

Bacterial growth and grazing on diatom aggregates: Respiratory carbon turnover as a function of aggregate size and sinking velocity

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Abstract

Bacterial growth, respiration, particulate organic carbon (POC), and particulate organic nitrogen (PON) were measured directly on differentially sized diatom aggregates incubated individually in suspension in order to study the coupling between these parameters under controlled conditions. After 3 d of incubation, bacteria, flagellates, and ciliates were present on aggregates in the ratio of 1,100:30:1. Bacterial generation times ranged from 0.4 to 2 d, but these short generation times did not result in an increase of bacterial abundance because bacteria were grazed approximately at similar rates. The entire microbial community respired 2.90 carbon units for each carbon unit incorporated by the bacteria. Bacterial production, community respiration, POC, and PON increased with increasing aggregate size, and respiration was proportional to POC and PON content. The POC specific respiration rate on aggregates was 0.083 d^{-1} , and 40% of the initial POC content was respired after 6 d. From simple calculations combining carbon-specific respiration rates and aggregate sinking velocities, it is concluded that a tight coupling between POC and microbial respiration may control the carbon fluxes of sinking diatom aggregates in the ocean.

Macroscopic organic aggregates $>0.5 \text{ mm}$ (marine snow) comprise a significant fraction of the sinking flux of particulate organic carbon (POC) in the ocean (Shanks and Trent 1979; Fowler and Knauer 1986). Sinking aggregates, therefore, are important for the export of carbon fixed within the euphotic zone to the deep ocean and to sediments (i.e., the biological carbon pump). In recent years, macroscopic aggregates $>0.5 \text{ mm}$ (marine snow) have, thus, received increased attention in order to understand fluxes of particulate organic matter in aquatic systems, and particle size spectra and size-specific sinking velocities have been determined within the euphotic zone (Alldredge and Gotschalk 1988; Jackson et al. 1997). The POC and particulate organic nitrogen (PON) content of aggregates is significantly related to aggregate size (Alldredge 1998). The extent to which biological processes are quantitatively linked to the particle size spectrum in aquatic environments, however, is poorly understood because of few experimental studies (Karl et al. 1988; Banse 1990).

Macroscopic organic aggregates are microhabitats highly enriched in phytoplankton, bacteria, and flagellates com-

pared to the surrounding water in lakes and rivers and in the sea (Caron et al. 1986; Alldredge and Silver 1988; Grossart and Simon 1993; Zimmermann and Kausch 1996). Attached microorganisms have been considered to control remineralization of the sinking fraction of POC, which decreases significantly with increasing depth below the euphotic zone in the ocean (Karl et al. 1988; Banse 1990). The carbon demand of the attached bacteria, as estimated from bacterial production rates, however, is often so low that months or years would be required for the bacteria to consume all organic carbon of aggregates after sinking out of the euphotic zone (Karl et al. 1988; Smith et al. 1992). Aggregates have often been pooled during incubation with radio tracers in order to achieve representative estimates of the bacterial production; however, it recently has been shown that the chemical microenvironment of aggregates is significantly different from the bulk water phase, and the diffusion time of radio tracers into aggregates is significantly increased when aggregates are sedimented in an incubation vial compared to when the aggregates are kept in suspension, as in the natural environment. Bacterial production measured on aggregates is therefore significantly higher when the aggregates are incubated individually and suspended, compared to pooled aggregates that are incubated under static conditions (Ploug and Grossart 1999). New studies of respiration rates on sinking marine snow and the size-dependent POC content of aggregates have furthermore indicated that a strong coupling between the aggregate POC and respiration rates on aggregates does indeed occur on sinking aggregates. The turnover time of aggregate carbon due to respiration was 8–9 d, only, and appeared independent on aggregate size. This is because POC on marine snow, as previously measured by Alldredge (1998), and respiration rates show the same dependency on

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aggregate size (Ploug et al. 1999). The quantitative importance of aggregate-attached bacteria to support protozoan growth and the remineralization processes on suspended aggregates may, therefore, be underestimated.

In the present study, the attached microbial biota, bacterial production and community respiration, POC, and PON were measured on suspended diatom aggregates of different sizes incubated under controlled conditions for a week. The coupling between bacterial production, community respiration, POC, and PON was analyzed directly during the decomposition process.

Materials and methods

Sampling and aggregate formation—Aggregates were formed from diatom cultures in order to have a homogeneous substrate for aggregate formation and degradation. Diatom cultures (*Skeletonema costatum*, *Thalassiosira weissflogii*, *Chaetoceros debilis*) were grown in continuous light on B-medium diluted eightfold (Hansen 1989). Silicate was added in a molar ratio of 1:1 relative to nitrate to a final concentration of 150 μM . Diatoms were harvested by filtration on a plankton net (10- μm mesh size) after 7 d of exponential growth and were frozen at -20°C . Immediately after thawing, the diatom slurry was added in the ratio of 1:50 to filtered (mesh size: 80 μm) surface water from Øresund, Denmark, and incubated in 1.4-L Plexiglas cylinders rotating at 2.5 rpm at in situ temperature (16°C) and in darkness (Shanks and Edmondson 1989). The phytoplankton biomass in Øresund was low because of intensive copepod grazing. Aggregates >1 mm diameter formed within 2 h of incubation, and 400 single aggregates were subsequently isolated in 10-ml test tubes with sterile water and placed on a plankton wheel (50 cm wide, 1.5 rpm) in the dark at in situ temperature in order to study the aggregate degradation process during the following week without further colonization and aggregation.

Respiration measurements—Oxygen gradients were measured directly in the diffusive boundary layer (DBL) of individual aggregates, which were kept in suspension above a net in a specially designed flow system with an upward-directed flow that opposed sinking velocity (Ploug and Jørgensen 1999). Flow velocities ranged between 0.58 and 2.0 mm s^{-1} , equal to 50 and 172 m d^{-1} . The flow system was filled with 5 L of surface water from Øresund, filtered through microfiber glass filters (GF/C, Whatman) to avoid smaller particles being collected in the net of the flow system. The oxygen measurements were done at in situ temperature (16°C). The position of the aggregate surface was determined visually by advancing a slender Clark-type microelectrode (Revsbech 1989) toward the aggregate until it touched the upper surface, as observed under a dissecting microscope. The oxygen gradient was measured with a spatial resolution of 50- μm step intervals in the DBL at steady state in darkness. Three to four series of measurements were performed for each aggregate. The oxygen microelectrode (Unisense, Denmark) had a 4- μm -wide sensing tip, a 90% response time of 0.8 s, and a stirring sensitivity of <0.3%. It was calibrated in air-saturated and anoxic water with so-

dium dithionite. The electrode signal was measured by a picoammeter connected to a strip chart recorder. The same aggregates (20 to 24 d^{-1}) were used for bacterial production measurements or POC/PON measurements immediately after oxygen measurements (see below).

Size measurements of aggregate—Dimensions of individual aggregates were measured in the flow system using a dissecting microscope with a calibrated ocular micrometer. The volume was calculated as ellipsoids: $V = 4\pi abc/3$, where a, b, and c are the half-axes in each dimension (Spiegel 1968).

Calculation of respiration rates—Respiration rates of the aggregate community in the dark determines the oxygen gradient within the DBL at the aggregate-water interface because the net oxygen exchange between the aggregate community and the surrounding water occurs through the DBL when mass transfer occurs by molecular diffusion within aggregates. Respiration rates in the aggregates were thus calculated from the O_2 concentration gradient in the DBL (Ploug et al. 1997). The aggregate surface area was calculated for ellipsoids as described by Maas (1994). Respiration rates were converted to carbon equivalents using a conversion factor of 1 mol O_2 to 1 mol CO_2 . The diffusion coefficient for oxygen in water at 15‰ salinity and 16°C is $1.83 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ (Broecker and Peng 1974).

Bacterial production rates—Bacterial production rates were measured using the microcentrifugation method (Smith and Azam 1992). Incorporation of [^3H]-thymidine (^3H -TdR; Fuhrman and Azam 1980) and [^{14}C]-leucine (^{14}C -Leu; Kirchman et al. 1985, Simon and Azam 1989) into the ice-cold trichloroacetic acid precipitate was measured with a dual label approach (Chin-Leo and Kirchman 1988). ^3H -TdR (75 Ci mmol^{-1}) and ^{14}C -Leu (312 mCi mmol^{-1} , both from Amersham) were added to samples and a formalin-killed control and were incubated at in situ temperature for 1 h in the dark.

Bacterial production rates on aggregates were up to one order of magnitude higher when individual aggregates were suspended during incubations on the plankton wheel, compared to when they were sedimented on the bottom of the vial (Fig. 1). All aggregates of the present study were thus incubated singly on the plankton wheel during the measurements of bacterial production in order to simulate the natural flow field experienced by sinking aggregates. Bacterial production rates were measured directly after respiration measurements with the same aggregate (10 to 12 d^{-1}) and on 10–12 additional aggregates with known sizes. Single aggregates were incubated in test tubes filled with 10 ml of sterile filtered bulk water and were kept in suspension by rotating the test tube on the plankton wheel. Final concentrations of the radiotracers were 120 nM, which saturated the uptake systems of both tracers (data not shown). Bacterial cell multiplication was calculated from ^3H -TdR incubation assuming an isotope dilution factor of four and a conversion factor of 2×10^{18} cells mol^{-1} ^3H -TdR (Simon 1990). Bacterial carbon production (BCP) by attached bacteria was calculated from ^{14}C -Leu incorporation assuming an intracel-

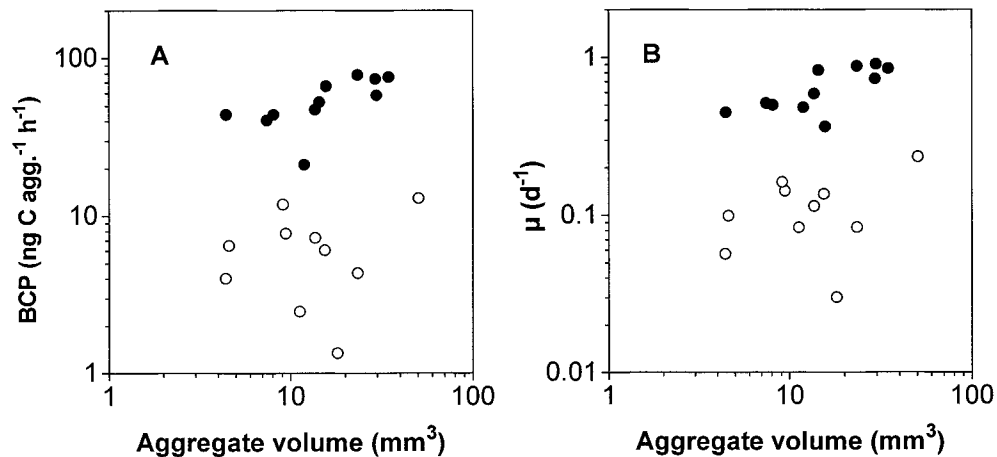


Fig. 1. (A) Bacterial carbon production measured by leucine incorporation rate and (B) growth rate measured by thymidine incorporation rate in suspended (closed symbols) and sedimented (open symbols) aggregates.

lular isotope dilution factor of two (Simon and Azam 1989). The apparent (minimum) bacterial growth efficiency (BGE) was determined from $(BCP / (BCP + \text{community respiration}))$ measured on the same aggregate. Examination of the aggregates under the microscope and measurements of oxygen gradients in light and dark revealed that the diatoms were dead.

Enumeration of bacteria and protozoa—Bacteria were counted on aggregates after 4'6'diamidino-2-phenolindole (DAPI) staining by epifluorescence microscopy (Porter and Feig 1980). In order to remove attached bacteria from single aggregates, these were treated with ultrasonication in 2 mM Na-pyrophosphate (Velji and Albright 1986) prior to filtration onto 0.2- μm Nucleopore membranes. Flagellates and cil-

iates were counted on unfixed DAPI-stained and filtered samples. Ten aggregates with known sizes were examined each day.

POC and PON measurements—Aggregates (10 to 12 d⁻¹) with a known respiration rate and size were gently transferred from the flow system and into precombusted vials with a wide bore pipette, without disrupting the aggregates, and stored frozen until POC and PON measurements. Immediately after thawing, aggregates were transferred into the tin cups used for HCN analysis and dried over night at 65°C. POC and PON were measured on a Carlo-Erba HCN analyzer.

Results

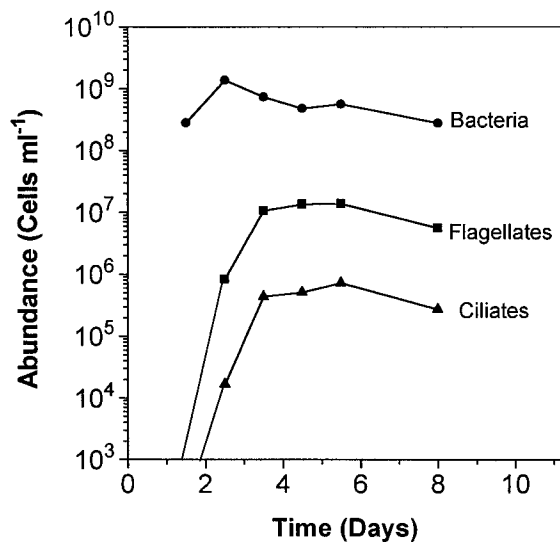


Fig. 2. The abundance of bacteria, flagellates, and ciliates on aggregates during time. The concentration of cells were calculated from the average number and aggregate size. Each data point represents the average value of 10 aggregates.

Bacterial growth was high during the first and second day of the experiment when protozoan abundance was low (Fig. 2), and the bacterial abundance increased on the aggregate (agg) as well as in the surrounding water. The surrounding water in the vials was sterile when the colonized aggregates were added to the vials on day 0.5, but the abundance of bacteria in the surrounding water increased to a maximum of $4.6 \times 10^5 \text{ ml}^{-1}$ on day 2.5, after which it decreased to $<3.7 \times 10^5 \text{ ml}^{-1}$ (data not shown). The maximum abundance of attached bacteria also occurred on day 2.5 with 1.4×10^9 bacteria $(\text{ml agg})^{-1}$, while abundance of flagellates and ciliates were $<1,000$ protozoa $(\text{ml agg})^{-1}$. Bacterial abundance decreased as the abundance of flagellates and ciliates increased during the second and third day, respectively, of the experiment. The bacteria, flagellates, and ciliates on the aggregates occurred in a ratio of 1,100:30:1, on average, after the exponential growth of protozoa had ceased.

Bacterial carbon production and respiration ranged between 0.4 and $4.0 \mu\text{g C agg}^{-1} \text{ d}^{-1}$ in 1.5–2.5-d-old aggregates, when the protozoan abundance was low (Fig. 3A,C). Respiration rates depended on aggregate size rather than on BCP, which was more variable. In 3.5–8-d-old aggregates when protozoa were abundant, the BCP and community res-

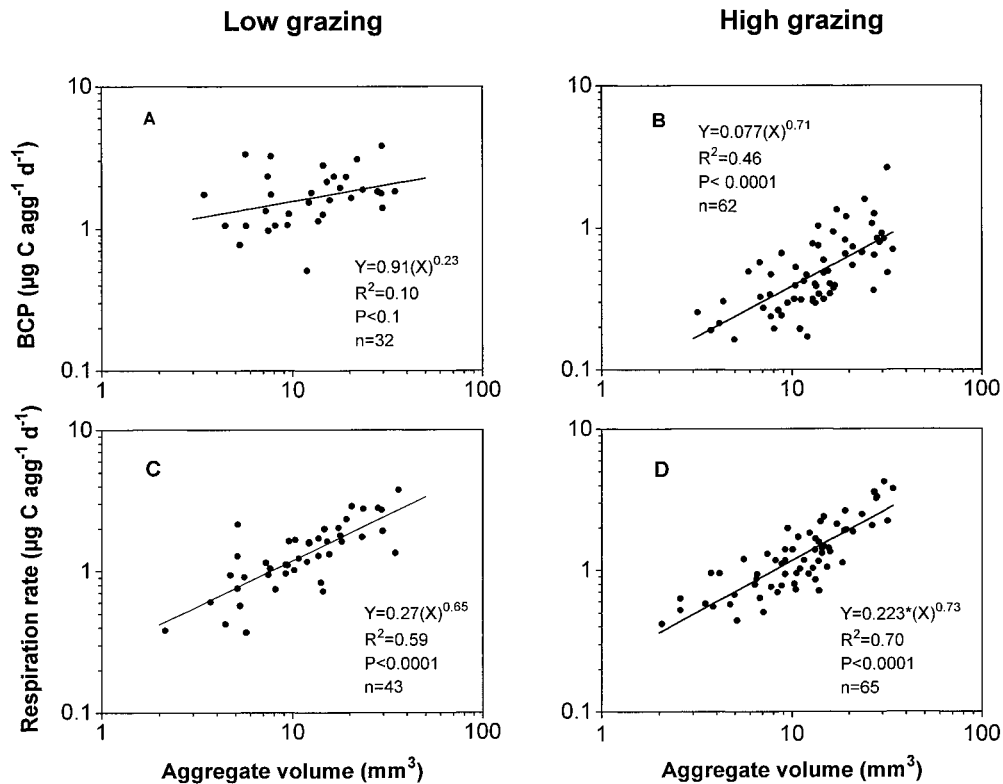


Fig. 3. (A, B) Bacterial carbon production and (C, D) respiration as a function of aggregate size and during different stages in the decomposition process. Low grazing refers to days 1.5 and 2.5, whereas high grazing refers to days 3.5 to 7.5.

piration both increased with aggregate volume to the power of 0.71 to 0.73 (Fig. 3B,D). Bacterial carbon production on 3.5–8-d-old aggregates ranged between 0.15 and $3.0 \mu\text{g C agg}^{-1} \text{d}^{-1}$, and the community respiration was on average 2.90-fold higher than bacterial carbon production for a given aggregate size. Hence, bacterial production decreased more on small aggregates than on larger ones over time, whereas respiration remained relatively unchanged.

Bacterial cell multiplication rates were calculated from the thymidine incorporation rates, and it increased more with aggregate size rather than with number of bacteria per aggregate on 3.5–8-d-old aggregates (Fig. 4A,B). The generation time of the bacteria, however, showed no significant dependency on aggregate size and it ranged between 0.4 and 2 d (Fig. 4C). The apparent (minimum) net growth efficiency of the bacteria was calculated from combined measurements of leucine incorporation and community respiration rates on the same aggregate, and it ranged between 0.1 and 0.5, with an average value of 0.26 (Fig. 4D). Bacterial numbers decreased slightly over time, although their growth rates were relatively high (Fig. 2). Thus, the bacteria were grazed by flagellates at approximately the same rate as they divided, and a significant fraction of aggregate carbon content must be channeled through the food web in order to support microbial growth.

The POC and PON content of 3.5–8-d-old aggregates when BCP and respiration showed a similar dependency on aggregate size is shown in Fig. 5. The POC content (μg

agg^{-1}) increased with increasing aggregate volume (mm^3) as $\text{POC} = 3.25V^{0.66}$, and the PON ($\mu\text{g agg}^{-1}$) content increased with increasing aggregate volume (mm^3) as $\text{PON} = 0.42V^{0.67}$. The C:N ratio was 7.7 and invariant with aggregate size.

Respiration was measured on the same aggregate as POC and PON in order to study directly the coupling between these parameters during the aggregate decomposition process (Fig. 6). The respiration could be described by power functions, where R is the respiration rate ($\mu\text{g C agg}^{-1} \text{d}^{-1}$): POC ($\mu\text{g C agg}^{-1}$), $R = 0.083(\text{POC})^{0.98}$ (Fig. 6A); PON ($\mu\text{g N agg}^{-1}$), $R = 0.60(\text{PON})^{0.99}$ (Fig. 6B). The respiration was, thus, proportional to the POC content, as well as to the PON content, of the aggregate because the power was ~ 1 , and it varied largely within the narrow C:N ratio shown in Fig. 5. The POC-specific respiration rate was $0.083 \pm 0.034 \text{ d}^{-1}$. The carbon turnover time was calculated from the POC content divided by the respiration rate measured in the single aggregates. The turnover time ranged between 5 and 35 d, with an average value of 13.7 d, and it was independent on aggregate size (Fig. 6C), because the respiration rate on average was proportional to the POC content of the aggregate.

When aggregate POC is the substrate for respiration and respiration is the major sink for POC, the respiration and POC can, thus, be described by the first-order process.

$$-dC/dt = kC \quad (1)$$

where $-dC/dt$ is the substrate decrease per unit time (R), C

High grazing

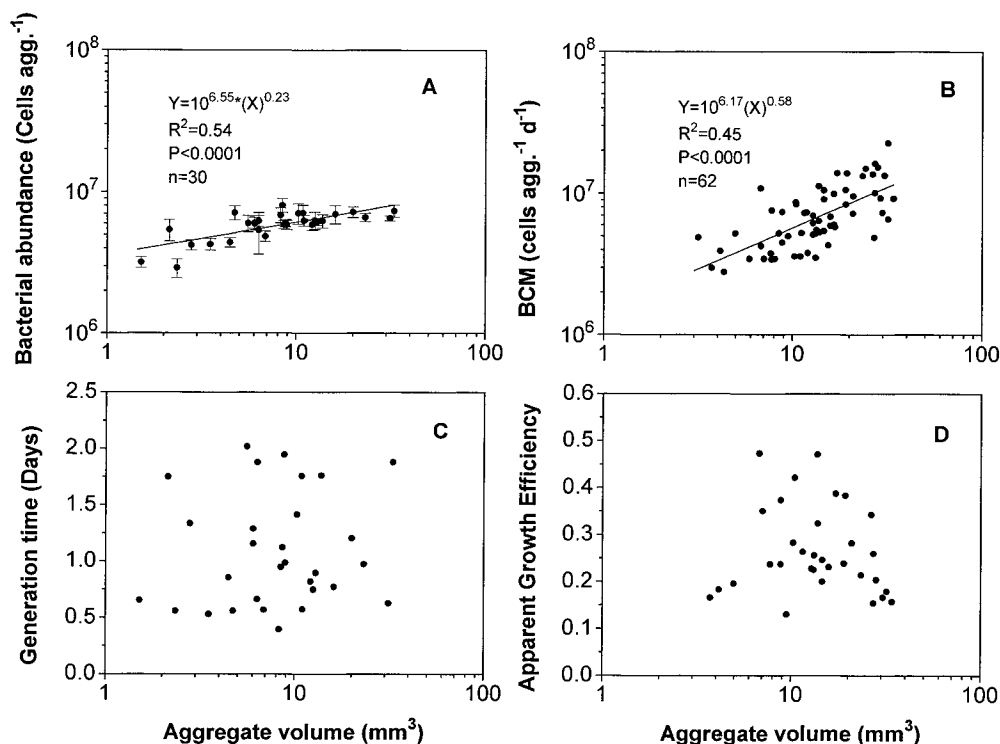


Fig. 4. (A) Bacterial abundance, (B) bacterial cell multiplication rate (BCM), (C) generation time, and (D) apparent growth efficiency as a function of aggregate size.

is the substrate concentration (POC), and k is the carbon-specific respiration rate (R/POC). Solving Eq. 1 yields $POC_t = POC_0 \exp(-kt)$. The POC decreased exponentially over time with $k = 0.106 \pm 0.028 \text{ d}^{-1}$, and $T_{1/2}$ was 6.5 d (i.e., $\ln(0.5)/(-0.106)$). The proportionality constant determined in Fig. 6A. was 78% of that determined from the decrease in POC over time, but the difference was within the uncertainty range of the measurements. The POC and the cumulated carbon respired, as measured directly on the same aggregates over time, is shown in Fig. 7. The decrease in POC was balanced by the carbon respired during time, which accounted for 40% of the initial POC in the aggregate after 6 d of respiration. The average aggregate volume measured on 60 randomly sampled aggregates per day decreased by 37% from day 1.5 to day 5.5. Using the relation between aggregate POC and size (Fig. 5) for these aggregates with known age showed a similar decrease in aggregate POC over time.

Discussion

The succession of bacteria, flagellates, and ciliates on our diatom aggregates was similar to that observed in earlier studies (Biddanda and Pomeroy 1988; Artolozaga et al. 1997). A similar high abundance of bacteria, as well as of flagellates and ciliates, has also been observed on field-sampled marine snow and detrital particles, which can contain high concentrations of amoebae as well. The enrichment of flagellates on aggregates compared to the surrounding water

is often higher than that for bacteria, and aggregates may promote growth of some bacterivorous species as previously suggested (Caron et al. 1986; Turley and Mackie 1994). Bacteria associated with aggregates have been shown to be specialized to utilize the microenvironment with high concentrations of organic matter. A high fraction of bacteria on aggregates are motile on surfaces and capable of hydrolyzing less degradable substrates through ectoenzymatic hydrolysis (Smith et al. 1992; Grossart and Simon 1998). As determined by in situ hybridization techniques, the bacterial community on our aggregates changed, and filamentous bacteria became increasingly more abundant on aggregates during grazing by protozoa (Grossart and Ploug in press). The bacterial generation time ranged between 0.4 and 2 d, and the apparent net growth efficiency was relatively high during grazing by protozoa on the aggregates. Similar short generation times and high growth efficiencies have recently been demonstrated on riverine aggregates, which were incubated with radio tracers while being kept in suspension, similar to the present study (Ploug and Grossart 1999; Grossart and Ploug 2000).

The biomass and growth rates of flagellates and ciliates were not determined in the present study, and respiration by protozoa may contribute significantly to community respiration. The carbon flow and respiration by protozoa during bacterial growth and grazing by flagellates can be calculated for a simple food web where bacteria, flagellates, and ciliates have a net growth efficiency of 33%, and bacteria and fla-

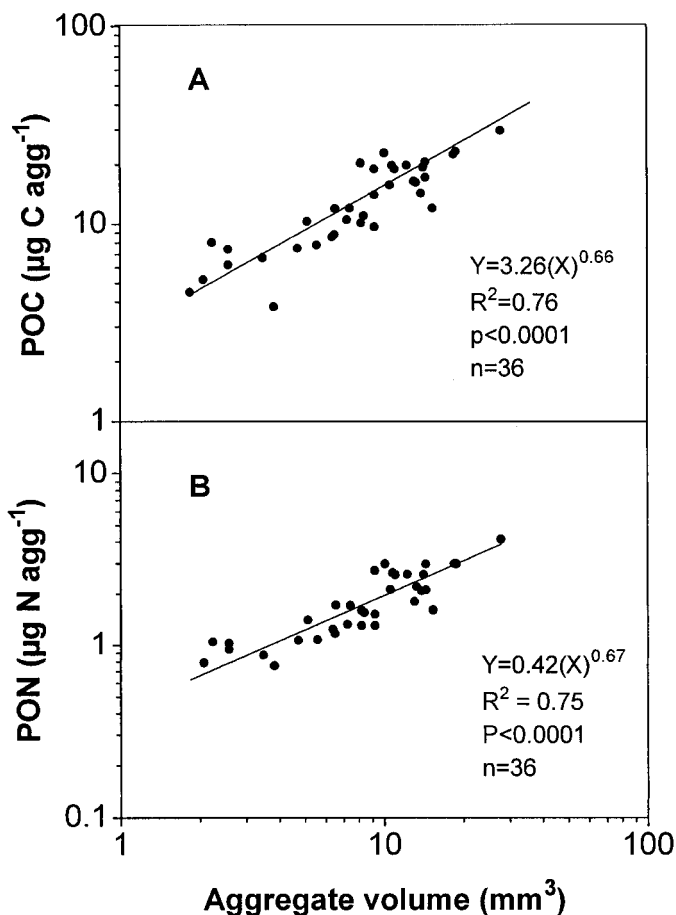


Fig. 5. (A) POC and (B) PON as a function of aggregate size.

gellates are grazed at the same rate as they divide. For each cell division, bacteria need threefold more C than their cell-specific carbon content. One-third of this carbon is incorporated into new bacterial biomass while the other $\frac{2}{3}$ are respired. At steady state, the number of bacteria are constant during cell division because the bacteria are grazed by flagellates at the same rate as they divide, and the same is the case for the flagellates being grazed by ciliates. In this simple food web, $\frac{1}{3}$ of the C incorporated by the bacteria is incorporated by the flagellates, and only $\frac{1}{9}$ of the C incorporated by the bacteria is incorporated by the ciliates per bacterial cell division. For each C unit incorporated by the bacteria, two C units are respired by the bacteria, $\frac{2}{3}$ C units are respired by the flagellates, while $\frac{1}{9}$ C units are respired by ciliates. Thus, 2.88 C units are respired for each C unit incorporated by bacteria, which is close to 2.90, as determined in Fig. 3. As a consequence, 30.9% of the community respiration is due to respiration by protozoa. Because the apparent (minimum) net growth efficiency of the bacteria is $\text{BCP} : (\text{BCP} + \text{total respiration})$, this scenario will result in an apparent net growth efficiency for the bacteria of $1 : (1 + 2 + \frac{2}{3} + \frac{2}{9}) = 0.26$, which is similar to that measured. The actual food web, however, may likely be more complicated because ciliates also graze directly on bacteria and on the particles (Fenchel 1968).

Increasing cell-specific bacterial production and respira-

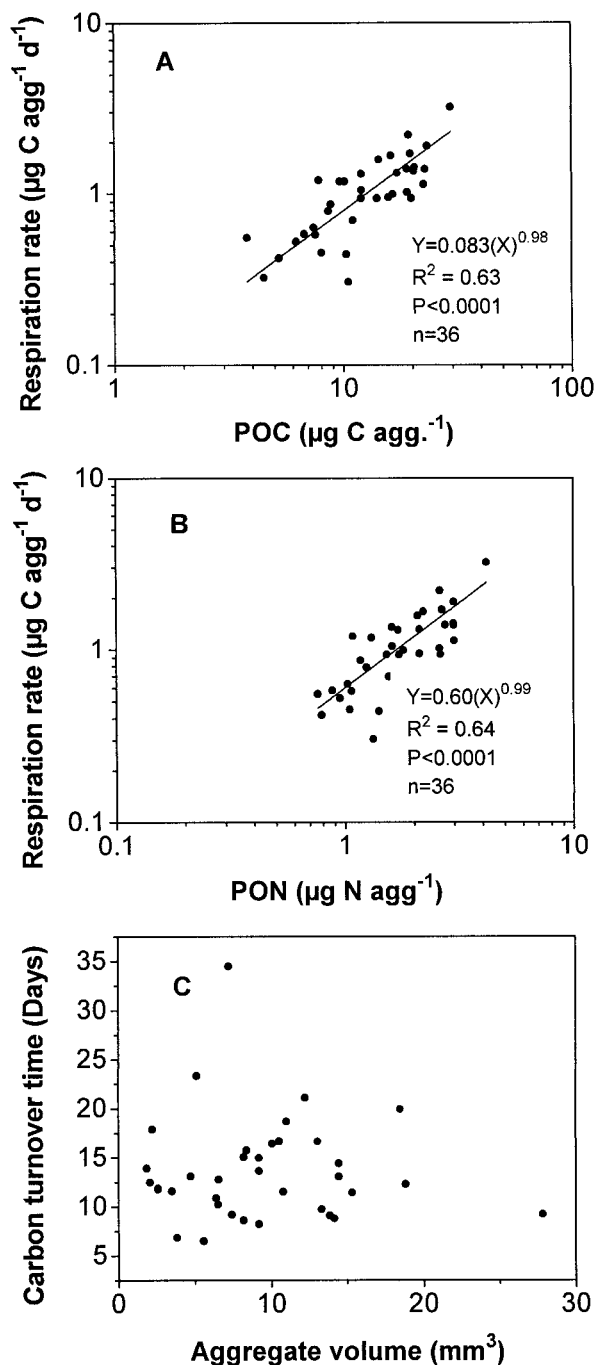


Fig. 6. (A) Respiration as a function of POC content, (B) respiration as a function of PON content, and (C) carbon turnover time versus aggregate volume.

tion with increasing aggregate size have been suggested to be due to more substrate and less competition in larger aggregates with less bacteria per aggregate volume (Allredge and Gotschalk 1990; Ploug et al. 1999). Bacterial production decreased during time more on smaller aggregates with less substrate compared to larger aggregates in the present study. Lab-generated aggregates are often more compact than aggregates formed in the ocean, and the size-specific carbon and nitrogen content were three- to fivefold higher than that

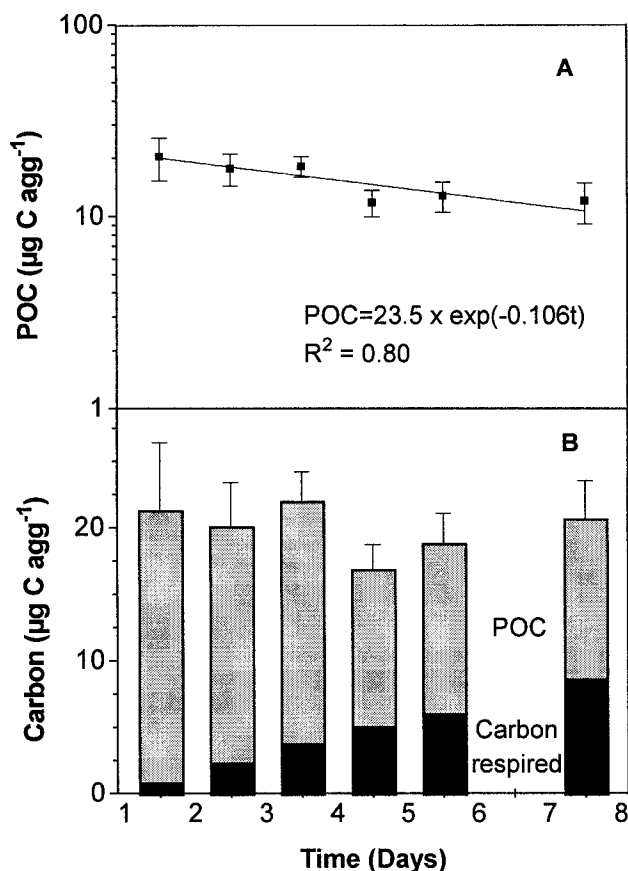


Fig. 7. (A) POC content per aggregate measured over time. (B) POC content per aggregate (gray) and cumulated carbon respiration per aggregate (black). Each point represents the mean value with the standard error of the mean value ($n = 10\text{--}12$ for days 1.5–5.5; $n = 6$ for day 7.5).

of field-sampled aggregates (Allredge 1998). Our aggregates, thus, provided more substrate for microbial growth than aggregates of similar sizes formed in the field. In >3-d-old aggregates, however, BCP and respiration were proportional to the aggregate POC, as well as to the PON content. POC-specific bacterial production rate was 0.029 d^{-1} on average but was more variable than POC-specific respiration rates. This variation was partly because POC-specific bacterial production decreased from 0.11 d^{-1} to 0.019 d^{-1} during the incubation time, and bacterial production was probably limited by the aggregate content of particulate combined amino acids (PCAA) (Grossart and Ploug in press). POC-specific bacterial production rates on pooled samples of diatom flocs collected in the field by SCUBA have ranged between 0.0007 and 0.0087 d^{-1} (Simon et al. 1990; Smith et al. 1992). Our average POC-specific BCP rates were, thus, 3- to 44-fold higher than these previous measurements, whereas our PCAA-specific BCP rate was 47-fold higher on average than those measured by Smith et al. (1992) (Grossart and Ploug in press). This difference may partly be explained by different colonization levels of microorganisms on the aggregates, but the bacterial production may also be underestimated in previous studies because the aggregates were incubated under static conditions. Our BCP

rates decreased up to 10-fold when single aggregates were incubated under static conditions (Fig. 1). The average POC-specific respiration rate measured in the present study was, in contrast, only 25–34% lower than those determined on marine snow collected in the field by SCUBA and measured by exactly the same methods as used in the present study (Ploug et al. 1999).

Previous studies have shown that attached bacteria hydrolyze the substrates in aggregates much faster than they can take up the end products, which then diffuse into the surrounding water and support the growth of free-living bacteria (Cho and Azam 1988; Smith et al. 1992; Grossart and Simon 1998). The decomposition process of aggregates composed of ^{14}C -labeled dead algae colonized by bacteria and flagellates resulted in carbon loss of 20–50% due to $^{14}\text{CO}_2$ production during the first 8 d after aggregate formation (Bidanda 1988). In that study, the respiratory loss of aggregate carbon due to bacteria attached to aggregates could not be distinguished from the fraction of aggregate carbon being hydrolyzed by attached bacteria but respired by free-living bacteria in the surrounding water. In the present study, the respiration was directly measured on the aggregates, where 40% of the aggregate carbon was respired during 6 d. Respiration on aggregates could explain 78% of the observed POC loss on the aggregates; only 22% of the POC loss (i.e., 0.023 d^{-1}) may be due to diffusion of hydrolysis products into the surrounding water. The POC-specific carbon loss due to release of amino acids on diatom aggregates measured by Smith et al. (1992) was 0.075 d^{-1} and, thus, 3.3-fold higher than that estimated in the present study but 10–40% lower than the POC-specific respiration rates measured here and on natural marine snow (Ploug et al. 1999).

Aggregates composed of chain-forming diatoms, as used in the present study, comprise a significant fraction of the flux of organic matter in the ocean. The POC and PON content of marine snow of different origin have been shown to be significantly related to aggregate size (Allredge 1998) similar to the respiration rates measured on natural marine snow with few diatoms (Ploug et al. 1999). In the present study it was directly demonstrated that respiration is proportional to POC and PON in diatom aggregates. Respiration on aggregates may thus be directly linked to aggregate size spectrum and proportional to cumulated aggregate carbon and its residence time in the water column. Sinking velocities of marine snow measured in situ varies largely between 20 and 200 m d^{-1} (Allredge and Gotschalk 1988). The sinking velocities of the aggregates of the present study ranged from 50 to 170 m d^{-1} . It has been suggested that the observed carbon fluxes in the ocean may be explained by a slow-sinking and a fast-sinking fraction of settling particles (Karl et al. 1988; Banse 1990). The POC in single aggregates sinking out of a euphotic zone can be calculated for different sinking velocities, assuming a carbon-specific respiration rate of 0.10 d^{-1} , which is the average of the value found in the present study and in field-sampled marine snow (Ploug et al. 1999). Slowly sinking particles would be degraded most within and below the euphotic zone, where their quantitative role in total carbon remineralization is high. The specific respiration rates of particles with sinking velocities of 20 m d^{-1} would be 0.005 m^{-1} , and only 50% of the initial

POC content would be left at $(\ln 0.5)/(-0.005) = 138$ m, whereas fast-sinking aggregates with sinking velocities of 200 m d^{-1} quantitatively add more to the flux at greater depth with specific degradation rates of 0.0005 m^{-1} , similar to values obtained in carbon flux studies in the ocean (Banse 1990). These are minimum estimates, as the dark respiration by algae, which was eliminated in the present study, implies higher carbon-specific respiration rates within and below the euphotic zone, and release of amino acids add to the carbon loss within the same order of magnitude as the respiration. In addition, aggregates are potential food sources for zooplankton and nekton (Lampitt et al. 1993; Green and Dagg 1997; Grossart et al. 1998), which may also be important for their residence time and their remineralization in the water column (Graham et al. 2000; Kjørboe 2000). However, the POC on single aggregates decreases exponentially with residence time (depth) in the water column if the aggregates are not ingested and defecated by higher trophic levels and no further carbon is produced or scavenged during sedimentation, as was the case in the present study.

Conclusion

Slow growth rates of bacteria attached to aggregates and poor efficiency in utilization of the aggregate as a substrate for growth is inconsistent with the fact that bacterial populations on aggregates obviously support growth of high numbers of protozoa and cannot explain the observed decrease in POC flux with depth in the sea due to respiration on sinking aggregates. The present study, using new approaches for bacterial production and respiration measurements, demonstrates a tight coupling between bacterial production, grazing by protozoa, community respiration, POC, and PON on sinking diatom aggregates. Hence, the role of bacterial production and community respiration on sinking aggregates should be reconsidered as an important factor to explain the observed decrease in POC flux with increasing depth in the ocean.

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