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Virus and bacteria dynamics of a coastal sediment: Implication for benthic carbon cycling

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Abstract

We measured microbial heterotrophic activity, bacteria, and virus-like particle (VLP) abundance in homogenized, undiluted, and anoxic enclosures of sediment collected at a coastal station. The bacterial growth rate and VLP net production increased along with the respiratory activity in response to temperature. This suggests that VLPs represent a dynamic component of benthic microbial communities and that the net production of viriobenthos is regulated by the metabolic activity of bacteria. The abundance, net production, and decay rate of VLPs were significantly higher than those encountered in most pelagic systems. However, the rates were lower than the very few available potential rates (three studies) of viriobenthic activity, which all were obtained applying different slurry approaches. Our measurements support the general observation that virus abundance and production correlate with the trophic status of the environment and show that microbial activity can regulate the viriobenthic production in undiluted, homogenized marine sediments. The virus-induced bacterial mortality corresponded to ~20% of bacterial net production and ~2% h^{-1} of the total bacterial population. This is moderate compared with the results of most pelagic studies, and the associated leakage of lysates (dissolved organic carbon) only amounted to 4–8% of the produced dissolved inorganic carbon. Despite high standing stocks and relatively high turnover rates, VLP-induced bacterial lysis represented only a minor shunt in the benthic carbon cycle at the investigated site.

Viruses are abundant in all aquatic environments, and their potential effect on prokaryotic population dynamics and element cycling received considerable attention during the past decade (Fuhrman 1999; Wommack and Colwell 2000; Weinbauer 2004). However, by far, most studies have been dedicated to the planktonic environment. It has been shown that virus infections are responsible for 20–50% of the prokaryotic mortality (Weinbauer 2004) and that the virus-induced lysis of bacteria is a major source of planktonic dissolved organic matter (DOM) production (e.g., Fuhrman 1999; Wommack and Colwell 2000; Middelboe et al. 2003*b*). Despite the well-documented importance of prokaryotic viruses for the pelagic environment, our present knowledge on the distribution and impact of viriobenthos is still very limited.

Sediments play a key role in the marine carbon cycle, and it is generally anticipated that, in coastal environments, 25–50% of the carbon degradation is mediated by benthic processes (Wollast 1991). On the global scale, marine sediments are the quantitatively most important sites for carbon burial, and the burial rate is ultimately regulated by the efficiency of microbial-driven diagenesis (Archer and Maier Reimer 1994). Understanding environmental processes that affect the activity of benthic microbial communities is thus essential for accessing local and global carbon cycling.

The relatively few benthic studies show high virus-like particle (VLP) abundances in sediments and that the number of benthic VLPs is positively correlated to the trophic status of the environment (e.g., Hewson et al. 2001; Danovaro et al. 2002). Generally, measurements of benthic viral abundance have ranged between 10^7 and 10^9 cm^{-3}, which is 10–100 times higher than the densities found in the overlying water column (e.g., Hewson et al. 2001; Weinbauer 2004). In parallel, it has been shown that along with the diagenetic activity, the bacteria and VLP abundance generally decrease with increasing sediment depth (Danovaro and Serresi 2000; Middelboe et al. 2003*a*). Recently, it was also documented that the VLP abundance of an estuarine sediment was strongly correlated to the microbial sulfate reduction rate (Middelboe et al. 2003*a*). These observations suggest that viriobenthos represent a dynamic component of marine sediments with implications for microbial activity and ecology. There exist only very few studies that have estimated the production and turnover rate of viriobenthos (Fischer et al. 2003; Hewson and Fuhrman 2003; Mei and Danovaro 2004), and they present a wide range of VLP production (1–46 $\times$ 10^7 cm^{-3} h^{-1}) and turnover rates (from 1–5 h [Hewson and Fuhrman 2003] to 1–4 d [Fischer et al. 2003]). Therefore, the conclusions on the importance of viriobenthos for benthic bacterial mortality and carbon cycling have differed markedly among these studies. The disparate conclusions can, to some extent, reflect differences in the local environments and thereby the abundance and activity of the microbial community. However, to some extent, the discrepancy may also reflect the very different approaches that were applied and the use of highly manipulated systems. Obviously, more studies are needed to quantitatively assess the importance of VLPs for benthic carbon cycling.

The present article represents the first attempt to quantify viriobenthic production and decay along with the microbial activity of undiluted, homogenized anoxic sediment samples. VLP turnover was monitored during temperature-induced stimulation and inhibition of the microbial activity, and our

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findings are quantitatively discussed in the context of benthic carbon mineralization.

Material and methods

Study site and sediment sampling—On 5 August 2003, 15 sediment cores were collected at 35 m water depth in central Øresund, Denmark (55°58′N, 12°48′E). The salinity was 30, and the in situ temperature was 10°C. The cores (internal diameter = 13.2 cm) were kept in bottom water at in situ temperature on board the ship until they were placed in a thermoregulated laboratory on land and kept at 10°C. They were then left in aerated bottom water for 1 week before further processing.

From each sediment corer, three subcores (inner diameter = 5.2 cm) were taken; of those, two were used for measuring the porosity and the organic carbon content, six were used to determine the total O_2 uptake rate and the microdistribution of O_2, three were used to quantify the vertical distribution of VLP and bacteria, and the remaining subcores were used for experimental work on the microbial community (see below).

Sediment characteristics and oxygen measurements—The porosity and the content of organic carbon were both measured at a depth resolution of 1.0 cm. The porosity was calculated from the specific density and weight loss after drying at 105°C for 24 h, and the organic carbon content was determined as the weight loss after combustion at 450°C for 24 h.

Oxygen microprofiles were measured at a depth resolution of 100 μm, as measured by a Clark-type microelectrode (Revsbech 1989). The sensors were positioned by a motorized micromanipulator, and the signals were measured by a pico ampmeter (Unisense) connected to a laptop computer. A total of eight profiles were measured in three different sediment cores, in which attached rotating bars ensured a well-mixed overlying water phase and an approximate diffusive boundary layer (DBL) thickness of 400 μm (Rasmussen and Jørgensen 1992). The diffusive O_2 uptake was calculated from the linear concentration gradient within the DBL applying Fick's first law of diffusion (Rasmussen and Jørgensen 1992).

The total exchange rate of O_2 and dissolved inorganic carbon (DIC) was measured the next day after all six sediment cores were capped. The lid was perforated by a small hole, which allowed the O_2 microsensor tip to be inserted consecutively in the respective cores, and a linear decrease in O_2 was confirmed in all cases. During measurements, the rotating bars ensured a similar DBL thickness as during the profiling measurements. Samples of DIC were collected at the start and end of the incubation and were preserved in 7-ml gas-tight glass vials (Exetainer; Labco) spiked with 200 μl saturated $HgCl_2$ until analysis. The DIC concentration was measured on an infrared gas analyzer (ADC-225-MK3) after acidification. The total exchange rates were calculated from the measured concentration changes, accounting for the volume of enclosed water.

Würgler bag experiments—Approximately 30 subcores were selected and placed in a glove bag under an oxygen-free N_2 atmosphere. Because the study focused on the dynamic of anoxic sediments, the upper centimeters that contained the oxic horizon were discarded, and the depth interval from 1–5 cm of all cores was pooled and carefully but thoroughly mixed. Larger obstacles like shells, burrow linings, and macrophyte rests were removed. The mixed sediment was divided into four equally sized gas-tight Würgler-bags (Hansen et al. 2000). Two bags (called 10A and 10B) were placed at 10°C, and the two remaining bags were placed at 5°C (5A) and 13°C (13A), respectively. During a period of 8 d, samples for measuring the DIC concentration and samples for bacteria and VLP enumeration were collected from each bag on a regular basis (for DIC, every ~15 h; for bacteria and VLP abundance, ~6 h; *see Figs. 3, 4 below*). All samplings were conducted under an oxygen-free N_2 atmosphere. After 4 d, mitomycin C was added to a final concentration of 1 μg ml^{-1} to one of the two control bags (bag 10B).

On each sampling occasion, 9.5–10.5 g of sediment (with a precision of 0.005 g) was transferred to a 50-ml centrifuge tube, and 4 ml of virus-free (0.02 μm filtered) bottom water and 1 ml of glutaraldehyde (3% final concentration) were added. The samples were further processed within 24 h, to extract the VLP and bacteria population. We basically followed the procedure of Danovaro et al. (2001), adding sodium pyrophosphate to a final concentration of 5 mmol L^{-1}, followed by 2 × 1 min of sonication before centrifuging for 5 min at 700 *g*. The supernatant was collected in 15-ml centrifuge tubes. The sediment samples were subsequently washed twice with 2 ml of virus-free water, and the extracted volume was pooled with the above mentioned supernatant. The additional washing increased the amount of VLP and bacteria extracted from the sediment (Danovaro et al. 2001; Middelboe et al. 2003*a*). This procedure collected the majority of the extractable VLPs, and only 10% of the extracted VLPs were released during the second wash (Middelboe et al. 2003*a*). The treatment was less efficient toward the bacteria population, where 20% of the extractable bacteria were released during the second wash (Middelboe et al. 2003*a*). Within 24 h, 100 μl of the VLP-bacteria extract was filtered onto a 0.02-μm Anodisc filter and stained with SYBR-Green I and II (Noble and Fuhrman 1998; Weinbauer et al. 1998). The discovery of VLPs with single-stranded DNA in sediments (Middelboe et al. 2003*a*) led us to combine the two stains, because both double- and single-stranded DNA, as well as RNA, are labeled by this procedure. One alternative procedure could be to use SYBR Gold staining, which is supposed to stain all nucleic acids (Chen et al. 2001). On each slide, 300–600 bacteria and VLPs were counted in 10–20 fields using epifluorescence microscopy. To evaluate the accuracy of the enumeration procedure, we made replicate determinations of viral and bacterial abundance in the sediment extract from the different bags at selected time points. It was concluded from replicate sampling (n = 7) that the VLP and bacterial abundances were determined with an average accuracy of ±6% and ±5%, respectively. This was close to the replicate determinations of a previous study made in similar sediment, where the average accuracy was

5% (Middelboe et al. 2003*a*). Therefore, we applied this estimate as a general accuracy for the enumeration procedure.

On some occasions, another 10 g of sediment was sampled in gas-tight vials for the determination of DIC, along with the samples for enumeration. These were centrifuged at 1200 *g* for 4 min, and the supernatant was treated as described for the DIC samples of the whole core incubation.

Vertical profiles of VLP and bacterial abundance—Three subcores were sectioned in 1-cm slices for quantifying the vertical distribution of VLP and bacterial abundance. Before extraction, the slices were thoroughly mixed, and the profiles therefore represent the average distribution below a sediment area of 50 cm^2. The same procedure for extraction and enumeration as that described above was applied.

Testing extraction and conservation of VLPs and bacteria—Along with the incubation procedures, reliable extraction procedures are essential for producing trustworthy data. The extraction procedure that we used has been evaluated previously, and it was concluded that the vast majority of the extractable VLPs and bacteria were released from the sediment (Danovaro et al. 2001; Middelboe et al. 2003*a*). However, bacteria were less efficiently extracted than the VLP, and virus : bacteria ratio (VB ratio) may thus have been overestimated. Furthermore, it cannot be excluded that especially silty sediments host a fraction of VLPs and bacteria that not are extractable by the present procedure (Gough and Stahl 2003; Hewson and Fuhrman 2003). For the present sediments, the amount of extractable VLP and bacteria increased linearly with the amount of sediment taken from a conserved homogenized sample, which shows that the extraction potential was not saturated in up to ~15 g of sediment by the applied approach (Fig. 1A).

Another critical point in VLP and bacteria enumeration is to what extent the conservation of sediments, extracts, and slides is efficient. To test this, we repeatedly measured the abundance of bacteria and VLPs in glutaraldehyde-fixed sediments, VLP-bacteria extracts, and SYBR Green–stained slides. Both VLP and bacterial abundance decreased over time in the glutaraldehyde-conserved sample (kept at +5°C), which underscores the importance of quickly processing samples, despite the generally applied conservation procedure (Fig. 1B). We recommend extracting VLPs and bacteria within 48 h after sediment conservation. However, even in extracted samples, their abundance decreased relatively quickly (Fig. 1C), so we recommend processing the extracts within 48 h. The relatively fast decay of VLPs and bacteria in glutaraldehyde-conserved samples (kept at +5°C) may be due to the proteolytic breakdown of bacteria and viruses and the subsequent loss of DNA, as was suggested by Gundersen et al. (1996). This observation must be accommodated in future studies. The SYBR Green–stained slides, however, remained stable, and we obtained constant VLP and bacterial abundances from slides kept at −18°C for up to 3 months (data not shown).

Results

Sediment characteristics—The porosity at the sediment surface was 0.95 v/v and gradually decreased to a constant

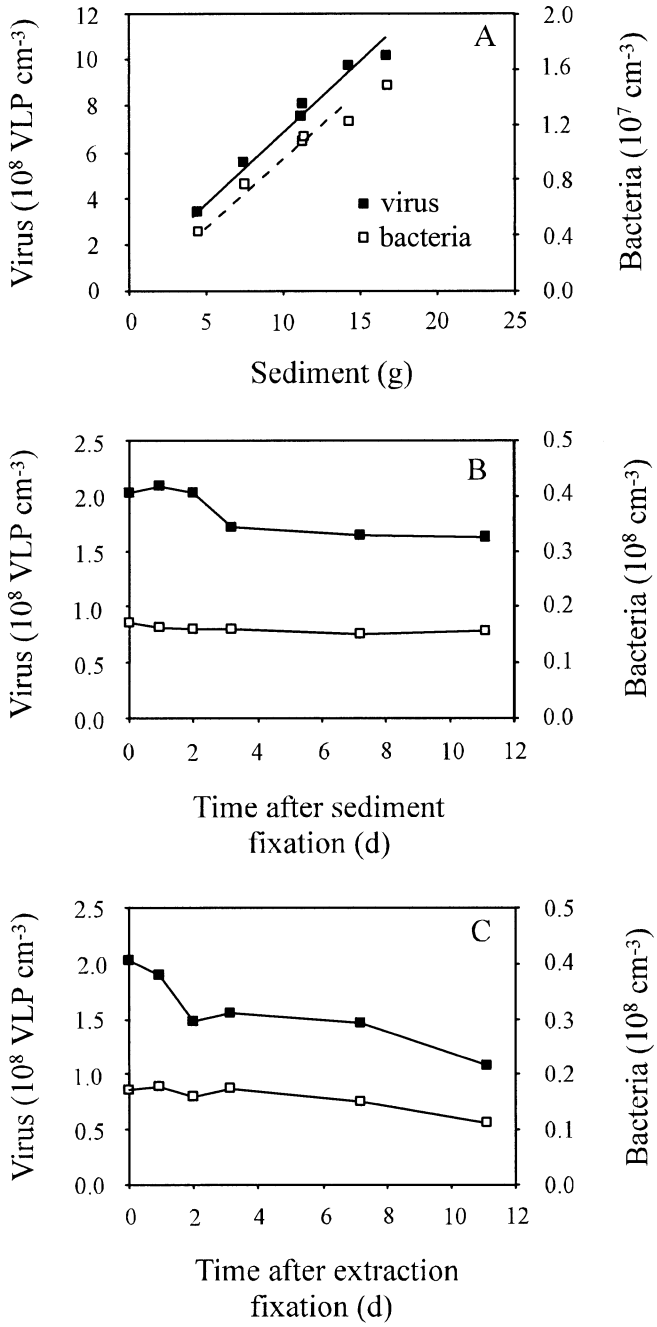

Fig. 1. (A) The extractable virus and bacterial abundance as a function of the amount of sediment used for extraction. (B, C) The amount of virus and bacteria in glutaraldehyde conserved samples kept at +5°C as a function of the number of storage days.

level of ~0.80 v/v at a sediment depth of 2.5 cm (Fig. 2A). The depth profile of the organic carbon content reflected enrichment at the sediment surface but also reached a more or less constant value of 7% at 2.5 cm (Fig. 2A). The VLP abundance decreased markedly from 3.8×10^8 cm^{-3} at the sediment surface to 2.0×10^8 cm^{-3} at 2.5 cm (Fig. 2B). Hereafter, the abundance only decreased slightly, reaching a minimum value of 1.5×10^8 cm^{-3} at the bottom of the core. The values are considerably higher than for the water col-

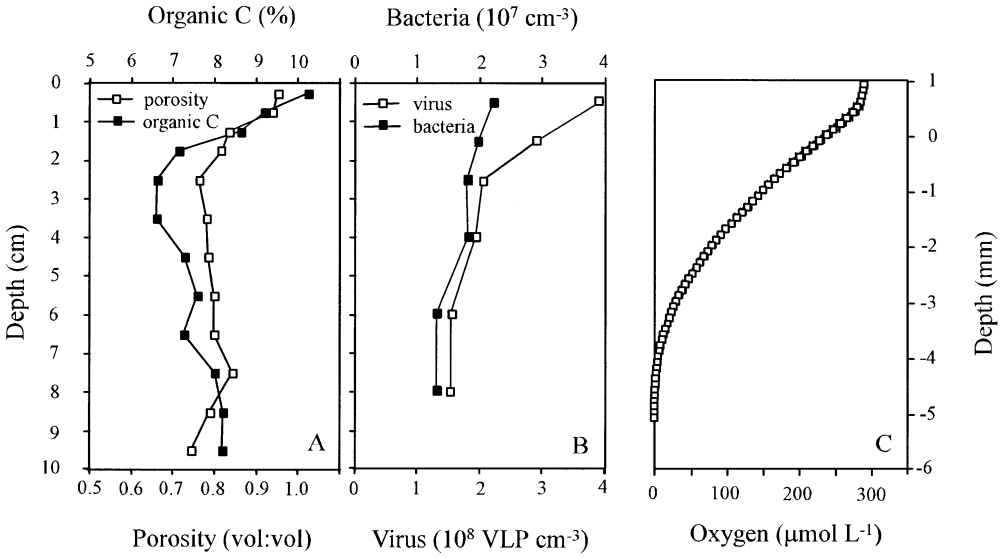

Fig. 2. (A) Vertical profiles of the porosity and organic carbon content, (B) the virus and the bacterial abundance profiles, and (C) the oxygen concentration profiles at the investigated site. The "0" value on the *Y*-axis indicates the position of the sediment-water interface.

umn in the same area (Riemann and Middelboe 2002), but both values and the distribution pattern match the results of previous benthic studies of coastal environments (e.g., Hewson at al. 2001; Middelboe et al. 2003*a*). The depth profile of the bacterial abundance closely followed the VLP abundance, with maximum values at the sediment surface and only a slight decrease from 2.5 cm and downward (Fig. 2B). The VB ratio decreased from 17 at the sediment surface to a constant value of ~11 at a sediment depth of 2.5 cm. This pattern also fits most benthic observations, and the values are intermediary to the published range for coastal sediments (e.g., Fischer et al. 2003; Mei and Danovaro 2004; Weinbauer 2004). The absolute numbers of bacteria were a factor of 10–50 lower than most total counts from marine sediments performed by dilution and 4′,6′ diamidino-2-phenylindole–staining procedures (e.g., Gough and Stahl 2003). However, the possibility exists that bacterial abundance in some of these earlier studies included stained VLP in their bacterial counts.

The average O_2 penetration depth at the investigated site was 4.8 ± 0.2 mm (Fig. 2C), whereas the calculated diffusive-mediated O_2 uptake was 12.9 ± 1.3 mmol m^{-2} d^{-1}. The total O_2 uptake that integrates the uptake of the entire sediment core area was 18.3 ± 5.3 mmol m^{-2} d^{-1}, which reflects a moderate fauna-mediated O_2 uptake of 30% [(18.3–12.9)/18.3] (Glud et al. 2003). The total benthic DIC release was 21.8 ± 6.2 mmol m^{-2} d^{-1}, which results in an apparent sediment respiratory quotient of ~1.2 (18.3/21.8).

Sediment bag incubations—We enclosed the anoxic sediment recovered from the 1–5 cm depth interval, and the DIC concentration of all enclosures increased linearly for ~40 h (Figs. 3A, 4A). The two sediment bags incubated at in situ temperature had DIC accumulation rates of 25.9 and 22.7 μmol L^{-1} h^{-1} (Fig. 3A), which were equivalent to area rates of 24.9 and 21.8 mmol m^{-2} d^{-1}, respectively. This was slightly higher than the intact core incubations that integrated the activity of the entire depth interval (0–12 cm) and indicates that homogenization increased the mineralization activity of the sediment.

The bacterial abundance in the two parallel bags incubated at in situ temperature increased from ~1.6 to ~3.0 × 10^7 cm^{-3} during the first 22 h, after which it declined moderately (Fig. 3B). This fits the observation of elevated mineralization following sediment homogenization and is, to our knowledge, the first documentation of stimulated bacterial growth following sediment homogenization. The VLP abundance followed the bacterial abundance closely, leading to an almost constant VB-ratio during the first half of the incubation (Fig. 3). For both bags incubated at in situ temperature, the net production of VLPs during the initial stage (0–22 h) was ~3.2 × 10^6 cm^{-3} h^{-1}, corresponding to an initial population doubling time of ~29 h (Table 1). The addition of mitomycin C to one of the two parallel bags (bag 10B) at 45 h resulted in a distinct decrease in the bacterial abundance of 3.6 × 10^6 cm^{-3}, whereas the VLP abundance momentarily increased by 5.2 × 10^7 cm^{-3}. Mitomycin C is commonly used to induce lysogenic cells (e.g., Weinbauer and Suttle 1996). The addition indicates that at least 14% of the bacteria population was lysogenically infected and that the average burst size of the infected cells was ~14 (calculated as the number of emerging VLP divided by the decrease in bacteria numbers (3.6 × 10^6/5.2 × 10^7)). This is very similar to direct measurements (15–18) performed by Mei and Danovaro (2004) but is significantly lower than estimates applied in the two other available benthic studies, 25–40 and 50–100 (Fischer et al. 2003; Hewson and Fuhrman 2003).

After cell lysis was induced, the bacterial abundance increased rapidly to the background level, which indicates stimulated growth and an extremely fast consumption of leaked DOC. Simultaneously the VLP abundance decreased by a decay rate of ~3.5 × 10^6 cm^{-3} h^{-1} (Table 1). Apart

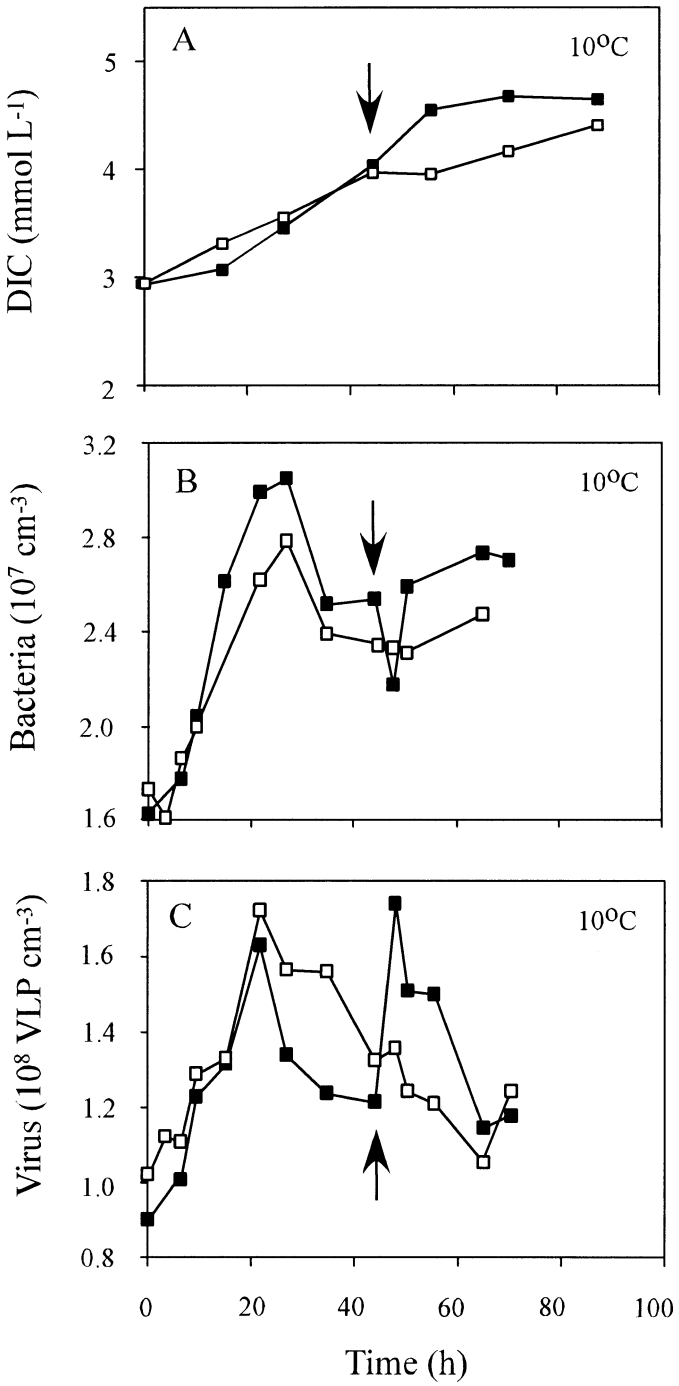

Fig. 3. (A) The DIC concentration, (B) the bacterial abundance, and (C) the virus abundance of homogenized anoxic sediment incubated at in situ temperature in two parallel Würgler-bags. The arrows indicate time of the addition of mitomycin C to one of the two bags (black symbols).

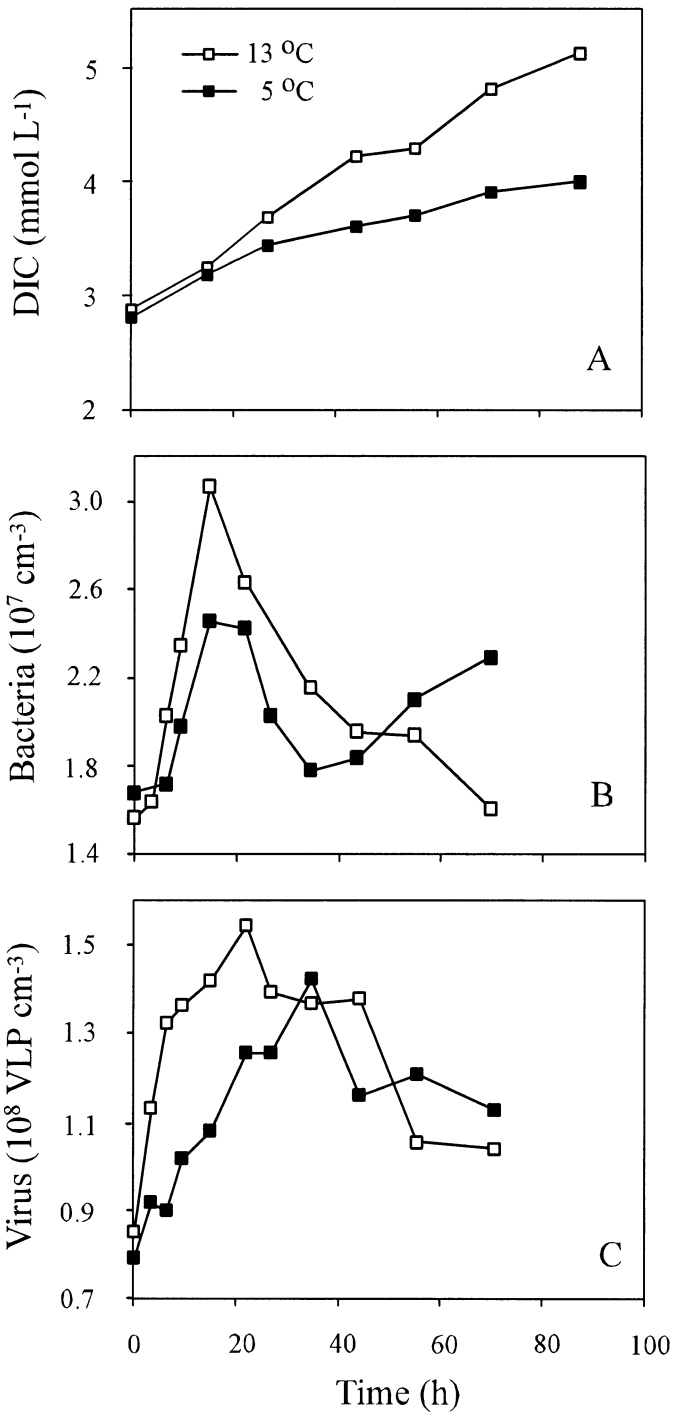

Fig. 4. (A) The DIC concentration, (B) the bacterial abundance, and (C) the virus abundance of homogenized anoxic sediment in two parallel Würgler bags incubated at 5°C and 13°C, respectively.

from the abrupt changes induced by the addition of mitomycin C to bag 10B, the development in the two parallel bags kept at the same condition was very similar. During the second half of the incubations, the mineralization rate was significantly reduced (Fig. 3A), and the bacterial abundance reached an elevated but constant level in both bags. After reaching the peak, the VLP abundance in the control bag (10A) gradually decreased, with an average decay rate of $\sim 1.6 \times 10^6$ cm^{-3} h^{-1} (Table 1).

The initial mineralization activity in the bag incubations was markedly affected by the temperature treatments (Fig. 4). During the first ~40 h, the DIC accumulation rate at 13°C was 30.9 μmol L^{-1} h^{-1}, and the corresponding value at 5°C

Table 1. Virus production and decay.

Incubation	Initial virus net production (VLP 10^6 cm^{-3} h^{-1})	Doubling time of initial population (h)	Net decay rate* (VLP 10^6 cm^{-3} h^{-1})	Population elimation time* (h)
10A	3.0	33	1.6	108
10B	3.4	25	3.5†	49†
5A	1.8	44	0.6	283
13A	6.7	13	1.1	141

* Calculated after the initial abundance peak was reached.
† Calculated after the addition of mitomycin.

only was 18.1 μmol L^{-1} h^{-1}. This difference in activity was reflected in the bacterial abundances. In the heated bag, the bacterial abundance increased rapidly and reached the maximum value of 3.1×10^7 cm^{-3} after only 15 h, after which the abundance gradually decreased to the initial value. The response of the bag incubated at 5°C was delayed and never reached the same maximum abundance as the heated sample. The VLP abundances roughly followed the bacterial abundance but with a delayed response, so that the peak was reached some hours later than the bacteria peak (Fig. 4). The VLP net production rate during the initial phase (0–22 h) was 6.7 and 1.8×10^6 cm^{-3} h^{-1}, corresponding to initial population doubling times of 13 and 44 h at 13°C and 5°C, respectively (Table 1). The VLP abundance decreased after the peak value, with an average decay rate of 1.1 and 0.6×10^6 cm^{-3} h^{-1} at the two respective temperatures (Table 1). As for the bags incubated at in situ temperature, the overall mineralization rate was reduced during the second half of the incubation.

Discussion

Bacteria-virus dynamics—Both the VLP and the bacteria net production were positively correlated to the ambient temperature and thereby to the microbial activity of the sediment enclosures (Table 1, Fig. 5). The net VLP production was, however, stimulated 5, 3, and 2 times more than the net bacteria production rates at 13°C, 10°C, and 5°C, respectively (Fig. 5). This was most likely the result of a positive relation between the bacteria growth rate and the VLP burst size (Middelboe 2000) and because stimulated metabolic activity can lead to the lysis of a larger fraction of lysogenic infected cells (e.g., Williamson et al. 2002). Nevertheless, the data strongly suggest that the viriobenthos net production was regulated by the inherent microbial activity, as has been observed in the pelagic environment (Weinbauer 2004).

Our net VLP production rates (Table 1) were substantially higher than those of most reports from pelagic, open-water environments (e.g., Steward et al. 1992), matched rates from eutrophicated water columns (e.g., Furmann and Noble 1995; Weinbauer 2004), and were ~10–100 times lower that what was reported in the few other available measurement of viriobenthos production: $1–16 \times 10^7$ cm^{-3} h^{-1} (Mei and Danovaro 2004), 12.2×10^7 cm^{-3} h^{-1} (mean from Fischer et al. 2003), and $0.4–4.6 \times 10^8$ cm^{-3} h^{-1} (Hewson and Fuhrman 2003)—the latter and highest range was, however, only reported as a potential rate. Unfortunately, no metabolic activity was measured during those studies, hampering any speculation about the reason for the observed differences. Most previous measurements were performed on oxic surface sediments, which are expected to have a relatively higher in situ respiration activity, even though the natural O_2 distribution was poorly defined and poorly reestablished during these incubations. The observed range for the existing database may thus indeed reflect a correlation to the inherent metabolic activity, as we observed in the present study. Because the metabolic activity seems to be a prime factor regulating the VLP production rate, it is a crucial parameter to include in future studies. The sediment that we studied exhibited relatively high mineralization rates for coastal shelf areas in general (e.g., Glud et al. 1998 and references therein) but were typical for a more shallow, eutrophic environment (e.g., Jørgensen 1996).

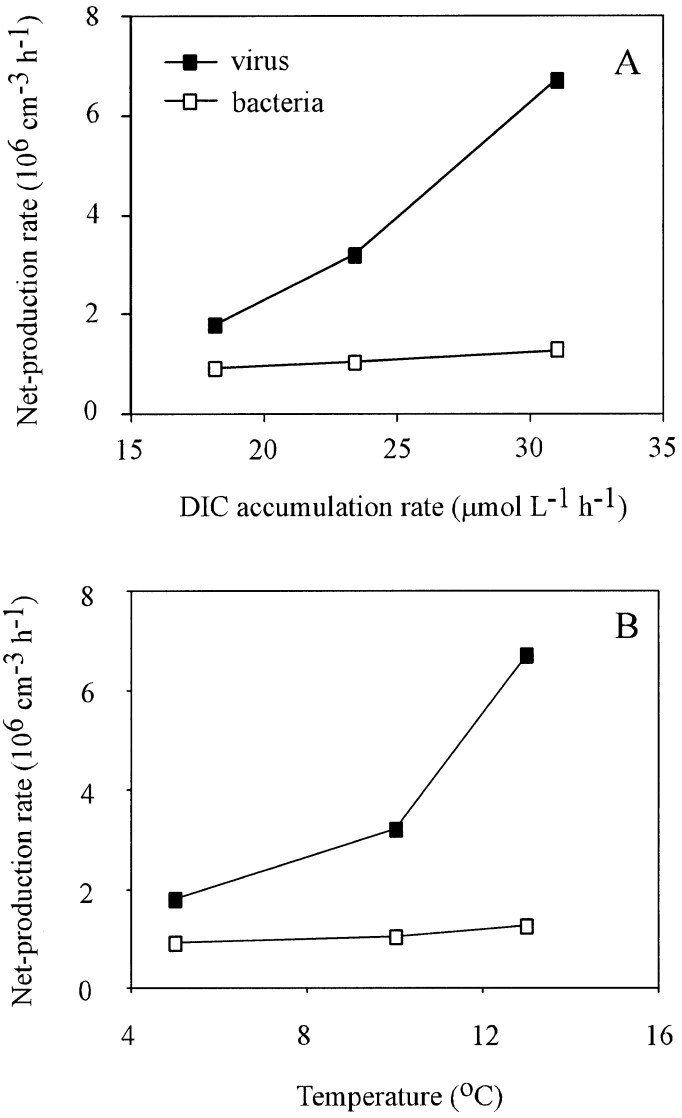

Fig. 5. The virus and bacteria net production as a function of (A) the carbon oxidation rate and (B) the incubation temperature of the anoxic sediment enclosures.

Würgler bag incubations have become a standard procedure for quantifying the total anaerobic mineralization rate of marine sediments (e.g., Canfield et al. 1993), even though sediment homogenization leads to elevated mineralization rates (e.g., Hansen et al. 2000). This is ascribed to increased solute and particle transport, the decay of enclosed fauna, and the break down of spatial heterogeneity (Hansen et al. 2000). Nevertheless, the approach is advantageous to other incubation approaches as dilution and slurrying, which are known to dramatically stimulate benthic heterotrophic activity (Hansen et al. 2000). The approach therefore still represents the best option to quantitatively assess the total anaerobic degradation at a given sediment horizon and provide a flexible cosmos for mechanistic studies of bacteria-VLP dynamics with only moderately manipulated conditions. The relatively low rates of viriobenthos production that we obtained may, to some extent, reflect the more gentle incubation procedure that stimulated microbial activity and, thereby the viriobenthos production, to a less extent, compared with previous slurry-based approaches (Fischer et al. 2003; Hewson and Fuhrman 2003; Mei and Danovaro 2004).

VLP-induced lysis is of quantitative importance for the prokaryotic mortality in the water column (Weinbauer 2004). Applying our estimated burst size (14) and the net VLP production at in situ temperature (Table 1), it follows that VLP-induced lysis caused an initial bacteria mortality rate of 2.3×10^5 cm^{-3} h^{-1}, corresponding to 21% of the net bacterial production (Fig. 5). The equivalent values from the bags incubated at 5°C and 13°C amounted to 14% and 38%, respectively, but, as was stated above, the burst size might be a function of temperature, which compromises direct comparison. However, VLP-induced mortality of the total bacteria population only amounted to 1.5% h^{-1} at in situ temperature (1.0% and 2.5% at 5°C and 13°C, respectively). This was less than was obtained for slurred sediment samples (4–14% h^{-1}, Hewson and Fuhrman 2003; and 3.3% h^{-1}, Mei and Danovaro 2004), which are the only other available marine studies.

Very low VLP-induced mortality rates of a freshwater sediment (~0.2% h^{-1}) was ascribed to very low VB ratios and low in situ particle transport coefficients (Fischer et al. 2003). The decrease in VLP abundance immediately after the addition of mitomycin C resulted in a maximum VLP decay rate of 3.5×10^6 cm^{-3} h^{-1}. Using the decrease that followed the initial peak in VLP abundance of the remaining incubations (time interval = 35–70 h) gave VLP decay rates of $0.6–1.6 \times 10^6$ cm^{-3} h^{-1} (Table 1). There was no clear correlation to the temperature of the individual bags, even though bag 5A (incubated at +5°C) did give the lowest value. The net decay rates did not solely represent the breakdown of VLPs but included those that disappeared as a result of adsorption to bacteria hosts. The present net decay rates represent, to our knowledge, the only marine estimates and are somewhat lower than those estimated from a KCN-inhibited freshwater sediment, where an average VLP gross decay rate of 12×10^7 cm^{-3} h^{-1} was reported (Fischer et al. 2003), but our rates match estimates from eutrophicated water columns (e.g., Fischer and Velimirov 2002). Nevertheless, our net rates suggest a relatively fast turnover of the interstitial VLP population leading to the complete elimination of the viriobenthos population after 49–108 h at in situ temperature (Table 1) if VLP production ceases. It is, however, to be expected that different subpopulations have different decay rates, and the mitomycin experiment did indicate a faster decay of newly produced VLPs and that older VLPs decay at a substantially lower pace (Table 1).

The present work clearly documents that VLPs can represent a dynamic component of the benthic microbial community with potential implications for bacteria mortality. Increased metabolic activity induces cell lysis and, expectedly, an increase in the interstitial pool of labile DOC. This so-called viral loop has proved to be of significant importance for C-cycling in the pelagic environment (e.g., Wilhelm and Suttle 1999; Middelboe et al. 2003*b*) but has so far only been quantitatively assessed in two benthic studies performed in very different environments (Fischer et al. 2003; Hewson and Fuhrman 2003).

Implication for the interstitial carbon cycling—The microbial activity of coastal marine sediments undergoes a large seasonal variation, partly as a result of temperature changes but mainly due to variations in input of labile organic material. In a yearly study of sediments very similar to the ones investigated here, the in situ aerobic microbial activity varied by a factor of ~13 (Glud et al. 2003), whereas the laboratory determined sulfate reduction rate in some horizons of the same sediment varied by a factor of >6 (Thamdrup et al. 1994). The periods with elevated activity were associated with sedimentation events, and the material was rapidly consumed as a consequence of labile carbon limitation. Labile organic material has been shown to have a very fast turnover time in marine sediments (e.g., Hansen and Blackburn 1992). From pelagic studies, it is known that the microbial respiration of noninfected cells can increase as a result of increased DOC availability from VLP-induced cell lysis (Middelboe and Lyck 2002). Even though our study only represents a single selected sampling site, it demonstrates that a similar scenario could occur in sediments and that a sudden increase in microbial activity stimulates the lysis of bacteria by VLPs, presumably with a concurrent increase of the labile DOC pool and thereby a subsequent bust in the activity of noninfected cells.

From the bags incubated at in situ temperature, we estimated a bacteria mortality rate of 2.3×10^5 cm^{-3} h^{-1}. Under the assumption of an average prokaryotic cell carbon content of 50–100 fg C (Simon et al. 1990), cell lysis amounted to a labile DOC production of ~1.0–1.9 nmol cm^3 h^{-1}, which is equivalent to 4.1–7.9% of the simultaneously measured DIC production.

The values are based on net VLP production rates and therefore do not account for any concomitant VLP decay; furthermore, they are very dependent on the applied value of the cell carbon content. Nevertheless, they indicate that the viral loop in benthic communities is of less quantitative importance for C-cycling than what has been encountered in the pelagic environment. The somewhat surprising finding does, however, align with the conclusion of Fischer et al. (2003), who found that only 6% of the bacterial secondary production in freshwater sediment could be ascribed to VLP-induced DOC production. The only other quantitative ben-

thic marine study found VLP induced production of 7.5–38 nmol cm^{-3} h^{-1}, which is somewhat higher than ours, but, as was previously mentioned, they were obtained from a highly manipulated cosmos and were reported as potential values (Hewson and Fuhrman 2003). Still, the VLP-induced DOC production of that study only amounted to 6–11% of the average carbon sedimentation rate of the area (Hewson and Fuhrman 2003).

Obviously, much more work is required before conclusive statements can be made about the importance of viriobenthos for benthic C cycling. But the very few quantitative studies (four, including the present study) seem to suggest that, even though VLP abundance and production rates in sediments are relatively high, the impact they have on the benthic carbon cycling only seems to be moderate. However, coastal sediments are very diverse and dynamic; fauna constantly perturbate the sediment, resuspension events redistribute material, sedimentation of carbon-enriched aggregates creates short-lived hotspots, and benthic primary production locally changes the redox conditions and the nutrient concentration (e.g., Fenchel and Glud 2000; Wenzhöfer and Glud 2004). The presently available techniques for accessing the importance of viriobenthos only poorly reflect in situ conditions, and only few benthic communities have so far been investigated. Furthermore, extraction procedures are imperfect, and less extractable, attached microbes are not accounted for in most studies. It might be speculated, however, that these most likely represent a less dynamic and less active component of the benthic community. Optimized and standardized approaches for incubation and extraction have yet to be developed, and the applied techniques have to be thoroughly evaluated to facilitate any comparison between different studies.

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