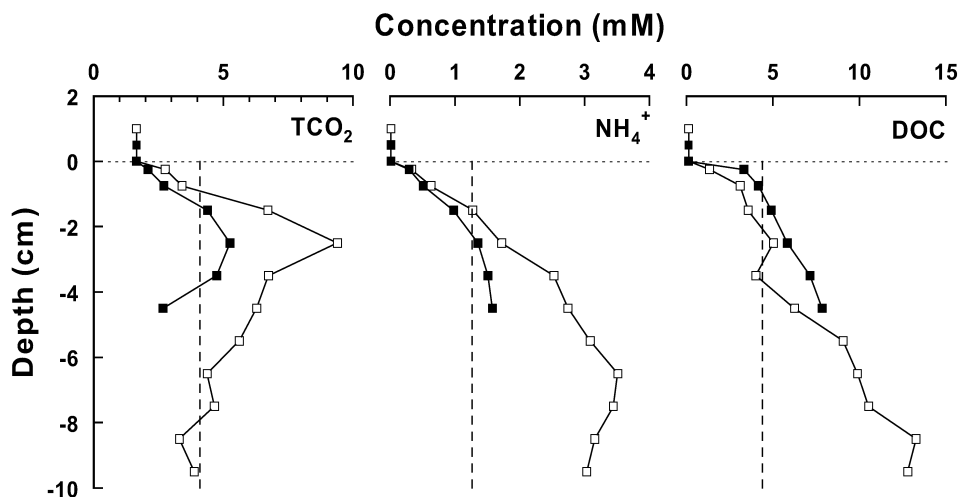
Figure 4. Experiment 1. Final porewater profiles of TCO_2, NH_4^+, and DOC in 5 cm (solid symbols) and 10 cm (open symbols) plugs.

Bacterial abundance in plug sediments varied from 1.5 to 2.5 10^9 cells cm^{-3}, with a decreasing depth trend (Fig. 5). The variability (not shown) was large and no significant differences were observed with depth and among different plug sizes.

c. Experiment 2—Effect of plug thickness on reaction rates in porewater

The overall trend of apparent reaction rates for plugs submerged in porewater (Fig. 6) was similar to that observed for plugs incubated in seawater (Fig. 2). $R_{app}(TCO_2)$ was 3–5 times higher in 0.1 cm plugs than in 1 cm plugs, and were generally higher in the first than in the second incubation series. $R_{app}(TCO_2)$ increased again after the addition of DOC to the porewater in the third incubation series. The correction for solute changes due to water phase and biofilm activity generally depressed $R_{app}(TCO_2)$. The effect was more pronounced for 0.1 cm plugs (22–46%) than 1 cm plugs (10–19%).

NH_4^+ production showed almost the same pattern, with 3–4 times higher $R_{app}(NH_4^+)$ in 0.1 cm plugs than in 1 cm plugs. $R_{app}(NH_4^+)$ generally decreased throughout the 3 successive incubation series to 41% in 0.1 cm plugs, and 19% in 1 cm plugs. Accordingly, $R_{app}(NH_4^+)$ was not significantly affected by the addition of DOC. Correction for water phase and biofilm activity, on the other hand, caused a general 30–70% decrease in $R_{app}(NH_4^+)$.

$R_{app}(DOC)$ varied considerably, both within and among incubation series, and shifted between release and uptake in both plug sizes. DOC release was only observed for uncorrected plugs of the first incubation series. Otherwise, the sediment consumed DOC from the overlying water. Rates were generally higher for 0.1 cm plugs than 1 cm plugs. Correction for water phase and biofilm activity always resulted in higher DOC uptake. No DON was present in any of the samples as all TDN consisted entirely of NH_4^+.

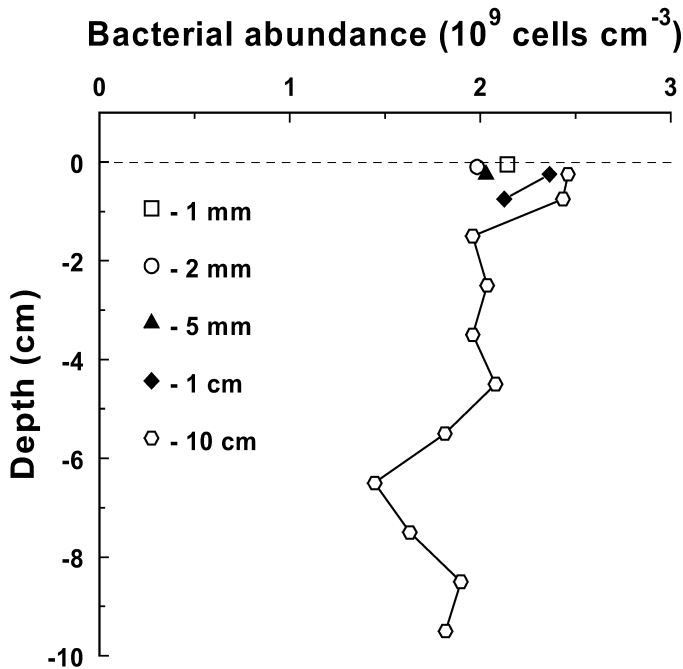## Bacterial abundance (10^9 cells cm^{-3})

Figure 5. Experiment 1. Final vertical distribution of bacterial abundance in plugs of various thickness. Each datapoint represents the average of 6 counts (error bars not shown).

Final bacterial abundance in the porewater overlying sediment plugs and in the control chamber ranged from 3.9 to 5.4 10^6 cells mL^{-1}, and was comparable to the bacterial numbers found initially in the porewater (Table 1).

d. *Experiment 3—Effects of biofilm and water phase activity on plug rates*

The apparent reaction rates in plugs of Experiment 3 showed the same diffusion scale dependent relationship (Fig. 7) as observed in the previous plug experiments. However, there appeared to be an attenuation of particularly $R_{app}(TCO_2)$ and $R_{app}(DOC)$ during the three successive incubations with respect to absolute rates, but not the diffusion scale dependence. Thus, $R_{app}(TCO_2)$ was always 3–5 times higher in 0.1 cm plugs than 1 cm plugs. The diffusion scale dependence of $R_{app}(NH_4^+)$ from 0.1 to 1 cm was 1.7–2.6 times but appeared more linear than previously observed, particularly for incubation 1 and 2. DOC and DON were consumed by the sediment in a pattern that mirrored TCO_2 and NH_4^+ production.

Estimated reaction rates, R_{sj}, in the closed jar experiment were 319, 74.9, 4.44 and 7.98 nmol cm^{-3} day^{-1} for TCO_2, NH_4^+, DOC and DON, respectively. R_{sj} was of similar magnitude to, but always lower than R_{app} in the 1 cm plugs for TCO_2 and NH_4^+ (480–1027 and 105–140 nmol cm^{-3} day^{-1}, respectively). R_{sj} for DOC and DON were not compa-
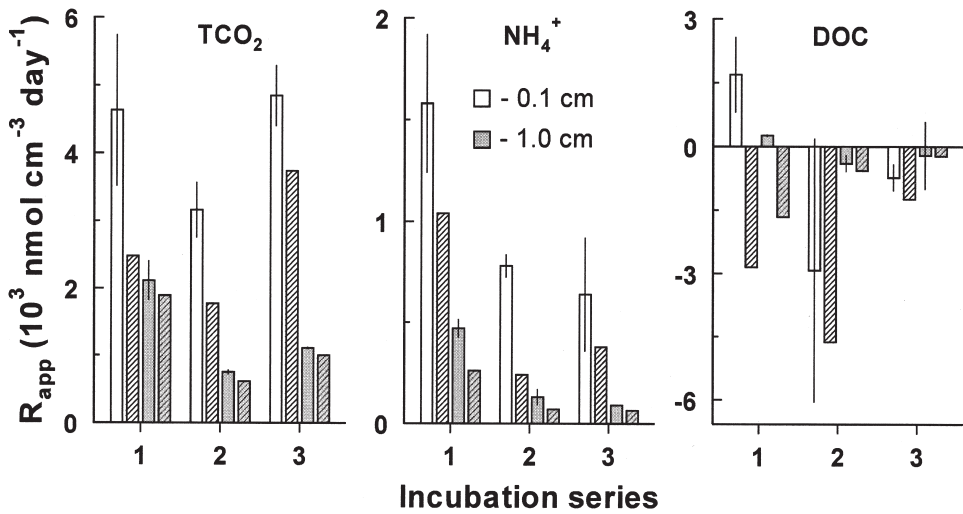
Figure 6. Experiment 2. Apparent volume specific reaction rates (R_{app}) in 0.1 cm (open bars) and 1 cm (gray bars) plugs from three successive incubation series with porewater as the overlying water phase. The overlying water was enriched by 7 mM DOC in the third incubation series. Hatched bars represent rates corrected for reactions in the water phase (and biofilms). Positive values represent solute production. Error bars indicate S.E. ($n = 3$, not shown for corrected rates).

rable to R_{app} determined from plugs as R_{sj} for both compounds showed a slow, but continuous production.

Solute transformations in the overlying water (R_w) were generally small and varied in magnitude for TCO_2 and NH_4^+ with no specific pattern during successive incubations (Fig. 8). Actually, the water activity was on several occasions close to zero. The average $R_w(TCO_2)$ and $R_w(NH_4^+)$ were 5.9 and 0.16 nmol cm^{-3} day^{-1}, respectively. DOC on the other hand, was produced at a rate of 35–78 (average 45) nmol cm^{-3} day^{-1} with highest rates in water overlying the thinner plugs of the first incubation. No DON was present, as all TDN consisted entirely of NH_4^+.

Reaction rates in the biofilm (R_b) along the walls of the emptied incubation chambers showed no specific pattern between incubations and plug thicknesses, except

Table 1. Experiment 2. Bacterial abundance in porewater before (unused) and after (0.1 cm, 1 cm and control) plug incubations. The standard error (SE) of counts is presented ($n = 6$).

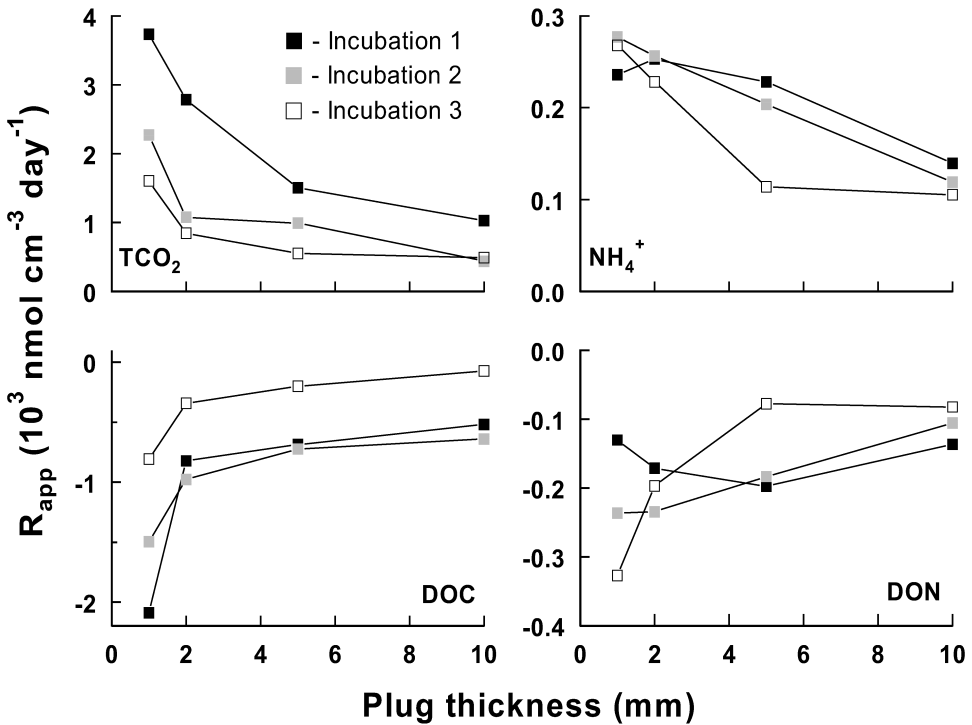|	Cells 10^6 cm^{-3}	SE
Unused porewater	4.42	0.24
0.1 cm	3.86	0.25
1 cm	5.10	0.54
Control	5.40	0.58

Figure 7. Experiment 3. Apparent volume specific reaction rates (R_{app}) of TCO_2, NH_4^+, DOC and DON as a function of plug thickness. Results are shown for three successive incubations. Positive values represent solute production.

for elevated rates during the first incubation of 0.5 cm plugs (Fig. 8). $R_b(TCO_2)$ in the biofilm ranged between 50 and 140 (average 97) nmol cm^{-2} day^{-1}, while $R_b(NH_4^+)$ varied from 4 to 16 (average 8) nmol cm^{-2} day^{-1}. $R_b(DOC)$ was -70 to -160 (average: -109) nmol cm^{-2} day^{-1}), whereas no DON was detected.

e. Experiment 4—Bacterial distribution along walls

A pronounced vertical distribution of bacteria was evident on slides inserted into the sediment (Fig. 9). Bacterial abundance was up to 7 times higher immediately above the sediment-water interface than below. The average bacterial numbers ranged from 2 10^5 cells cm^{-2} below the sediment to 15–20 10^5 cells cm^{-2} just above the sediment. Farther up in the water column, the abundance decreased slightly to 10–15 10^5 cells cm^{-2}. The distribution of bacteria on slides from 3 cm plugs was more consistent and with less variation between individual counts than from 0.5 cm plugs. Bacterial density above the sediment-water interface appeared to increase as the experiment progressed, whereas no such temporal pattern was evident below the interface. The time dependent changes in bacterial numbers were, however, not statistically significant.
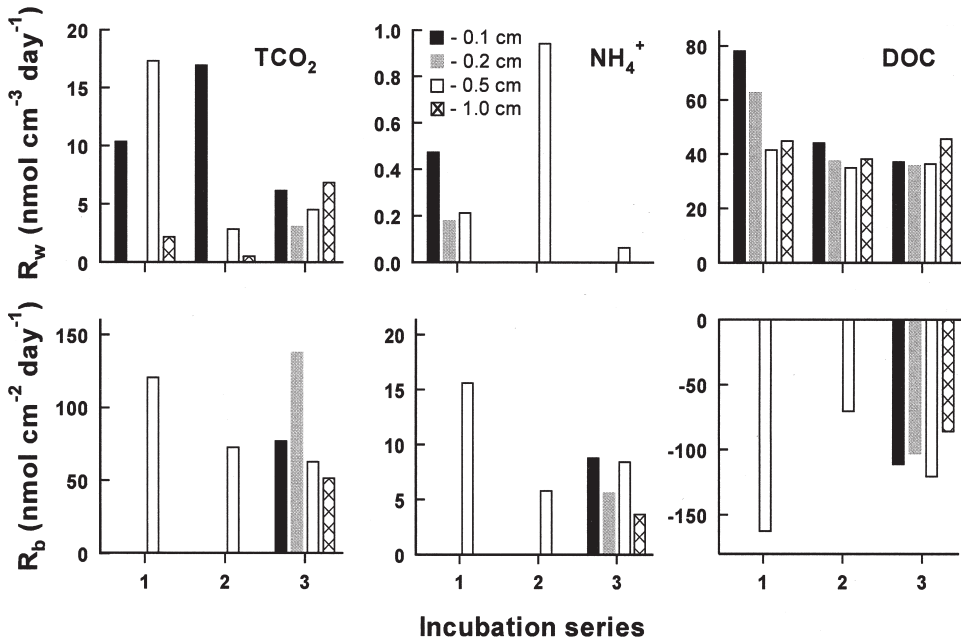
Figure 8. Experiment 3. Reaction rates in the overlying water phase (R_W) and biofilms along chamber walls (R_b) from three successive incubation series in clean bottles and chambers without sediment, respectively. The biofilm activity is corrected for water phase activity. Biofilm activity was not determined in 0.1, 0.2 and 1 cm plug chambers during the first two incubation series. Positive values represent solute production.

4. Discussion

a. Methodological considerations

Sieving and homogenization of sediment destroy the original chemical profiles, and may dramatically alter microbial biomass and metabolic activity in the sediment (Findlay *et al.,* 1990). It has been suggested that as much as 85 days are needed to restore chemical and microbial profiles in disturbed sediment microcosms (Gilbert *et al.,* 1995). Such long restoration time is not realistic for laboratory microcosms, because most of the available organic substrates will be exhausted. The pre-incubation time in the present study was, therefore, chosen to be 2–14 days, and the results obtained cannot be extrapolated to *in situ* conditions but should solely be considered for comparative purposes. However, pre-incubation is vital for our anoxic experiments which are aimed for examining only hydrolysis/fermentation and SO_4^{2-} reduction. All available pools of other electron acceptors (Fe and Mn oxides and NO_3^-) must, therefore, be depleted from the sediment during pre-incubation.

Pre-incubation also allows the chemical profiles in the sediment plugs to approach a diffusion-controlled state, particularly in the thinner plugs (Kristensen and Hansen,
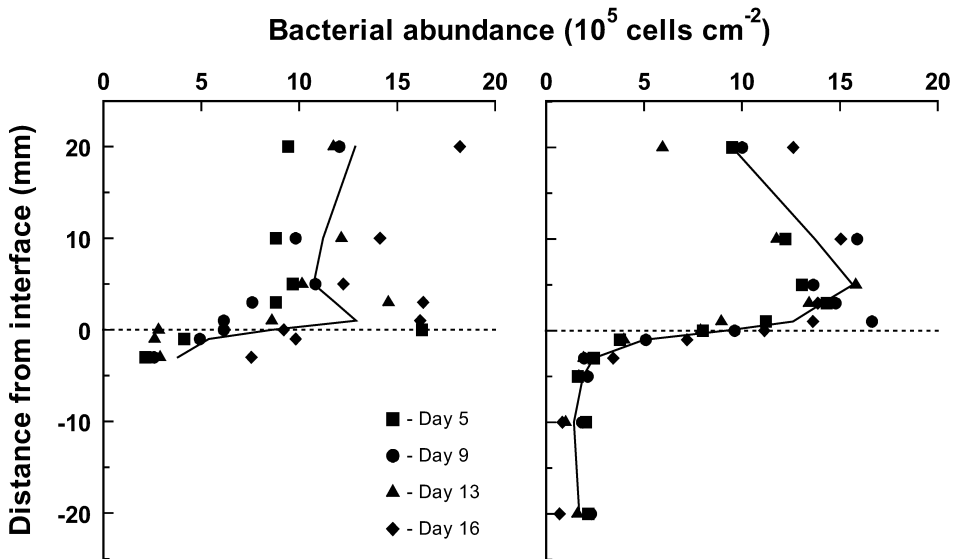
Figure 9. Experiment 4. Vertical distribution of bacterial abundance on slides inserted vertically into
    0.5 cm plugs (left) and 3 cm plugs (right). The solid line represents the floating average of all
    counts. The dashed line indicates the sediment-water interface.

1995). According to Aller and Mackin (1989), steady state can be assumed in our 5 cm plugs for both HCO_3^- and NH_4^+ after 4 weeks of pre-incubation, while the 10 cm plugs need longer time to reach steady state. This prediction seems to be valid for NH_4^+ in Experiment 1 as indicated by the shape of the porewater profile in 5 cm plugs (Fig. 4). However, the subsurface peak of TCO_2 in these plugs implies that steady state is not achieved for this solute. DOC profiles, on the other hand, show a steady and almost linear increase from top to bottom indicating that no reactions and only diffusion affects DOC in the sediment. Thus, the DOC consuming SO_4^2 reduction process is most likely inhibited by SO_4^{2-} availability in the deeper sediment due to low salinity ($\sim 5\permil$) at Kærby Fed at the time of sediment sampling. This is substantiated by the difference between $R_{app}(TCO_2)$ of the 10 cm plugs and $R_{sj}(TCO_2)$ of the SO_4^{2-} enriched jars (238 vs. 547 $nmol\,cm^{-3}\,day^{-1}$) in Experiment 1. Inhibition of SO_4^{2-} reduction only depresses DOC consumption and TCO_2 production, but not NH_4^+ production that is produced by fermentation and as such, uncoupled from carbon oxidation (Kristensen and Hansen, 1995). It is therefore, in our opinion, justified to ignore solute accumulation in the porewater of plugs ≤ 5 cm when R_{app} is estimated by Eq. (1). The contribution from porewater accumulation is also small in 10 cm plugs. If sediment reaction rates (R_{app}) in these plugs are corrected for linear accumulation of porewater TCO_2 and NH_4^+ during the entire experimental period, it will only affect $R_{app}(NH_4^+)$ significantly, bringing it in compliance with rates estimated from 5 cm plugs and jars (Fig. 2).

A significant fraction of the TCO_2 generated in plug chambers may originate from DOC oxidation in the water phase and biofilms rather than DOC produced within the sediment as indicated by a high apparent DOC uptake in thin plugs. Since all plugs shared the same water reservoir, the thick plugs may have acted as a DOC source to the thin plugs. When TCO_2 production is corrected for DOC uptake the increase in volume specific reaction between thin and thick plugs of Experiment 1 reduces from a factor of 15 to 5.5. When a similar correction is made for 0.1 cm plugs in Experiment 3, the relative increase in volume specific TCO_2 production compared with jars reduces from 12, 7 and 5 to 5, 2.5 and 2.5 in incubation 1, 2 and 3 respectively.

b. The role of porewater based inhibitors on diffusion scale dependent reaction rates

At first glance, our plug experiments substantiate an apparent inverse relationship between diffusion distance and volume specific reaction rates (R_{app}) of TCO_2 and NH_4^+ (Fig. 2, 6 and 7) as previously shown by Aller and Aller (1998). Heterotrophic carbon oxidation in closed anoxic sediments may be affected negatively by several factors, such as metabolite inhibition (e.g. S^{2-} and H_2) or product inhibition by negative feedback (Koster, 1988; Schink, 1988; Canfield, 1994). Differential accumulation of inhibiting substances in plugs of different thickness is therefore a likely explanation for the observed diffusion scale dependent changes in volume specific sediment reaction rates. However, porewater from Kærby Fed sediment is apparently low in inhibitory components as shown by the high reaction rates in plugs incubated in porewater (Experiment 2, Fig. 6). Both the magnitude and diffusion scale dependence of $R_{app}(TCO_2)$ in these plugs are comparable to those obtained in 0.1 and 1 cm plugs incubated in artificial seawater (Experiment 1 and 3). The decreasing rates observed through time are instead caused by depletion of labile organic substrates (Kristensen *et al.,* 1995; Hulthe *et al.,* 1998). This is substantiated by the restoration of carbon oxidation to the initial level after addition of labile DOC (acetate).

The lack of inhibition of microbial reactions in thin plugs exposed to porewater may be due to counteractive stimulation of heterotrophic activity fueled by the much higher DOC available in porewater compared with artificial seawater (1.5 mM compared with 0.1 mM). The lack of inhibition could also be caused by handling and freezing of porewater prior to the incubation, which may have removed inhibitory substances. Freezing and thawing procedures are known to affect the chemical composition of water samples (Chapman and Mostert, 1989; Dore *et al.,* 1996). These latter mechanisms were not investigated here, but deserve more attention in future studies.

c. Are experimental artifacts responsible for the diffusion scale dependence of reactions?

The lack of any experimentally proven mechanisms for the apparent diffusion scale dependent sediment reaction rates led us to shift our focus to possible experimental errors. The most obvious problem in our experiment is the involvement of processes occurring in the water phase and in biofilms along chamber walls above the sediment. The available knowledge on water column and biofilm processes as a potential source of error in

measurements of sediment-water fluxes in microcosms is scarce, but particularly biofilms are known to have high metabolic activity. Thus, it has been estimated that a 1200 μm thick wastewater biofilm can consume 5000 nmol O_2 cm^{-2} day^{-1} (Santegoeds *et al.,* 1998). Similarly, a 350 μm thick trickling filter biofilm in tap water without organic amendments is capable of consuming 300 nmol O_2 cm^{-2} day^{-1} (Horn and Hempel, 1997).

Although reaction rates in the overlying water and biofilms along walls in our plug chambers are relatively low, they are nevertheless sufficient to alter the estimated volume specific reactions in the sediment significantly. Corrections based on TCO_2 production of 20 to 60 nmol cm^{-3} day^{-1} caused by reactions in the water and biofilm in Experiment 2 results in 20–50% lower carbon oxidation in 0.1 cm plugs and 10–20% lower in 1 cm plugs (Fig. 6). Similarly, correction for water phase and biofilm NH_4^+ production of 5–10 nmol cm^{-3} day^{-1} reduces N mineralization in 0.1 cm plugs by 30–50%. Thus, thin plugs are obviously most vulnerable to this type of error since estimates of volume specific reaction rates for these are based on small concentration changes in the overlying water.

Artifacts due to microbial activity in the water phase and biofilms on walls were examined more closely in Experiment 3. The microbial activity of water (Fig. 8) is relatively low with TCO_2 and NH_4^+ production in the range of 0–16 and 0–0.9 nmol cm^{-3} day^{-1}. There is no obvious correlation between plug thickness and TCO_2 production in chamber water. However, the DOC production of 35–78 nmol cm^{-3} day^{-1} is puzzling, since no known source of DOC should be present in the water. It is possible that DOC is released by starved and dying bacterial cells. A similar "nutrient flush" has been observed by bacterial communities in response to dramatic changes in the surrounding environment (Aller, 1994). Microbial activity in biofilms on chamber walls is more pronounced and consistent than, and in some cases strongly counteract (e.g. DOC), processes in the water phase (Fig. 8). Thus, biofilm solute transformations normalized to chamber surface area range between 51 and 140, 3.9 and 16 and −70 to −160 nmol cm^{-2} day^{-1} for TCO_2, NH_4^+ and DOC, respectively. Experimental artifacts due to microbial activity in the water and along walls are therefore not a phenomenon restricted to situations where porewater was used the water phase (Experiment 2), but seem to be a general source of error in the present type of incubation.

Biofilm activities measured in emptied chambers may be different from those obtained in chambers with sediment. New wall surfaces are exposed when the sediment is removed. The wall surface area of empty chambers previously containing 0.1, 0.2, 0.5 and 1 cm plugs is increased by 17, 18, 20 and 22.5%, respectively. The role of these additional surfaces is, however, difficult to evaluate. Visual observations from chambers containing sediment revealed a distinct biofilm near the sediment-water interface that extended a few mm above, but not below the sediment-water interface. The bacterial counts (Fig. 9) confirm that bacteria in the biofilm are not distributed evenly. In chambers containing the 3 cm plugs, bacterial abundance is 7 times higher just above the interface than 1 cm into the sediment. Biofilms along chamber walls are therefore restricted to the water exposed surfaces above the sediment, particularly near the interface. If wall rates are proportional to
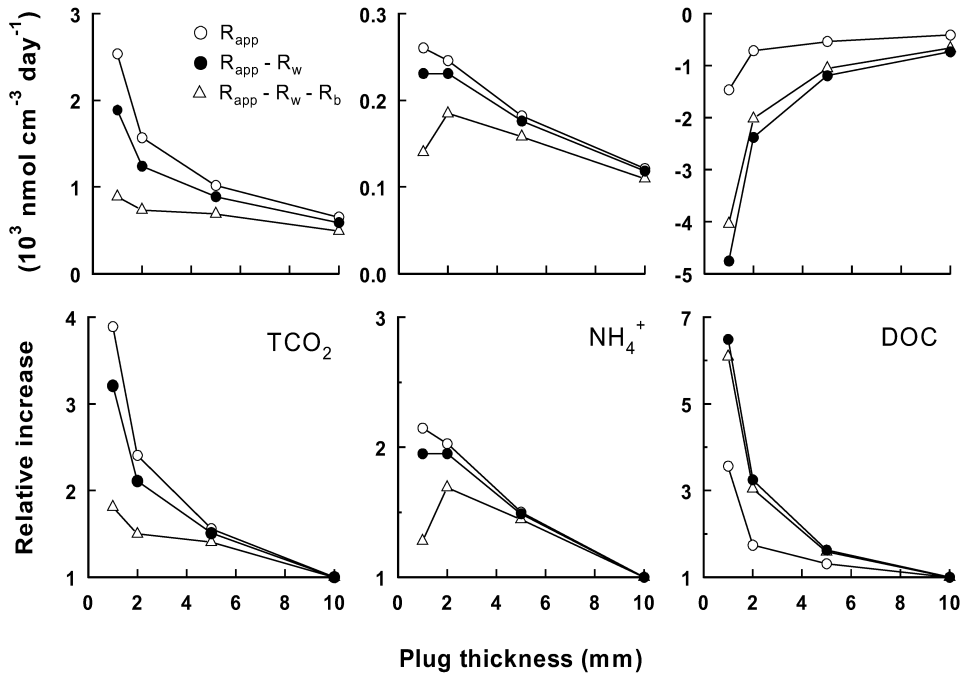
Figure 10. Experiment 3. Upper panel: Volume specific sediment reaction rates of TCO_2, NH_4^+ and DOC as a function of plug thickness. Lower panel: Increase in reaction rates as a function of plug thickness relative to the rate in 1 cm plugs. Open circles represent the measured apparent rate (R_{app}), while closed circles represent apparent rates corrected for water phase reactions ($R_{app} - R_W$) and triangles represent apparent rates corrected for both water phase and biofilm reactions ($R_{app} - R_W - R_b$). Positive values indicate solute production.

bacterial abundance, however, the new area exposed by removing sediment in Experiment 3 will result in underestimates of the mean area specific biofilm activity by no more than 3–4%. Our correction of plug rates with area specific biofilm activities measured in emptied chambers therefore seems to be justifiable. We have no specific explanation for the high bacterial abundance just above the sediment-water interface, except for a possible upward migration of interface bacteria, which are fed by nutrients diffusing out of the sediment.

If the measured water and biofilm contributions of Experiment 3 are converted to volume specific sediment rates and subtracted from the rates in the corresponding plugs, the diffusion scale dependent effect on reaction rates for TCO_2 and NH_4^+ is reduced considerably (Fig. 10). It appears, therefore, that much of the apparent diffusion scale dependent changes in mineralization rates for our sediment can be explained by experimental artifacts due to microbial activity in the water phase and biofilms along walls of incubation chambers. DOC consumption is increased much when corrected for the water contribution, which is caused by the surprising and

unexplainable high DOC generation in the water (Fig. 8). Corrections must therefore be applied with caution, since the water and biofilm rates are calculated from small changes in solute concentrations. The sensitivity can probably be increased by employing longer incubation periods, but this will enhance the risk of exhausting the already low levels of substrate available in the water. The present approach to determine errors caused by processes in water and biofilms are therefore a compromise between experimental reliability and analytical precision. Anyway, the general consistency of the primarily biofilm data indicates that experimental errors contribute to the estimated high sediment reaction rates in thin plugs.

A more general evaluation of water and biofilm effects on the estimated diffusion scale dependence of sediment reactions can be made using simple volumetric considerations. The basic parameter measured in plug experiments is the concentration change, $\Delta C/\Delta t$ (μM day^{-1}, equivalent to R_{app}), of a given solute in the water phase, which is controlled by reactions within the sediment (R_{sp}, nmol cm^{-3} day^{-1}), in biofilms along chamber walls not covered with sediment (R_b, nmol cm^{-2} day^{-1}) and in the water phase (R_w, nmol cm^{-3} day^{-1}) as well as by the sediment plug thickness (j, cm) and chamber dimensions (radius, r (cm) and water column height, h (cm)). Accordingly, the concentration change can, by proper conversion of units, be described as:

$$\Delta C/\Delta t = [R_{sp}\pi r^2 j + R_b(\pi r^2 + 2\pi rh) + R_w\pi r^2 h]/\pi r^2 h \tag{5}$$

which reduces to:

$$\Delta C/\Delta t = R_{sp}j/h + R_b(r + 2h)/rh + R_w. \tag{6}$$

For our purpose here, we must consider the following two end-member cases:

Case 1. The apparent diffusion scale dependence of sediment reaction rates is caused by experimental artifacts. The volume specific sediment reaction rate, R_{sp-fix}, is therefore independent of sediment thickness. The apparent diffusion scale dependence is derived exclusively from biofilm and water phase reactions according to:

$$\Delta C/\Delta t = R_{sp-fix}j/h + R_b(r + 2h)/rh + R_w. \tag{7}$$

Thus, Eq. (7) describes the concentration change of solute in the water phase, when specific reactions in sediment, water and biofilms are all independent of sediment thickness.

Case 2. The diffusion scale dependence of sediment reaction rates is a true effect. The apparent volume specific reaction rate, R_{app}, increases with decreasing diffusion distance. The biofilm and water phase reactions are in this case assumed negligible and can be ignored according to:
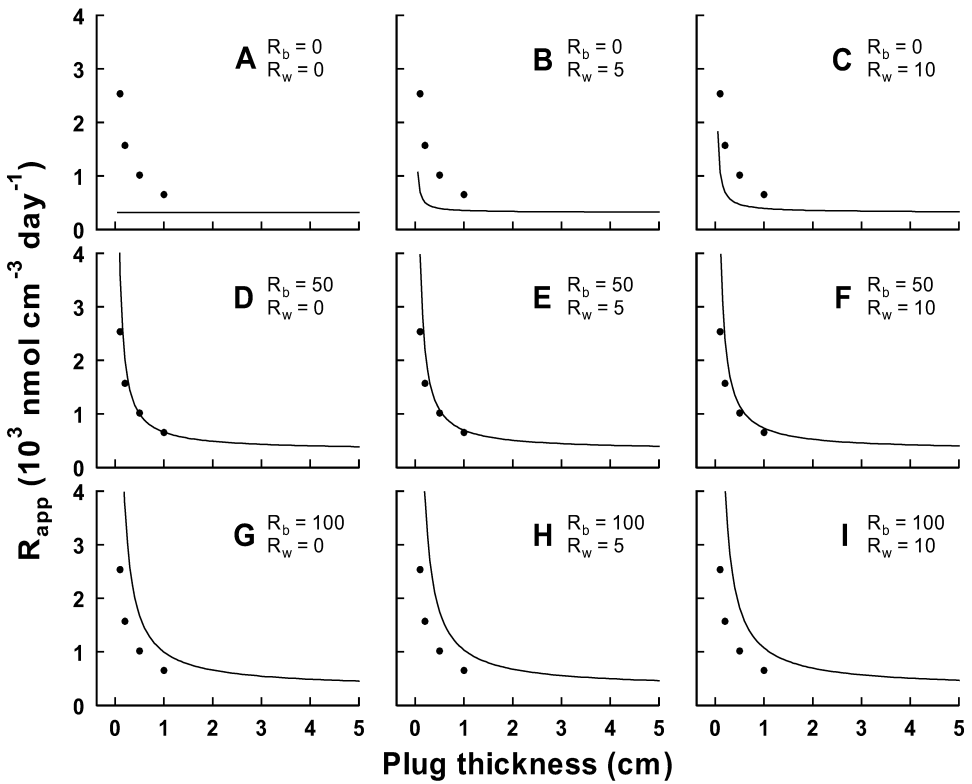
$$\Delta C/\Delta t = R_{app}j/h. \tag{8}$$

Figure 11. Experiment 3. R_{app} estimated from Eq. (9) as a function of plug thickness, j, for a range of biofilm activities, R_b (nmol cm^{-2} day^{-1}) and water phase activities, R_W (nmol cm^{-3} day^{-1}). R_{sp} was assumed independent of plug thickness and kept constant: $R_{sp\text{-}fix} = 319$ nmol cm^{-3} day^{-1}. The symbols represent the measured apparent $R_{app}(\mathrm{TCO_2})$ from Experiment 3.

By substitution of Eq. (8) into (7) and inserting the fixed values of r and h, we get an expression that describes the apparent thickness dependent volume specific reaction rates as a function of diffusion distance j entirely from thickness independent reaction terms:

$$R_{app} = R_{sp\text{-}fix} + R_b 6.77/j + R_w 7.5/j. \tag{9}$$

Any apparent diffusion scale impact of reactions in water and biofilm on the volume specific sediment $\mathrm{TCO_2}$ production can be evaluated from Eq. (9). For this purpose, we have chosen a range of realistic scenarios (Fig. 11), where R_b and R_w varies from 0 to 100 nmol cm^{-2} day^{-1} and 0 to 10 nmol cm^{-3} day^{-1}, respectively, while $R_{sp\text{-}fix}$ is set to 319 nmol cm^{-3} day^{-1} ($=R_{sj}$ in Experiment 3). These values largely cover the range found in Experiment 3 (Table 2 and Fig. 8). Independent of magnitude, R_b and R_w

Table 2. Experiment 3. Values of R_b and R_w, from the third incubation series as a function of plug thickness. $R_{p\text{-}fix}$ is equivalent to R_{sj} obtained from closed jar incubations.

	j	$R_{sp\text{-}fix}$	R_b	R_w
	cm	nmol cm^{-3} day^{-1}	nmol cm^{-2} day^{-1}	nmol cm^{-3} day^{-1}
CO_2	0.1	319	75.6	6.2
	0.2	319	137.8	3.0
	0.5	319	62.7	4.5
	1	319	51.5	6.8
	cm	nmol cm^{-3} day^{-1}	nmol cm^{-2} day^{-1}	nmol cm^{-3} day^{-1}
NH_4^+	0.1	74.9	9.1	—
	0.2	74.9	5.8	—
	0.5	74.9	8.7	0.4
	1	74.9	3.8	—

above zero always forces R_{app} to increase as j approaches 0. The asymptotic value of R_{app} as j increases is $R_{sp\text{-}fix}$. The best fit to the measured values of R_{app} in Experiment 3 is obtained with $R_b = 50$ nmol cm^{-2} day^{-1} and $R_w = 0$–5 nmol cm^{-3} day^{-1} (Fig. 11D, E), which are comparable to the measured average R_b of 66 nmol cm^{-2} day^{-1} (excluding two very high outliers) and R_w of 5.9 nmol cm^{-3} day^{-1}. Within the ranges chosen here, R_b has much greater impact on R_{app} than R_w. Furthermore, the curvature driven by R_b is almost identical to that of the measured values from Experiment 3 (Fig. 11D), while the R_w primarily affects thin plugs (low j, Fig. 11C). Accordingly, most of the apparent diffusion scale dependent change in volume specific reaction rates observed in the present experiments is caused by experimental artifacts due to bacterial activity in particularly biofilms along chamber walls.

No previous attempts have, to our knowledge, been made to quantify potential errors introduced by reactions other than those occurring within the sediment when sediment-water fluxes are measured by standard core incubation techniques. We can, therefore, only base our conclusions on the present study. In any case, the results suggest that most of the apparent diffusion scale dependent changes in R_{app} are caused by extra-sedimentary bacterial activity (e.g. biofilms along chamber walls), as these reactions primarily affect the estimated solute flux during incubation of relatively thin plugs (<1 cm). Our conclusion is supported by (1) direct evidence for a distinct biofilm of up to 20×10^5 cells cm^{-2} on chamber walls after only 5 days of incubation, (2) successful detection of TCO_2 and NH_4^+ generation in biofilm and water, and (3) successful simulation of the apparent diffusion scale dependence of R_{app} based on reactions occurring only in the biofilm and water phase. We still trust, though, that microbial activity is enhanced near the sediment-water interface of anaerobic sediments, as Aller and Aller (1998) showed elegantly for a variety of processes using different techniques. However, the steepness of the increase in reaction rates estimated from flux measurements as a function of decreasing sediment thickness will

in most cases be overestimated due primarily to thickness independent biofilm activity along walls.

REFERENCES

Aller, R. C. 1978. Experimental studies of changes produced by deposit feeders on pore water, sediment, and overlying water chemistry. Am. J. Sci., *278,* 1185–1234.

——1982. The effect of macrobenthos on chemical properties of marine sediment and overlying water, *in* Animal-Sediment Relations, P. L. McCall and M. J. S. Tevesz, eds., Plenum Publishing Company, 53–102.

——1994. Bioturbation and remineralization of sedimentary organic matter: effects of redox oscillation. Chem. Geol., *114,* 331–345.

Aller, R. C. and J. Y. Aller. 1998. The effect of biogenic irrigation intensity and solute exchange on diagenetic reaction rates in marine sediments. J. Mar. Res., *56,* 905–936.

Aller, R. C., J. Y. Aller and P. F. Kemp. 2001. Effects of particle and solute transport on rates and extent of remineralization in bioturbated sediments, *in* Organism-Sediment Interactions, J. Y. Aller, S. A. Woodin and R. C. Aller, eds., University of South Carolina Press, Columbia, 315–333.

Aller, R. C. and J. E. Mackin. 1989. Open-incubation, diffusion methods for measuring solute reaction rates in sediments. J. Mar. Res., *47,* 411–440.

Aller, R. C. and J. Y. Yingst. 1985. Effects of the marine deposit-feeders *Heteromastus filiformis* (polychaeta), *Macoma baltica* (Bivalvia) and *Tellina texana* (Bivalvia) on averaged sediment solute transport, reaction rates and microbial distributions. J. Mar. Res., *43,* 615–645.

Andresen, M. and E. Kristensen. 2002. The importance of bacteria and microalgae in the diet of the deposit-feeding polychaete *Arenicola marina.* Ophelia, *56,* 179–196.

Banta, G. T., M. Holmer, M. H. Jensen and E. Kristensen. 1999. The effect of two polychaete worms, *Nereis diversicolor* and *Arenicula marina,* on aerobic and anaerobic decomposition in a sandy marine sediment. Aquat. Microb. Ecol., *19,* 189–204.

Bower, C. E. and T. Holm-Hansen. 1980. A salicylate-hypochlorit method for determining ammonia in seawater. Can. J. Fish. Aquatic Sci., *37,* 794–798.

Canfield, D. E. 1994. Factors influencing organic matter preservation in marine sediments. Chem. Geol., *114,* 315–329.

Chapman, P. and S. A. Mostert. 1989. Does freezing of nutrient samples cause analytical errors? S. Afr. Mar. Sci., *9,* 239–247.

Christensen, J. P., A. H. Devol and W. M. Smethie. 1984. Biological enhancement of solute exchange between sediments and bottom water on the Washington continental shelf. Cont. Shelf Res., *3,* 9–23.

Dore, E. D., T. Houlihan, D. V. Hebel, G. Tien, L. Tupas and D. M. Karl. 1996. Freezing as a method of sample preservation for the analysis of dissolved inorganic nutrients in seawater. Mar. Chem., *53,* 173–185.

Fenchel, T. 1996. Worm burrows and oxic microniches in marine sediments. 1. Spatial and temporal scales. Mar. Biol., *127,* 289–295.

Findlay, R. H., M. B. Trexler, J. B. Guckert and D. C. White. 1990. Laboratory study of disturbance in marine sediments: response of a microbial community. Mar. Ecol. Prog. Ser., *62,* 121–133.

Furukawa, Y., S. J. Bentley and D. L. Lavoie. 2001. Bioirrigation modeling in experimental benthic mesocosms. J. Mar. Res., *59,* 417–452.

Gilbert, F., P. Bonin and G. Stora. 1995. Effect of bioturbation on denitrification in a marine sediment from the West Mediterranean littoral. Hydrobiol., *304,* 49–58.

Hall, P. O. J. and R. C. Aller. 1992. Rapid, small-volume, flow injection analysis for ΣCO_2 and NH_4^+ in marine and freshwaters. Limnol. Oceanogr., *37,* 1113–1119.

Holmboe, N. and E. Kristensen. 2002. Ammonium adsorption in sediments of a tropical mangrove forest (Thailand) and a temperate Wadden Sea area (Denmark). Wetland Ecol. Man., *10,* 453–460.

Horn, H. and D. C. Hempel. 1997. Growth and decay in an auto-/heterotrophic biofilm. Water Res., *31,* 2243–2252.

Hulthe, G., S. Hulth and P. O. J. Hall. 1998. Effect of oxygen on degradation rate of refractory and labile organic matter in continental margin sediments. Geochim. Cosmochim. Acta, *62,* 1319–1328.

Jørgensen, B. B. 1977. Bacterial sulfate reduction within reduced microniches of oxidized marine sediments. Mar. Biol., *41,* 7–17.

Koster, I. W. 1988. Microbial, chemical and technological aspects of the anaerobic degradation of organic pollutants, *in* Biotreatment Systems, D. L. Wisen, ed., Vol 1. CRC Press, Boca Raton, FL, 285–316.

Kristensen, E. 1985. Oxygen and inorganic nitrogen exchange in a *Nereis virens* (Polychaeta) bioturbated sediment-water system. J. Coast. Res., *1,* 109–116.

——2000. Organic matter diagenesis at the oxic/anoxic interface in coastal marine sediments, with emphasis on the role of burrowing animals. Hydrobiol., *426,* 1–24.

——2001. Impact of polychaetes (*Nereis* spp. and *Arenicula marina*) on carbon biogeochemistry in coastal marine sediments. Geochem. Trans., *2,* 92–104.

Kristensen, E., S. I. Ahmed and A. H. Devol. 1995. Aerobic and anaerobic decomposition of organic matter in marine sediment: Which is fastest? Limnol. Oceanogr., *40,* 1430–1437.

Kristensen, E., F. Ø. Andersen and T. H. Blackburn. 1992. Effect of benthic macrofauna and temperature on degradation of macroalgal detritus: the fate of organic carbon. Limnol. Oceanogr., *37,* 1404–1419.

Kristensen, E., A. H. Devol and H. E. Hartnett. 1999. Organic matter diagenesis in sediments on the continental shelf and slope of the Eastern Tropical and temperate North Pacific. Cont. Shelf Res., *19,* 1331–1351.

Kristensen, E. and K. Hansen. 1995. Decay of plant detritus in organic-poor sediment: production rates and stoichiometry of dissolved C and N compounds. J. Mar. Res., *53,* 675–702.

——1999. Transport of carbon dioxide and ammonium in bioturbated (*Nereis diversicolor*) coastal, marine sediments. Biogeochem., *45,* 147–168.

Kristensen, E., K. D. Kristiansen and M. H. Jensen. 2003. Temporal behaviour of manganese and iron in a eutrophic coastal sediment exposed to water column anoxia. Estuaries, *26,* 690–699.

Krom, M. D. and R. A. Berner. 1980. Adsorbtion of phosphate in anoxic marine sediments. Limnol. Oceanogr., *25,* 797–806.

Mackin, J. E. and R. C. Aller. 1984. Ammonium adsorbtion in marine sediments. Limnol. Oceanogr., *29,* 250–257.

Porter, K. G. and Y. S. Feigh. 1980. The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr., *25,* 943–948.

Santegoeds, C. M., A. Schramm and D. de Beer. 1998. Microsensors as a tool to determine chemical microgradients and bacterial activity in wastewater biofilms and flocs. Biodegradation, *9,* 159–167.

Schink, B. 1988. Principles and limits of anaerobic degradation: environmental and technological aspects, *in* Biology of Anaerobic Organisms, A. J. B. Zehnder, ed., Wiley, NY, 771–846.

Sun, M-Y., R. C. Aller, C. Lee and S. G. Wakeham. 1999. Enhanced degradation of algal lipids by benthic macrofaunal activity: Effect of *Yoldia limatula.* J. Mar. Res., *57,* 775–804.
Tratnyek, P. G., T. E. Reilkoff, A. W. Lemon, M. M. Scherer, B. A. Balko, L. M. Felk and B. D. Henegar. 2001. Visualizing redox chemistry: Probing environmental oxidation-reduction reactions with indicator dyes. Chem. Educator, *6,* 172–179.
Zar, J. H. 1999. Biostatistical Analysis, Prentice Hall, 4th ed., 663 pp.