

Impact of bioturbation by *Arenicola marina* on microbiological parameters in intertidal sediments

Wolfgang Reichardt

Institut für Meereskunde, Universität Kiel, Düsternbrooker Weg 20, D-2300 Kiel, Federal Republic of Germany

ABSTRACT: The impact of bioturbation by the lugworm *Arenicola marina* on sediment microflora and biochemical activities was investigated in intertidal sediments of the North Sea. Burrow walls and fecal casts were compared with surface and subsurface sediment surrounding the burrows. At the main study site burrow walls contained twice as much organic matter as the sediment surface. Viable counts of aerobic proteolytic and chitinolytic bacteria peaked at external and internal boundary layers (sediment surface and burrow walls). Burrow walls showed maximal bacterial production (incorporation of tritiated thymidine into DNA), maximal microheterotrophic activity (incorporation and mineralization of glucose and acetate) and highest levels of certain hydrolytic enzymes (alkaline phosphatase and sulfatase). Rates of chemoautotrophic CO₂ fixation in both burrow walls and surrounding subsurface sediment were 3 to 4 times higher than at the sediment surface, suggesting a short-circuited CO₂ turnover driven by an elevated catabolism. In a second, more polluted study area, burrow walls were again the main site of microheterotrophic activity, but only minor differences were noted for CO₂ fixation. Biogeochemical consequences of bioturbation by *A. marina* could be understood essentially as a shifting of catabolic and anabolic microbial activity peaks from the top to subsurface layers, where burrow walls showed the most conspicuous effects. Nevertheless, protease peaks in the fecal casts indicated also that this shifting pattern can be reversed for certain parameters.

INTRODUCTION

It is widely assumed that macrofaunal burrowing in marine sediments has a stimulatory effect on microbial growth and metabolic activities (Hargrave 1970, Aller & Yingst 1978, Henriksen et al. 1980, Hylleberg & Henriksen 1980, Morrison & White 1980, Yingst & Rhoads 1980, Hines & Jones 1985, Kikuchi 1986, Reichardt 1986a), although exceptions to this rule are evident (Bianchi & Levinton 1981, Hanson & Tenore 1981, Pearson 1982, Alongi 1985a, Alongi & Hanson 1985).

In organically enriched environments, macrofaunal control of microbial production and activity may become negligible (Bianchi & Levinton 1981, Newell 1982, Alongi & Hanson 1985), indicating the weakening of an important food chain linkage (Tenore & Hanson 1980, Newell 1982). This influence of elevated nutrient levels has been noted, in particular, for pioneering surface-deposit-feeders. In contrast to deep-dwelling 'equilibrium macrobenthos' the meiofauna and microflora associated with those early colonizers has, like the latter, been shown to exhibit con-

siderable resilience to physical disturbances (Christian et al. 1978, Alongi 1985a).

Arenicola marina, one of the most conspicuous macrofaunal inhabitants of the Wadden Sea sediment, is a funnel-type surface-deposit-feeder (Krüger 1966) and occurs in pristine as well as polluted intertidal sediments of the North Frisian Wadden Sea. *A. marina* burrows are enrichment sites for unique meiofaunal assemblages (Reise 1981, 1987, Scherer 1985). A direct impact of *A. marina* on the microbial communities in Wadden Sea sediment can be postulated (Reise 1985), but quantitative evidence for stimulating effects on microbial production and biogeochemical activities is lacking.

Most investigators of infauna-microflora relationships have focussed primarily on microbial production and related food chain aspects (e.g. Hanson & Tenore 1981, Levinton & Bianchi 1981, Alongi 1985a, b, Alongi & Hanson 1985, Kemp 1987). Less attention has been paid to the biocatalytic potential of a macrofauna-controlled microflora (e.g. Hargrave 1970, Aller & Yingst 1978, Hylleberg & Henriksen 1980).

Polychaete-dominated sediments have been noted for their increased production of CO₂ (Kikuchi 1986). Hanson & Tenore (1981) suggested that significant losses of carbon from food webs occurred in the absence of reversed processes such as CO₂-fixation by chemoautotrophic bacteria (Hanson & Tenore 1981). Therefore this investigation deals with CO₂-production (mineralization) by microheterotrophs as well as CO₂-fixation by chemoautotrophic microbial communities. Instead of comparing large sediment areas with and without significant bioturbation (Hines & Jones 1985), a small-scale approach was chosen to exclusively analyse microbial activities in sediment zones that were directly affected by the bioturbating animal (burrow walls, fecal casts) and in adjacent zones serving as controls. This also included CO₂ dark fixation as a measure of primary synthesis of organic matter by the sediment microbiota or, essentially, chemoautotrophic bacterial production. Finally, assays for extractable enzyme activities were designed to provide further biochemical clues concerning the degradation of organic matter. However, in this case differentiation between microbial and non-microbial sources was impossible.

Since the chosen study area (Westerhever Sand) appeared rather pristine, complementary investigations were carried out at a more polluted site (List) to examine the impact of higher organic loading on microbial release and assimilation of CO₂.

Materials and Methods

Sampling. Study sites were situated at Westerhever Sand (WHS), an intertidal sand flat off the Eiderstedt peninsula (54° 22.0' N, 8° 38.4' E), and at the northern tip of the island of Sylt (Königshafen-List; 54° 51.7' N, 8° 3.4' E) in the North Frisian Wadden Sea (North Sea). Samples from burrow sites of the lugworm *Arenicola marina* were collected within sampling areas of approximately 10 000 m² during June and July 1987 at water temperatures between 13 and 17 °C.

During low tide, burrows with dark fecal casts were dug out, to obtain sets of 4 different zones of sediment from each of $n=6$ (List) or $n=10$ (WHS) sampled burrow sites. The following 4 zones were sampled: (1) fecal casts; (2) an approximately 2 mm thick layer of the sediment surface; (3) an approximately 2 mm thick brownish surface layer of the vertical tail shaft and beginning parts of the gallery (Reise 1981); and (4) the reduced (black or greyish) subsurface sediment surrounding the burrows at 1 to 5 cm depths.

For most analytical procedures, samples were collected in sterile 1.0 cm³ sawn-off syringes for distributing 0.1 cm³ aliquots. Undisturbed subsamples, with

intact surfaces as used for CO₂ fixation measurements, were obtained as 1 cm² subsample discs. These were punched out from 2 to 3 mm thick sediment layers using a cork borer and scalpellum. Each disc was transferred into the fitting holes of tissue culture multi-well plates (Linbro).

Laboratory analyses. Samples were processed within 30 min of sampling in laboratories located within a short walking distance of the sampling sites. Redox measurements in combined samples were done using an Ingold platinum electrode. Organic matter content was determined as ash-free dry weight after combustion (24 h at 500 °C) of dried (65 °C) combined (0.5 cm³) subsamples. Pooled, combusted samples were further used for grain-size fractionation according to the Wentworth scale. Protein concentrations were analysed in 0.1 cm³ subsamples using a modification of Lowry's method (Herbert et al. 1971) with bovine serum albumin as reference standard. Photometric absorbance at 750 nm was measured after centrifugation (5000 × *g*, 15 min) of the reaction mixture.

Direct bacterial counts were based on a modification of the acridine orange epifluorescent technique by Rublee & Dornseif (1978), except that dispersion was accomplished by ultrasonication (3 × 5 s at approximately 100 W).

Viable counts of bacteria involved in the decomposition of detrital biopolymers were represented by proteolytic and chitinolytic bacteria (Reichardt 1988). The latter group was included for the particular importance of chitin as structural polysaccharide in macro- and meiofauna and for the potential role of this compound as a microbially digestible food source in benthic grazing.

Incorporation of tritiated thymidine into bacterial DNA was determined by incubating of 0.1 cm³ of sediment with 20 μl (370 × 10⁶ kBq) methyl-(³H)-thymidine (740 × 10³ kBq μmol⁻¹) for 30 min at in situ temperature, using samples containing 6 % formaldehyde as blanks. After 2 subsequent washes and centrifugation with 10 ml of 4 % formaldehyde, the concentration of thymidine incorporated into bacterial DNA was determined according to Findlay et al. (1984).

Rates of CO₂ dark fixation were measured in multi-well plates containing undisturbed surface samples of 2 to 3 mm thickness (see above). These were incubated for 30 min in the dark at in situ temperature with 200 μl (74 kBq) of ¹⁴C-labeled sodium bicarbonate (specific activity: 2194 kBq μmol⁻¹) in 1.5 mM non-radioactive NaHCO₃, pH 10.2). Parallel subsamples were poisoned with 20 mM iodoacetamide, an inhibitor of ribulose-1,5-bisphosphate carboxylase (RubisCo), to obtain, as far as possible, a blank for non-chemoautotrophic and RubisCo-independent CO₂ fixation (Reichardt unpubl). Incubation was terminated by adding 0.1 ml of concen-

Table 1. Grain size distribution in combusted sediments from 4 sampling zones at burrow sites of *Arenicola marina* (Westerhever Sand and List). Percent dry weight in combined subsamples. FC: fecal casts; S: sediment surface; RS: reduced subsurface sediment; BW: burrow walls

| Grade limits (μm) | Westerhever Sand | | | | List | | | | |
|-----------------------------------|------------------|------|------|------|------|------|------|------|--|
| | FC | S | RS | BW | FC | S | RS | BW | |
| > 1000 | – | – | – | – | 6.7 | 3.8 | 1.5 | 1.4 | |
| 500–1000 | 5.8 | 0.9 | 3.5 | 1.0 | 55.7 | 62.6 | 47.9 | 41.3 | |
| 250–500 | 21.6 | 22.9 | 14.5 | 16.2 | 31.5 | 31.6 | 43.1 | 49.5 | |
| 125–250 | 65.1 | 70.8 | 63.1 | 68.8 | 4.1 | 1.6 | 4.5 | 6.3 | |
| 63–125 | 7.5 | 5.4 | 17.0 | 12.5 | 1.4 | 0.3 | 2.2 | 1.4 | |
| < 63 | – | – | 1.9 | 1.5 | 0.6 | 0.1 | 0.8 | 0.1 | |

trated formaldehyde and 1 N H_2SO_4 sufficient to lower the pH to 2.0. The sediment was taken up in 10 ml of filtered seawater, centrifuged (15 min at $6000 \times g$) and the pellets dried at 60°C . Weighed amounts were combusted using a Packard ^{14}C -sample oxidizer for liquid scintillation counting (Beckmann LS 100). Quench corrections were based on external standard and channels ratios. Total carbonate concentrations available in the assay were calculated from carbonate alkalinity of the pore water (Gargas 1980).

Microheterotrophic activity was determined as simultaneous incorporation into macromolecular pools and mineralization to CO_2 of ^{14}C -labeled glucose or acetate. Each sediment sample (0.1 cm^3) was incubated for 30 min at in situ temperature with $100 \mu\text{l}$ aliquots of ^{14}C (U)glucose (11.1 kBq; $163 \text{ kBq } \mu\text{mol}^{-1}$) and ^{14}C (U)acetate (11.1 kBq; $89 \text{ kBq } \mu\text{mol}^{-1}$) dissolved in sterile artificial seawater. Potential mineralization rates were measured according to Reichardt & Morita (1982); potential incorporation rates were obtained from the same assay after acidification, centrifugation, and combustion in a Packard ^{14}C -sample oxidizer (see previous paragraph).

Enzyme assays were run with sediment extracts in ice-cold tris-HCl-buffered 2% triton-X-100 (Reichardt 1986b). In assays for alkaline phosphatase and sulfatase (2 to 4 h at 20°C) the corresponding nitrophenylesters served as substrates (Oshrain & Wiebe 1979, Degobbis et al. 1986). Scleroprotease activity was meas-

Table 2. Redox potential (E_h , mV) and total organic matter (OM; mg cm^{-3} of ash-free dry weight) in 4 sampling zones at *Arenicola marina* burrow sites at Westerhever Sand (WHS) and List. (Mean values of $n = 3$ pooled samples)

| Sampling zone | E_h | | OM | |
|-----------------------------|-------|------|------|------|
| | WHS | List | WHS | List |
| Fecal casts | 365 | 295 | 11.0 | 14.9 |
| Sediment surface | 425 | 290 | 8.9 | 12.1 |
| Reduced subsurface sediment | 150 | 130 | 13.2 | 23.5 |
| Burrow walls | 370 | 260 | 16.8 | 19.7 |

ured with the particulate 'hide powder azure' (Sigma) substrate (Reichardt 1986b).

RESULTS

Physicochemical parameters and bacterial densities

The 2 study sites were characterized by different grain size distributions: fine sand prevailing at Westerhever Sand (WHS), and coarse to medium sand at List (Table 1). As indicated by ash-free dry weights, the coarser-grained sediment at List was more reduced and contained higher amounts of organic matter accumulating below the surface at both sites (Table 2). Whereas the less polluted sediment (WHS) showed its maximum organic matter content in the burrow walls (F -test, $p = 0.05$), organic matter concentrations of the more polluted sediment (List) peaked in the reduced subsurface section.

At WHS, protein concentrations and ash-free dry weight showed similar patterns. These parameters were not reflected by densities of (aerobic and facultative)

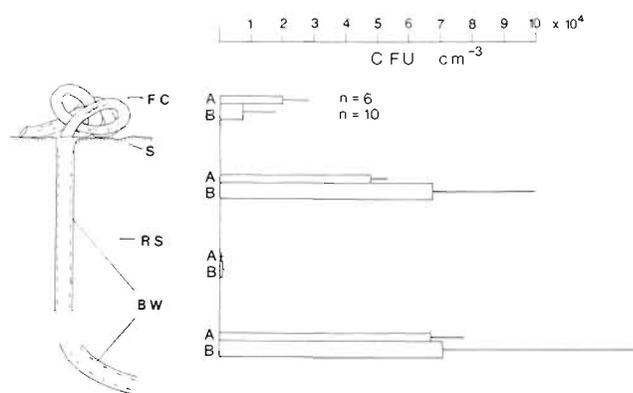


Fig. 1. Viable counts (aerobic colony forming units, CFU) of chitin-degrading bacteria in: fecal casts (FC), surface layer (S), reduced subsurface layer (RS), and burrow walls (BW) of *Arenicola marina* study sites at List (A) and Westerhever Sand (B). Bars mark standard deviations of a given number (n) of individual burrow sites sampled

Table 3. Protein content (mg cm^{-3}), proteolytic viable counts (CFU), and acridine orange epifluorescent direct counts of bacterial cells (AODC) in 4 sampling zones at *Arenicola marina* burrow sites in intertidal flats at Westerhever Sand. Means \pm standard deviation for given number (n) of samples

| Sampling zone | Protein ($n = 4$) | CFU $\times 10^6 \text{ cm}^{-3}$ ($n = 5$) | AODC $\times 10^9 \text{ cm}^{-3}$ ($n = 5$) |
|-----------------------------|------------------------|--|---|
| Fecal casts | 1.53 \pm 0.38 | 1.12 \pm 0.81 | 4.9 \pm 0.7 |
| Sediment surface | 1.06 \pm 0.22 | 7.04 \pm 3.94 | 2.5 \pm 0.4 |
| Reduced subsurface sediment | 1.90 \pm 1.29 | 0.40 \pm 0.61 | 3.5 \pm 1.7 |
| Burrow walls | 2.27 \pm 1.30 | 4.89 \pm 3.30 | 4.6 \pm 6.7 |

tively anaerobic) proteolytic bacteria (Table 3). Viable counts for protein- and chitin-degrading bacteria (Table 3; Fig. 1) peaked significantly (F -test, $p = 0.05$) at the top sediment layer ('S') as well as in the burrow walls ('BW') without significant differences between 'S' and 'BW'.

Epifluorescent microscopic direct counts (AODC), in particular, were subjected to great variations (Table 3) and revealed no significant difference (F -test, $p = 0.05$). According to microscopical evidence, this was at least partly due to microscale heterogeneity of the distribution of bacterial cells. Especially in burrow wall samples, cells were often found in clumps adhering to pellicular substrata.

Anabolic bacterial activities

Rates of (iodoacetamide-inhibitable) CO_2 dark fixation peaked in the subsurface layers of both study sites, where they showed a higher degree of variability than at the sediment surface (Fig. 2). Differences between burrow walls and adjacent sediment were not significant (F -test, $p = 0.05$). In the less polluted area (Fig. 2; 'B') fecal casts contributed to a local increase of CO_2 fixation on the sediment surface.

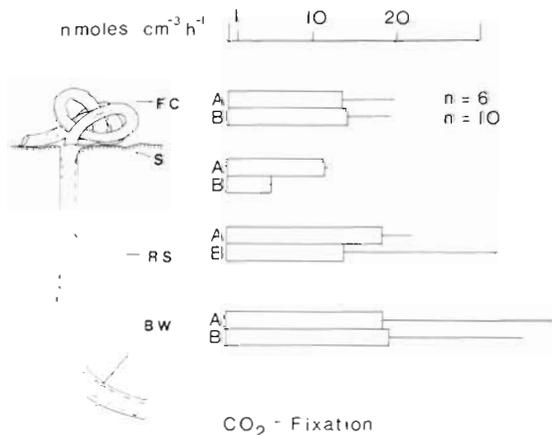


Fig. 2. Rates of (iodo-acetamide-inhibitable) CO_2 dark fixation in 4 selected sampling zones at burrow sites of *Arenicola marina*. Further details in Fig. 1

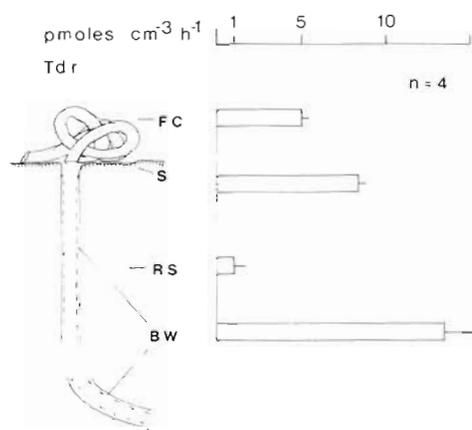


Fig. 3. Rates of incorporation of tritiated (methyl)thymidine into bacterial DNA in 4 selected sampling zones at burrow sites of *Arenicola marina* (Westerhever Sand). Further details in Fig. 1

In contrast to CO_2 fixation as a measure of largely chemolitho-autotrophic bacterial biosynthesis, incorporation of tritiated (methyl)-thymidine (tdr) into bacterial DNA peaked significantly (F -test, $p = 0.05$) in the burrow walls (Fig. 3). This maximum of bacterial production exceeded rates in subsurface sediments surrounding the burrows by roughly one order of magnitude. Fecal casts revealed significantly higher rates than subsurface sediment (where this material originated from); however, in contrast to CO_2 fixation (Fig. 2; 'B'), rates of tdr incorporation at the surface were higher than in fecal cast material (F -test, $p = 0.05$).

Microheterotrophic utilization of glucose and acetate

Relative activities of incorporation and mineralization of glucose declined steeply from the surface toward subsurface layers at the less polluted site (Fig. 4; 'B'). This was much less pronounced at the more polluted site (Fig. 4; 'A'). In both cases, however, the burrow walls were most active in mineralizing glucose and incorporating it into microbial cells (F -test, $p = 0.05$). A similar pattern of microheterotrophic activ-

ity was noted for acetate utilization at the less polluted study site (Fig. 5). The differences between surface sediment and fecal casts were rather insignificant for both substrates (Figs. 4 and 5).

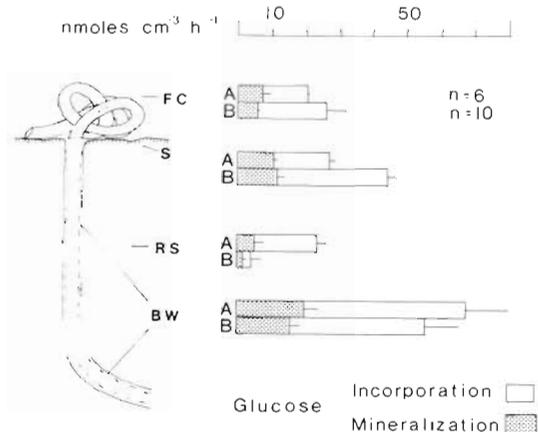


Fig. 4. Potential rates of utilization of ¹⁴C-(U)glucose in 4 selected sampling zones at burrow sites of *Arenicola marina*. Further details in Fig. 1

Enzyme activities

Activities of extractable alkaline phosphatase and sulfatase followed essentially the same trend as microheterotrophic utilization of glucose and acetate. Fecal casts, however, turned out to be zones with the strongest enzymatic potential to degrade particulate (sclero-)proteins (Fig. 6).

DISCUSSION

Evidence for bacterial enrichment?

Disturbances are considered as mechanisms to keep parts of any ecosystem at a high state of productivity (Rhoads et al. 1978). Bioturbation of the sea floor by burrowing macroinvertebrates comprises physical as well as chemical changes in those zones of the sediment that are affected by metabolic activities of the infauna. Correspondingly, microbial enrichment processes in bioturbated marine sediments have been attributed to both physical disturbances (Eckman 1985, Findlay et al. 1985), and infaunal metabolism (Driscoll 1975, Yingst & Rhoads 1980, Alongi & Hanson 1985, Reichardt 1986a, 1988). If bacterial enrichment is linked to certain macroinvertebrates, specific responses of these to physical disturbances also need to be considered (Woodin 1978, Thistle 1981). Since burrows of *Arenicola marina* are known as enrichment sites for

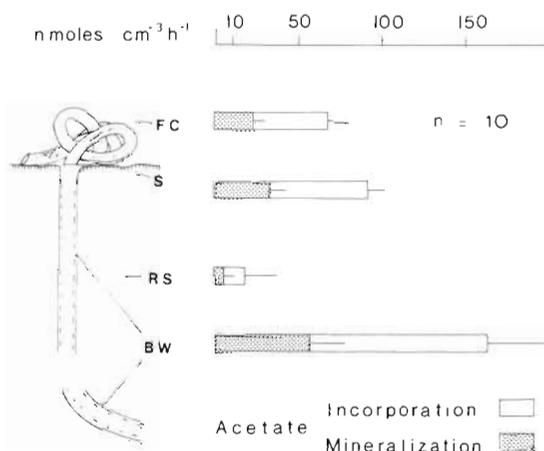


Fig. 5. Potential rates of utilization of ¹⁴C-acetate in 4 selected sampling zones at burrow sites of *Arenicola marina* (Westerhever Sand). Further details in Fig. 1

meiobenthic bacteriovores (Reise 1981, 1987, Scherer 1985), a rapid turnover of bacterial biomass can be anticipated.

Differences between bacterial cell densities obtained for sediment zones affected by the lugworm (burrow walls and fecal casts) and those serving as unaffected controls (sediment surface and reduced bulk sediment) were rather weak (Table 3). This lack of evidence for strong enrichment may be explained by meiofaunal and microfaunal grazing pressure. However, bacterial counts were subject to high statistical errors, especially in the burrow walls, caused by bacterial aggregates formed on flakes from the burrow lining material. Total cell densities in the range of 10⁹ cm⁻³ are common for intertidal sediments (e.g. Rublee & Dornseif 1978, DeFlaun & Mayer 1983). As a static measure, total cell counts can mirror only net enrichment of an extremely

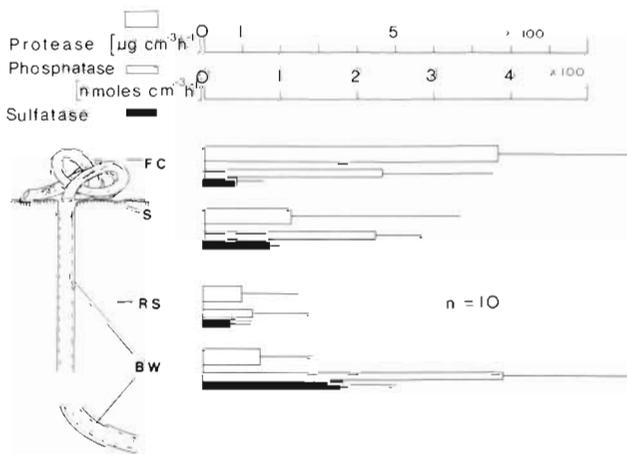


Fig. 6. Activities of hydrolytic enzymes after extraction with triton-X-100 from 4 selected sampling zones at burrow sites of *Arenicola marina* (Westerhever Sand). Further details in Fig. 1

heterogeneous group of organisms. In contrast to selective viable counts, total counts cannot provide any insight into microbial functions in nutrient cycling.

It is likely that bacterial distribution patterns were directly linked to the metabolic activities of the polychaete. Thus, passage through the guts of sediment-feeding macrofauna is known as a selective force that reduces viable cell counts of certain bacteria (Wavre & Brinkhurst 1971). In the present study, viable counts for aerobic biopolymer-degrading bacteria in fecal casts were also reduced as compared to burrow walls and sediment surface, but not in relation to sub-surface sediment surrounding the burrows (Table 2; Fig. 1). Coincident peaks for viable counts of proteolytic bacteria at the sediment surface and burrow walls suggested an intrusion from 'external' surfaces. It seems unlikely that the moderate decrease of redox potential (E_h) (Table 2) accounted entirely for the drastic decline of aerobic viable counts (which do include facultative anaerobes) in the reduced zones.

Thymidine incorporation

Production of bacterial biomass as measured by incorporation of tritiated (methyl)thymidine (tdr) into bacterial DNA peaked in the burrow walls. Similarly, sediment tubes of the capitellid polychaete *Capitella capitata* also showed significantly higher rates of tdr-dependent bacterial production than adjacent parts of the sediment, although bacterial densities were not different (Alongi 1985b). In this case, protozoan rather than meiofaunal grazing has been suggested as predominant mechanism controlling bacterial densities.

Macrofaunal grazing of sediment bacteria is well known in connection with the feed-back mechanism of 'gardening' (Hylleberg 1975). Still, estimates of bacterial contribution to macrofaunal diet are rare. Bacterivorous holothurians may eat 20 to 50 mg of bacterial carbon per day, which is equivalent to 10 to 40 % of the total bacterial production in their habitat (Moriarty et al. 1985). Yet, such short-circuited bacteria-macrofauna food chains seem to play a minor role. There is growing evidence against the notion of bacteria as a direct, principal food source of deposit-feeding marine infauna (Cammen 1980, Levinton & Bianchi 1981, Wetzel & Christian 1984, Alongi 1985b, Kemp 1987). Considering the abundance of meiofauna in *Arenicola* burrows (Reise 1981, 1987), it seems likely that, in this case too, macrofaunal grazing on bacteria can be ruled out as the primary mode of feeding in this study.

Based on a conversion factor of 1.1×10^{18} cells per mole of tdr incorporated into bacterial DNA (Riemann et al. 1987), the maximum bacterial production the burrow walls of *Arenicola marina* could account for is

7.6×10^7 cells $\text{cm}^{-3} \text{h}^{-1}$. Underestimates arising from greater densities of non-reactive anaerobes (Moriarty 1984) may be expected, in particular, for the more reduced zones. On the other hand, in a similar case of superficially oxidized burrow walls of *Nereis diversicolor*, the adjacent reduced sediment showed no significant differences in terms of phospholipid fatty acid biomarkers for anaerobic and sulfate-reducing bacteria. Incidentally, the same applied also to microfaunal biomarkers (Reichardt 1987).

Carbon dioxide dark fixation

As sites of steep oxygen gradients ensuring a continuous supply of potential electron donors, burrow walls may stimulate chemolithoautotrophic bacteria (Yingst & Rhoads 1980, Reichardt 1986a). Furthermore, measuring chemoautotrophic CO_2 fixation rates could also provide a production estimate of a selected group of bacteria.

At both study sites, burrow walls of *Arenicola marina* showed 1.6 to 3.7 times higher CO_2 fixation rates than the corresponding surface sediments (Fig. 2). However, measurements in the burrow walls were subject to high variability, and the results obtained were not significantly different from the surrounding sediment. This contrasted with earlier investigations in which burrow walls of *Nereis diversicolor* proved to be the most active sites of CO_2 dark fixation. This process was mainly driven by chemolithotrophic oxidation of reduced sulphur which followed a gradient within the burrow wall layers; the innermost, nearly anoxic layer showing maximal activity (Reichardt 1986a). Considerably thicker burrow wall samples were examined in the case of *A. marina*. Therefore existing activity gradients within the brown 2 to 4 mm thick walls (Reise 1981) might have been overlooked.

According to the literature, evidence for enhanced chemoautotrophic activities in polychaete burrows is mainly related to nitrification, an aerobic process (Henriksen et al. 1980, Sayama & Kurihara 1983, Kristensen et al. 1985). On the other hand, diffusive oxygen fluxes from aerated water into the sediment tend to decline most rapidly (Jørgensen & Revsbech 1985), and anaerobic microbial activities may predominate even in the outer burrow walls of deposit-feeding polychaetes (Aller & Yingst 1978). Considering the often negligible differences between CO_2 -fixation rates in burrow linings and the reduced sediment surrounding them, it becomes likely that (facultative) anaerobes were responsible for most of the chemoautotrophic activity. Certain sulphur-oxidizing thiobacilli (e.g. Timmer-ten Hoor 1975) as well as *Beggiatoa* (Nelson et al. 1986) belong to this group.

Microheterotrophic activities

Close-circuited bacteria-based food chains would require CO₂-consuming chemoautotrophs to maintain their carbon flow (Hanson & Tenore 1981). Microheterotrophic mineralization of DOM constitutes a major source of CO₂. Potential CO₂ production from glucose or acetate in the burrow walls proceeded 3 to 8 times faster than in the ambient sediment (Figs. 4 and 5). In addition to these microheterotrophic activities, CO₂ output from the polychaete itself must be also taken into account (Kikuchi 1986).

The present investigation deals with potential rates of CO₂ evolution from glucose and acetate as model substrates for dissolved organic matter (DOM). Thus, only a relative measure was obtained instead of real in situ mineralization rates. Measured rates of CO₂ production from the 2 substrates employed should be referred to as 'apparent' mineralization to avoid misleading conclusions about the in situ carbon flow (King & Berman 1984). The percentage of CO₂ of the total amount of glucose incorporated ranged from 26 to 39%. This corresponded with similar measurements in coastal marine sediments (Novitsky & Kepkay 1981, Novitsky 1983). Yet, estimates based on mass balances may be twice as high (King & Berman 1984). Further potential underestimates of real in situ CO₂ production resulted from measuring only net rates.

Heterotrophic utilization of glucose and acetate revealed quite similar distribution patterns (Figs. 4 and 5). They also coincided with bacterial production estimates based on incorporation of tdr into DNA (Fig. 3). Great differences of microheterotrophic activities occurring between burrow walls and surrounding sediment (Figs. 4 and 5) did not correspond with total organic matter or protein contents (Tables 2 and 3). This may indicate that the heterotrophic microflora in the burrow walls was particularly adapted to large fractions of easily available DOM.

Activities of hydrolyzing enzymes

Microbial substrates accumulating in burrow linings are partly excretion products of the burrow inhabitants (Johannes 1964, Zola 1967, Defretin 1971, Daly 1973). Enzymes which can hydrolyze such compounds are not confined to microbial sources (Zottoli & Carriker 1974, Reichardt 1986b). Excretion of the 3 enzymes investigated in the present study by *Arenicola marina* is possible (Johannes 1964, Oshrain & Wiebe 1979), and for proteases most likely (Zottoli & Carriker 1974).

Contrasting activity patterns obtained for alkaline phosphatase and sulphatase on one side, and sclero-protease on the other, were most striking (Fig. 6).

Proteases are hypothesized to play a role in purging inner polychaete tube surfaces (Zottoli & Carriker 1974). In the present study, distribution patterns for sclero-protease activities pointed to the gut of the polychaete as the main enzyme source. Activities in the fecal casts reached 5 to 3-fold higher levels than in burrow walls and sediment surface, respectively. This may indicate the existence of a potent output mechanism for an essential biocatalyst which is based on bioturbation-driven transport from subsurface zones to the primary accumulation site for sedimenting particles.

Alkaline phosphatase and sulphatase, on the other hand, peaked in the burrow walls. Although the biodegradative potential is usually concentrated in the burrow walls, the excretion of pertinent enzyme substrates by polychaetes should also be considered as a factor controlling the levels of these enzymes. For some polychaetes excretions of sulphomucopolysaccharides and phosphate-rich polysaccharides is documented (Zola 1967, Defretin 1971).

General aspects

Bell-jar experiments near one of the study sites (List) show a significant influence of *Arenicola marina* on the remobilization of nutrients (Asmus 1986). According to field studies of other shallow water sediments, bioturbation by polychaetes and bivalves may account for 3- to 5-fold increases in nutrient turnover that is mediated by bacteria (Hines & Jones 1985). The present study considered the impact of one predominant bioturbator on microbial and biogeochemical activities. This also implied a smaller scale approach that had already proven useful in tracking meiobenthic populations (Reise 1987).

As to be expected (Aller & Yingst 1978, Yingst & Rhoads 1980, Reichardt 1986a), most of the biogeochemical parameters showed peaks in the burrow walls. Steep E_h gradients at these 'microsites' have been cited as major determinants of bacterial, and particularly, chemoautotrophic growth (Yingst & Rhoads 1980, Reichardt 1986a). Nevertheless, the influence of other environmental factors should not be underestimated, as for example diffusive permeabilities and molecular sieve effects of mucoid burrow linings (Aller 1983), and the accumulation of organic matter at the burrows (Tables 2 and 3). As sediment volumes served as a reference basis for all the data presented, impacts of porosity were not taken into account. However, dry weight contents per cm³ differed only by 0.1 to 1.7% between the 4 sediment sections examined. Hence, putting the volume-based data on a dry weight basis would not lead to substantial changes of the relative differences.

Stimulation of anabolic processes in the burrow walls was mainly documented by elevated rates of tdr incorporation into bacterial DNA. Further considerations of the role of bacterial production in burrow wall-linked food webs, however, were beyond the scope of this study which focused on microbial biogeochemical activities. Nevertheless, abundance of bacterivorous meiofauna in *Arenicola marina* burrows (Reise 1981, 1987) indicates the existence of an efficient 'small food web' (Kuipers et al. 1981). In a previous investigation (Reichardt 1987), intensive grazing on bacteria in burrow walls of *Nereis diversicolor* was indicated by elevated levels of poly- β -hydroxybutyrate. At the same time, phospholipid fatty acid biomarkers for bacteria and microfauna (protozoa) reflected no increments of the standing crops. This may suggest that meiofaunal grazing played an important role in those benthic environments.

Whereas a positive feed-back mechanism between deposit-feeding burrowing macrofauna and bacterial decomposers (Yingst & Rhoads 1980) seems to function only when the detritus supply is low (Hanson & Tenore 1981), higher detritus availability may cause an uncoupling (Bianchi & Levinton 1981, Alongi & Hanson 1985). A comparison of the 2 study areas supports this view at least with respect to anabolic activities represented by CO₂ dark fixation. On the other hand, heterotrophic activities in the sewage-affected study area indicated macrofaunal control. At both places, subsurface peaks of major anabolic and catabolic processes could be explained by bioturbation-driven downward shifting of microbial and enzymatic activities. Elevated scleroprotease levels in the fecal casts indicated further that, for at least certain processes, the direction was reversed.

The relative significance of physical perturbation and nutrient availability in bioturbated sediments for microbial communities are still under discussion (Aller & Yingst 1978, Christian et al. 1978, White 1985, Alongi 1985a). The present study emphasizes the importance of lugworm burrows as transport and accumulation sites for organic matter, thus favoring a short-circuited CO₂ turnover between heterotrophic and chemoautotrophic microbial communities.

Acknowledgement. This investigation was financially supported by a grant from Deutsche Forschungsgemeinschaft (Re 271/14-1). I thank Prof. S. A. Gerlach and 2 anonymous reviewers for valuable criticism.

LITERATURE CITED

- Aller, R. C. (1983). The importance of the diffusive permeability of animal burrow linings in determining marine sediment chemistry. *J. mar. Res.* 41: 299–322
- Aller, R. C., Yingst, J. Y. (1978). Biogeochemistry of tube dwellings: a study of the sedentary polychaete *Amphitrite ornata* (Leidy). *J. mar. Res.* 36: 201–254
- Alongi, D. M. (1985a). Effect of disturbance on population dynamics and trophic interactions among microbes and meiofauna. *J. mar. Res.* 43: 351–364
- Alongi, D. M. (1985b). Microbes, meiofauna, and bacterial productivity on tubes constructed by the polychaete *Capitella capitata*. *Mar. Ecol. Prog. Ser.* 23: 207–208
- Alongi, D. M., Hanson, R. B. (1985). Effect of detritus supply on trophic relationships within experimental benthic food webs. II. Microbial responses, fate and composition of decomposing detritus. *J. exp. mar. Biol. Ecol.* 88: 167–182
- Asmus, R. (1986). Nutrient flux in short-term enclosures of intertidal sand communities. *Ophelia* 26: 1–18
- Bianchi, T. S., Levinton, J. S. (1981). Nutrition and food limitation of deposit-feeders. II. Differential effects of *Hydrobia toteni* and *Ilyanassa obsoleta* on the microbial community. *J. mar. Res.* 39: 547–556
- Cammen, L. M. (1980). Ingestion rate: an empirical model for aquatic deposit feeders and detritivores. *Oecologia (Berl.)* 44: 303–310
- Christian, R. R., Bancroft, K., Wiebe, W. J. (1978). Resistance of the microbial community within salt marsh soils to selected perturbations. *Ecology* 59: 1200–1210
- Daly, J. M. (1973). Behavioural and secretory activity during tube construction by *Platynereis dumerilii* Aud. & M. Edw. (Polychaeta: Nereidae). *J. mar. biol. Ass. U. K.* 53: 521–529
- DeFlaun, M. F., Mayer, L. M. (1983). Relationships between bacteria and grain surfaces in intertidal sediments. *Limnol. Oceanogr.* 28: 873–881
- Defretin, R. (1971). The tubes of polychaete annelids. In: Florkin, M., Stotz, E. H. (eds.) *Comprehensive biogeochemistry*. Elsevier, Amsterdam, p. 713–747
- Degobis, D., Homme-Maslowska, E., Orto, A., Donazzolo, R., Pavoni, B. (1986). The role of alkaline phosphatase in the sediments of Venice lagoon in nutrient regeneration. *Estuar. coast. Shelf Sci.* 22: 425–437
- Driscoll, E. G. (1975). Sediment-animal-water interaction, Buzzards Bay, Massachusetts. *J. mar. Res.* 33: 275–302
- Eckman, J. A. (1985). Flow disruption by an animal-tube mimic affects sediment bacterial colonization. *J. mar. Res.* 43: 419–435
- Findlay, S. E. G., Meyer, J. L., Edwards, R. T. (1984). Measuring bacterial production via rate of incorporation of (³H)thymidine into DNA. *J. Microbiol. Meth.* 2: 57–72
- Findlay, R. H., Pollard, P. C., Moriarty, D. J. W., White, D. C. (1985). Quantitative determination of microbial activity and community nutritional status in estuarine sediments: evidence for a disturbance artifact. *Can. J. Microbiol.* 31: 493–498
- Gargas, E. (1980). A manual for phytoplankton primary production studies in the Baltic, 2nd edn. *Baltic Marine Biologists Publ.* No. 2
- Hanson, R. B., Tenore, K. R. (1981). Microbial metabolism and incorporation by the polychaete *Capitella capitata* of aerobically and anaerobically decomposed detritus. *Mar. Ecol. Prog. Ser.* 6: 299–307
- Hargrave, B. T. (1970). The effect of a deposit-feeding amphipod on the metabolism of benthic microflora. *Limnol. Oceanogr.* 15: 21–30
- Henriksen, K., Hansen, J. I., Blackburn, T. H. (1980). The influence of benthic infauna on exchange rates of inorganic nitrogen between sediment and water. *Ophelia* (Suppl. 1): 249–256
- Herbert, D., Phipps, P. J., Strange, R. E. (1971). Chemical analysis of microbial cells. In: Norris, J. R., Ribbons, D. W.

- (eds.) Methods in microbiology 5B. Academic Press, London, p. 209–344
- Hines, M. E., Jones, G. E. (1985). Microbial biogeochemistry and bioturbation in the sediments of Great Bay, New Hampshire. *Estuar. coast. Shelf Sci.* 20: 729–742
- Hylleberg, J., Henriksen, K. (1980). The central role of bioturbation in sediment mineralization and element re-cycling. *Ophelia* (Suppl. 1): 1–16
- Hylleberg, J. (1975). Selective feeding by *Abarenicola vagabunda* and a concept of gardening in lugworms. *Ophelia* 14: 113–137
- Johannes, R. E. (1964). Uptake and release of dissolved organic phosphorus by representatives of a coastal marine ecosystem. *Limnol. Oceanogr.* 9: 224–234
- Jørgensen, B. B., Revsbech, N. P. (1985). Diffusive boundary layers and the oxygen uptake of sediments and detritus. *Limnol. Oceanogr.* 30: 111–122
- Kemp, P. F. (1987). Potential impact on bacteria of grazing by a macrofaunal deposit-feeder, and the fate of bacterial production. *Mar. Ecol. Prog. Ser.* 36: 151–161
- Kikuchi, E. (1986). Contribution of the polychaete, *Neanthes japonica* (Izuka), to the oxygen uptake and carbon dioxide production of an intertidal mud-flat of the Nanakita estuary, Japan. *J. exp. mar. Biol. Ecol.* 97: 81–93
- King, G. M., Berman, T. (1984). Potential effects of isotopic dilution on apparent respiration in ^{14}C heterotrophy experiments. *Mar. Ecol. Prog. Ser.* 19: 175–180
- Kristensen, E., Jensen, M. H., Andersen, T. K. (1985). The impact of polychaete (*Nereis virens* Sars) burrows on nitrification and nitrate reduction in estuarine sediments. *J. exp. mar. Biol. Ecol.* 85: 75–91
- Krüger, F. (1966). Messungen der Pumpfähigkeit von *Arenicola marina* L. im Watt. *Helgoländer wiss. Meeresunters.* 11: 70–91
- Kuipers, B. R., DeWilde, P. A. W., Creutzberg, F. (1981). Energy flow in a tidal flat ecosystem. *Mar. Ecol. Prog. Ser.* 5: 215–221
- Levinton, J. S., Bianchi, T. S. (1981). Nutrition and food limitation of deposit-feeders. I. The role of microbes in the growth of mud snails (Hydrobiidae). *J. mar. Res.* 39: 531–545
- Moriarty, D. J. W. (1984). Measurements of bacterial growth rates in some marine systems using the incorporation of tritiated thymidine into DNA. In: Hobbie, J. E., Williams, P. LeB. (eds.) *Heterotrophic activity in the sea*. Plenum Publ. Corp., New York, p. 217–231
- Moriarty, D. J. W., Pollard, P. C., Hunt, W. G., Moriarty, C. M., Wassenberg, T. J. (1985). Productivity of bacteria and microalgae and the effect of grazing by holothurians in sediments on a coral reef flat. *Mar. Biol.* 85: 293–300
- Morrison, S. J., White, D. C. (1980). Effects of grazing by Gammaridean amphipods on the microbiota of allochthonous detritus. *Appl. environ. Microbiol.* 40: 659–671.
- Nelson, D. C., Revsbech, N. P., Jørgensen, B. B. (1986). Microoxic-anoxic niche of *Beggiatoa* spp.: microelectrode survey of marine and freshwater strains. *Appl. environ. Microbiol.* 52: 161–168
- Newell, R. C. (1982). The energetics of detritus utilization in coastal lagoons and nearshore waters. *Oceanologica Acta* 32: 347–355
- Novitsky, J. A. (1983). Heterotrophic activity throughout a vertical profile of seawater in Halifax Harbor, Canada. *Appl. environ. Microbiol.* 45: 1753–1760
- Novitsky, J. A., Kepkay, P. E. (1981). Patterns of microbial heterotrophy through changing environments in a marine sediment. *Mar. Ecol. Prog. Ser.* 4: 1–7
- Oshrain, R. L., Wiebe, W. J. (1979). Arylsulfatase activity in salt marsh soils. *Appl. environ. Microbiol.* 38: 337–340
- Pearson, T. H. (1982). The Loch Eil project: assessment and synthesis with a discussion of certain biological questions arising from a study of the organic pollution of sediments. *J. exp. mar. Biol. Ecol.* 57: 93–124
- Reichardt, W. (1986a). Polychaete tube walls as zoned microhabitats for marine bacteria. *Actes Colloques IFREMER* 3: 415–425
- Reichardt, W. (1986b). Enzymatic potential for decomposition of detrital biopolymers in sediments from Kiel Bay. *Ophelia* 26: 369–384
- Reichardt, W. (1987). Microbiological aspects of bioturbation. *Proceedings 22nd European Marine Biology Symposium (Barcelona, 1987)*, Investigacion Pesquera (Suppl.)
- Reichardt, W. (1988). Impact of the Antarctic benthic fauna on the enrichment of biopolymer degrading psychrophilic bacteria. *Microb. Ecol.* 15: in press
- Reichardt, W., Morita, R. Y. (1982). Influence of temperature adaptation on glucose metabolism in a psychrotrophic strain of *Cytophaga johnsonae*. *Appl. environ. Microbiol.* 44: 1282–1288
- Reise, K. (1981). Gnathostomulida abundant alongside polychaete burrows. *Mar. Ecol. Prog. Ser.* 6: 329–333
- Reise, K. (1985). Tidal flat ecology. *Ecological Studies* 54, Springer Verlag, Berlin
- Reise, K. (1987). Spatial niches and long-term performance in meiobenthic Plathelminthes of an intertidal lugworm flat. *Mar. Ecol. Prog. Ser.* 38: 1–11
- Riemann, B., Bjørnsen, P. K., Newell, S., Fallon, R. (1987). Calculation of cell production of coastal marine bacteria based on measured incorporation of (^3H)thymidine. *Limnol. Oceanogr.* 32: 471–476
- Rhoads, D. C., McCall, P. L., Yingst, J. Y. (1978). Disturbance and production on the estuarine seafloor. *Am. Sci.* 66: 577–586
- Ruble, P., Dornseif, B. E. (1978). Direct counts of bacteria in the sediments of a North Carolina salt marsh. *Estuaries* 1: 188–191
- Sayama, M., Kurihara, Y. (1983). Relationship between burrowing activity of the polychaetous annelid *Neanthes japonica* (Izuka) and nitrification-denitrification processes in the sediments. *J. exp. mar. Biol. Ecol.* 72: 233–241
- Scherer, B. (1985). Annual dynamics of a meiofauna community from the sulfide layer of a North Sea sand flat. *Microfauna Marina* 2: 117–161
- Tenore, K. R., Hanson, P. B. (1980). Availability of detritus of different types and ages to a polychaete macroconsumer, *Capitella capitata*. *Limnol. Oceanogr.* 25: 553–558
- Timmer-ten Hoor, A. (1975). A new type of thiosulfate oxidizing nitrate reducing microorganism: *Thiomicrospora denitrificans* sp. nov. *Neth. J. Sea Res.* 9: 344–350
- Thistle, D. (1981). Natural physical disturbances and communities of marine soft bottoms. *Mar. Ecol. Prog. Ser.* 6: 223–228
- Wavre, M., Brinkhurst, R. O. (1971). Interactions between some tubificid oligochaetes and bacteria found in the sediments of Toronto Harbour, Ontario. *J. Fish. Res. Bd Can.* 28: 335–341
- Wetzel, R. L., Christian, R. R. (1984). Model studies on the interactions among carbon substrates, bacteria and consumers in a salt marsh estuary. *Bull. mar. Sci.* 35: 601–614
- White, D. C. (1985). Quantitative physical-chemical characterization of bacterial habitats. In: Poindexter, J., Leadbetter, E. (eds.) *Bacteria in nature, II*. Plenum Press, New York, 177–203
- Woodin, S. A. (1978). Refuges, disturbance, and community

- structure: a marine soft-bottom example. *Ecology* 59: 274-284
- Yingst, J. Y., Rhoads, D. C. (1980). The role of bioturbation in the enhancement of bacterial growth rates in marine sediments. In: Tenore, K. R., Coull, B. C. (eds.) *Marine benthic dynamics*. Univ. of South Carolina Press, Columbia, p. 407-421
- Zola, H. (1967). Sugar phosphate polymers in polychaete tubes and in mineralized animal tissues. *Comp. Biochem. Physiol.* 21: 179-183
- Zottoli, R. A., Carrker, M. R. (1974). External release of protease by stationary burrow-dwelling polychaetes. *J. mar. Res.* 32: 331-342

This article was submitted to the editor; it was accepted for printing on March 2, 1988