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Microbial degradation of organic carbon and nitrogen on diatom aggregates

Hans-Peter Grossart[1]
Institute for Chemistry and Biology of the Marine Environment (ICBM), University of Oldenburg, P.O. Box 2503, D-26111 Oldenburg, Germany

Helle Ploug
Marine Biological Laboratory, University of Copenhagen, Strandpromenaden 5, DK-3000 Helsingør, Denmark

Abstract

The major pathways of transformation of particulate organic matter by heterotrophic bacteria are respiration and production of new biomass. Until today only a limited number of studies have measured simultaneously respiration and production by aggregate-associated bacteria. To study their role in the carbon cycle of aquatic systems we have formed model particles from diatoms (*Skeletonema costatum, Thalasiosira weissflogii, Chaetoceros debilis*) in roller tanks filled with natural seawater from Øresund, Denmark. Changes in bacterial community structure were analyzed by in situ hybridization and revealed members of the *Cytophaga/Flavobacterium* cluster and of the γ subclass of *Proteobacteria* to be the main actors. The combination of radiotracer and microsensor techniques allowed determination of bacterial protein production and community respiration on the same aggregate and hence the apparent growth efficiency. Apparent growth efficiency (bacterial production/[bacterial production + community respiration]) was 0.50 ± 0.03 (se) on 1.5–2.5 d old aggregates and independent of bacterial growth rate. The initial carbon-specific bacterial production and community respiration was 0.082 d^{-1} and 0.084 d^{-1}, respectively. Thereafter, the carbon-specific bacterial production decreased to 0.020 d^{-1}, whereas specific community respiration decreased to 0.057 d^{-1}. Hence, the apparent net growth efficiency decreased, partly as a result of grazing by protozoa, and it was much lower (0.23 ± 0.04) at the end of incubation. Bacterial production was best correlated to particulate amino acids, whereas community respiration was best correlated to particulate organic carbon (POC). Protease activity was correlated to bacterial production and particulate combined amino acid content, whereas β-glucosidase activity was better correlated to POC and community respiration than to particulate combined amino acid content. Turnover times of radiolabeled amino acids increased from 17.8 to 1,190 h during incubation and were tightly coupled to particulate combined amino acids and POC. Eighty-seven percent of the decrease in particulate organic nitrogen (PON) over time could be explained by turnover of particulate combined amino acids by aggregate-associated food web. Thus, transformation and remineralization of freshly produced particulate organic matter by aggregate-attached food web is significant and the vertical flux of particulate organic matter in the ocean is highly reduced during sedimentation.

Marine and lake snow (larger than 0.5 and 0.3 mm in diameter, respectively) are characterized as microzones highly enriched with nutrients (Shanks and Trent 1980; Grossart and Simon 1993). High concentrations of nutrients and heterotrophic flagellates and ciliates on these aggregates (Silver and Alldredge 1981; Caron et al. 1982) suggest high activities of aggregate-associated microbes. Whereas measured enzymatic hydrolysis rates are high, those of bacterial production are relatively low (Simon et al. 1990; Smith et al. 1992). This notion implies that large fractions of particulate organic matter are released into the surrounding water and supply free-living bacteria with dissolved organic matter and nutrients (Cho and Azam 1988; Smith et al. 1992; Grossart and Simon 1998). However, radiotracer measurements of bacterial production on aggregates that were kept in suspension during incubation as in the natural environment yielded much higher bacterial production rates (Ploug and Grossart 1999; Grossart and Ploug 2000) than previously reported.

Bacterial growth efficiencies are rather variable in the surrounding water (Del Giorgio and Cole 1998) and on aggregates (Grossart and Ploug 2000) and may reflect different qualities of bacterial substrates. Rates of respiration have been shown to be high relative to bacterial protein production when it is low. This indicates that maintenance energy and/or nutrient supply consume large portions of the total cellular energy budget. Respiration further increases with increasing bacterial production. But growth efficiency was maximal when bacterial production was highest, which suggests that a lower portion of the cellular energy is used for maintenance or nutrient supply. On average, bacterial growth efficiency on 1–3 d old aggregates (Grossart and Ploug 2000) is almost the same as of free-living bacteria growing on organic carbon freshly excreted by phytoplankton (Del Giorgo and Cole 1998). In contrast, increasing fractions of hard-to-degrade substrates such as lignin and humic material may explain decreasing growth efficiency on aggregates over time (Grossart and Ploug 2000).

High turnover times of particulate organic carbon (POC)

[1] Corresponding author (hgrossart@icbm.uni-oldenburg.de).

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are presumably the result of organic matter that is relatively poor in quality. The frequently observed high variability in composition of macroscopic organic aggregates (Alldredge and Silver 1988; Grossart and Simon 1998) results in very different POC turnover times when calculated by community respiration, bacterial production, or potential ectoenzyme activities (Smith et al. 1992; Grossart and Simon 1998; Parparov et al. 1998; Ploug et al. 1999). Much shorter turnover times of particulate combined amino acids on aggregates even when calculated by bacterial production (Smith et al. 1992; Grossart and Simon 1993, 1998) indicate that particulate combined amino acids are the main substrates for bacterial production (Rosenstock and Simon 1993). Since the actual carbon turnover of the aggregate-associated bacterial community depends on their production and respiration rates as well as enzymatic hydrolysis, it is necessary to simultaneously determine these parameters. Measurements of bacterial production contemporary with community respiration (Grossart and Ploug 2000) showed relatively short turnover times of carbon on riverine aggregates. Furthermore, high growth efficiencies of attached bacteria on 1–3 d old aggregates indicate that a large fraction of the organic matter on aggregates is directly used by aggregate-associated bacteria.

Yet it is unclear which fraction of the organic matter is directly used by bacteria on aggregates and which fraction is released into the surrounding water. The combination of radiotracer with microsensor techniques allows direct determination of growth efficiency of attached bacteria when grazing by protozoa is negligible (Ploug and Grossart 1999). Freeze-killed diatoms were incubated in roller tanks to form aggregates of a well-known age and composition and to study changes in bacterial production and community respiration in relation to changes of bacterial community structure and the amount of POC, particulate organic nitrogen (PON), as well as particulate combined amino acids of the aggregates during time. Thus, we were able to quantify the turnover of various organic components, e.g., carbon and nitrogen, and to study the major processes involved in microbial degradation of aggregates.

Materials and methods

Sampling and aggregate formation—Three cultures of diatoms (*Skeletonema costatum, Thalasiosira weissflogii, Chaetoceros debilis*) were grown in continuous light on eightfold diluted B1 medium. Silicate was added to a final concentration of 150 μM (molar ratio of 1 : 1 relative to nitrate). Seven days after reaching exponential growth the algae were harvested by filtration (10-μm plankton net), freeze-thawed, and kept frozen at -20°C until the start of experiments. The defrosted diatom slurry was mixed with prefiltered (80 μm) surface water from Øresund, Denmark, in a constant ratio of 1 : 50 and incubated in 1.4-liter plexiglass cylinders rotating at 2.5 rpm (Shanks and Edmondson 1989). The incubation was performed at in situ temperature (16°C) in the dark. Aggregates, >1 mm in diameter, occurred within 2 h of incubation. By using 10-ml test tubes filled with sterile water, we isolated 400 individual aggregates and their associated microbial community. Time series of various parameters were measured on suspended aggregates, which were incubated on a plankton wheel (rotating at 2.5 rpm) in darkness and at in situ temperature.

Enumeration of bacteria—Bacterial numbers on aggregates were counted after 4′,6-diamidino-2-phenylindole (DAPI) staining in an epifluorescence microscope (Porter and Feig 1980). Attached bacteria were removed from aggregates by using ultrasonication in 2 mM Na pyrophosphate prior to filtration onto 0.2-μm Nuclepore membranes.

Fluorescent in situ hybridization—Aliquots of aggregates (20 μl) were pipetted onto gelatin-coated Teflon microslides (P. Marienfeld KG) and dried at 46°C prior to fixation in 40 μl of fresh paraformaldehyde (4%) for 4 h at 4°C. We have used 16S rRNA oligonucleotide probes to determine the percentages of *Archaea* and *Bacteria*, members of the α, β, and γ subclass of *Proteobacteria*, of the Cytophaga-fexibacterium-bacterioides cluster (*Cytophaga*), and of sulfate-reducing bacteria. Specific sequences of oligonucleotide probes are given by Amann et al. (1995) for *Archaea*, *Bacteria*, α, β, and γ *Proteobacteria* as well as *Cytophaga*, whereas those of sulfate-reducing bacteria are given by Amann et al. (1990, SRB 385) and Rabus et al. (1996, SRB dB).

Respiration measurements—Individual aggregates were kept in suspension above a net in a vertical flow system by an upward directed flow that opposed their sinking velocities (Ploug and Jørgensen 1999) and allowed direct measurement of oxygen gradients in the diffusive boundary layer surrounding the aggregates. A slender Clark-type microelectrode was attached to a micromanipulator, and the position of the aggregate surface was observed under a dissecting microscope. Oxygen measurements were done at a spatial resolution of 50 μm. All measurements were performed at steady state in darkness and at in situ temperature. Dimensions of every single aggregate were measured in the flow system by using a dissecting microscope with a calibrated ocular micrometer. To avoid smaller particles being collected in the net of the flow system, the ambient seawater was filtered through microfiber glass filters (GF/F, Whatman). Filtering of seawater did not influence the respiration rates on aggregates (unpubl. data). The oxygen electrode had a 4-μm wide sensing tip, a 90% response time of 0.2 s, and a stirring sensitivity of <0.3%. Calibration was done in air-saturated and N_2-flushed water. A picoamperemeter connected to a strip chart recorder was used to detect the signal from the electrode. Immediately after oxygen measurements, aggregates were collected for bacterial production measurements (*see below*).

Calculation of respiration rates—Since the net oxygen exchange between the aggregate community and the surrounding water occurs through the diffusive boundary layer, respiration rates of the whole aggregate community in the dark determine the oxygen gradient within the diffusive boundary layer at the aggregate-water interface. Respiration rates in the aggregates were calculated from the measured oxygen fluxes through the diffusive boundary layer (Ploug et al. 1997) and the aggregate surface area for ellipsoids as

described by Ploug et al. (1999). A conversion factor of 1 mol O_2 to 1 mol carbon was used to convert to carbon equivalents. The diffusion coefficient for oxygen in water at salinities 15‰ and at 16°C is 1.83×10^{-5} cm^2 s^{-1} (Broecker and Peng 1974).

Bacterial production—Bacterial production on aggregates was determined directly after the respiration measurements. Bacterial production was measured by incorporation of [^{14}C] leucine (^{14}C-Leu; Kirchman et al. 1985; Simon and Azam 1989) and bacterial growth rate by incorporation of [^{3}H] thymidine (^{3}H-TdR; Fuhrman and Azam 1980) into the ice-cold trichloroacetic acid precipitate using the dual label approach (Chin-Leo and Kirchman 1988). Both radiotracers (^{3}H-TdR [75 Ci mmol^{-1}] and ^{14}C-Leu [312 mCi mmol^{-1}], Amersham) were added to three samples and a formalin-killed control and were incubated at in situ temperature for 1 h in the dark. Single aggregates were incubated in test tubes filled with 5 ml of sterile filtered seawater and kept in suspension by using a plankton wheel (2.5 rpm min^{-1}). Final concentrations of the radiotracers to ensure saturation of the uptake systems were 120 nM for both tracers. For calculation of growth rate we assumed an isotope dilution factor of 4 and a conversion factor of 2×10^{18} cells mol^{-1} ^{3}H-TdR, whereas bacterial production was determined from ^{14}C-Leu incorporation using an intracellular isotope dilution factor of 2 (Simon and Azam 1989). Bacterial growth efficiency was calculated from (bacterial production/[bacterial production + community respiration]) on each aggregate. Since bacterial respiration cannot be measured separately, the calculated apparent growth efficiency is greatly underestimated when respiration by organisms others than bacteria is high. The method also neglects the release of dissolved organic matter by bacteria, e.g., carbohydrates, which reduces calculated growth efficiencies even more. In contrast, net growth efficiency by bacteria is defined as bacterial production/(bacterial production + bacterial respiration).

Turnover rates of dissolved free amino acids and glucose—Turnover of dissolved free amino acids was determined by incubation of individual aggregates with a mix of ^{14}C amino acids (53.2 mCi [milliatom C]$^{-1}$, Amersham) at 0.1 nM final concentration. To determine turnover of glucose, ^{14}C glucose (304 mCi mmol^{-1}, Amersham) was added at 1 nM final concentration. The low concentrations of the radiolabel added ensured that the natural concentration of the substrate did not change significantly. Triplicates and one Formalin-killed control were incubated in horizontally rotating vials at in situ temperature and in the dark for 1 h. The aggregates were filtered onto 0.45-μm nitro-cellulose filter, rinsed with particle-free bulk water, and radioassayed. The turnover of ^{14}C-dissolved free amino acids and ^{14}C-glucose was calculated according to Wright and Burnison (1979).

Hydrolytic enzyme activities—Aminopeptidase and β-glucosidase activities of attached bacteria were measured with fluorogenic substrate analogs (Hoppe 1983) using L-leucine-methyl coumarinylamide and methyl-umbelliferyl-β-D-glucoside, respectively. Hydrolysis was measured on single suspended aggregates with 500-μM substrate, which assured maximum hydrolysis rates at in situ temperature (16°C) in the dark as determined by saturation kinetics. A sample killed with paraformaldehyde (4% fin conc.) served as a control. Fluorescence was measured at 300–400 nm excitation and 410–610 nm emission for both substrates in a fluorometer (TD 700, Turner Design). Activities of aminopeptidase and β-glucosidase of aggregate-associated bacteria were calculated after calibration with methyl-coumarinylamide and methyl-umbelliferyl, respectively.

Release of amino acids and ammonia—The release of dissolved free and dissolved combined amino acids, as well as ammonia, from the aggregate into the surrounding water was measured by rotating (2.5 rpm) individual aggregates in 10-ml glass syringes. The syringes were filled with 0.2-μm filtered bulk water. All incubations were performed at in situ temperature (16°C) in the dark. Subsamples were withdrawn periodically through a three-way valve at the tip of the syringes for subsequent analysis of dissolved free and dissolved combined amino acids, as well as ammonia (*see below*). The net release of substrates into the surrounding water was calculated by their increase in concentration during incubation.

Analysis of amino acids and ammonia—Dissolved free and dissolved combined amino acids, as well as ammonia, were determined by high performance liquid chromatography after precolumn derivatization with o-phtaldialdehyde. Analysis of ammonia by using HPLC and precolumn derivatization was not optimal since the sensitivity factor of ammonia in relation to our internal standard (α-amino butyric acid) was 0.17. Samples for dissolved free and dissolved combined amino acids, as well as ammonia analysis, were prefiltered through 0.2-μm tuffrin filters (Gelman Acrodisc; low protein binding capacity). Whereas dissolved free amino acids and ammonia were directly measured, dissolved combined amino acids and particulate amino acids were hydrolyzed prior to analysis in double-distilled 6 N HCl for 20 h at 110°C. Amino acid oxidation due to high nitrate concentration was prevented by adding 20 μl of ascorbic acid (2 mg ml^{-1}) prior to hydrolysis. Concentrations of dissolved combined amino acids and particulate combined amino acids in moles were calculated as amino acid equivalents.

CHN analysis—Particulate organic carbon (POC) and nitrogen (PON) content of aggregates were determined by CHN analysis (Carlo Erba) after the measurement of aggregate size or respiration rate, respectively. Single aggregates were transferred to tin cups, dried overnight at 60°C, and weighed on a microbalance before CHN analysis.

Results

Characterization of aggregates—Our model aggregates were predominantly composed of fresh phytoplankton detritus from the added diatom slurry (*Skeletonema costatum, Thalasiosira weissflogii, Chaetoceros debilis*). Particulate organic carbon (POC) and nitrogen (PON) were highest on day 1.5 (20.4 and 2.8 μg agg^{-1}, respectively). POC and PON

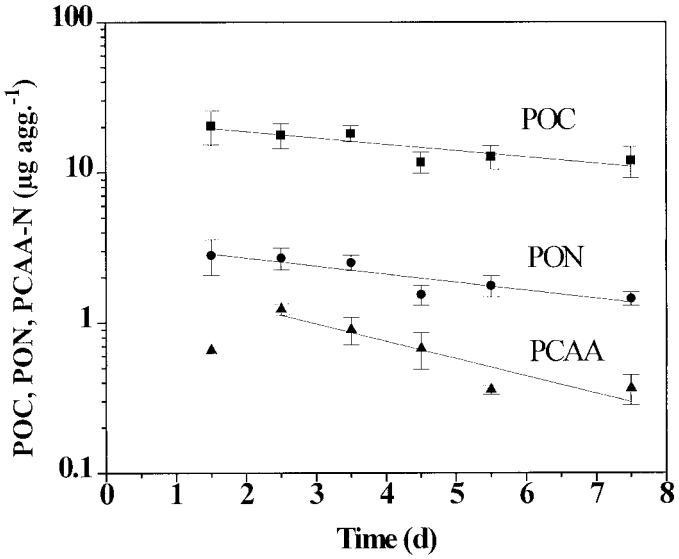

Fig. 1. Particulate organic carbon (POC), particulate organic nitrogen (PON), and particulate combined amino acids (PCAA) during time. Each data point represents the average value with the standard error of the mean ($n = 10$).

decreased exponentially during incubation: POC (μg agg^{-1}) = 22.62 $e^{-0.097t}$, $r^2 = 0.75$, and PON (μg agg^{-1}) = 3.45 $e^{-0.124t}$, $r^2 = 0.81$, respectively, and time is measured in days (Fig. 1). Thus, the half-time ($T_{1/2}$) of C and N was 7.1 and 5.4 d, respectively. However, nitrogen fixed in particulate combined amino acids was highest on day 2.5 (1.23 μgN agg^{-1}) in parallel to highest bacterial biomass because bacteria also used dissolved amino acids and ammonium from the surrounding water during the first day of incubation (data not shown). Nitrogen of particulate combined amino acids strongly decreased thereafter (PCAA-N [μg agg^{-1}] = 2.18 $e^{-0.266t}$, $r^2 = 0.86$, $n = 20$) with a $T_{1/2}$ of 2.6 d. Between days 2.5 and 5.5 particulate combined amino acid nitrogen decreased by more than 60% and particulate combined amino acids were, thus, preferentially lost from the aggregates. There was no further decrease in particulate combined amino acid nitrogen between days 5.5 and 7.5, which suggests that particulate combined amino acids became more refractory with time. However, mol percentages of 16 different particulate combined amino acids remained rather constant and did not show significant changes over time (data not shown).

Microbial colonization—The maximum of bacterial abundance on aggregates occurred on day 2.5 with 1.4×10^9 bacteria (ml agg)$^{-1}$. It decreased after 2.5 d in parallel to a strong increase in numbers of heterotrophic flagellates and ciliates on day 3.5 (1.2×10^7 flagellates [ml agg]$^{-1}$ and 4.5×10^5 ciliates [ml agg]$^{-1}$, respectively) when bacteria were grazed at approximately the same rate as they divided. In addition, size and shape of the aggregate-associated bacteria significantly changed during incubation from single rods to filamentous and colony-forming types indicating high grazing of attached bacteria by protozoa (Ploug and Grossart 2000).

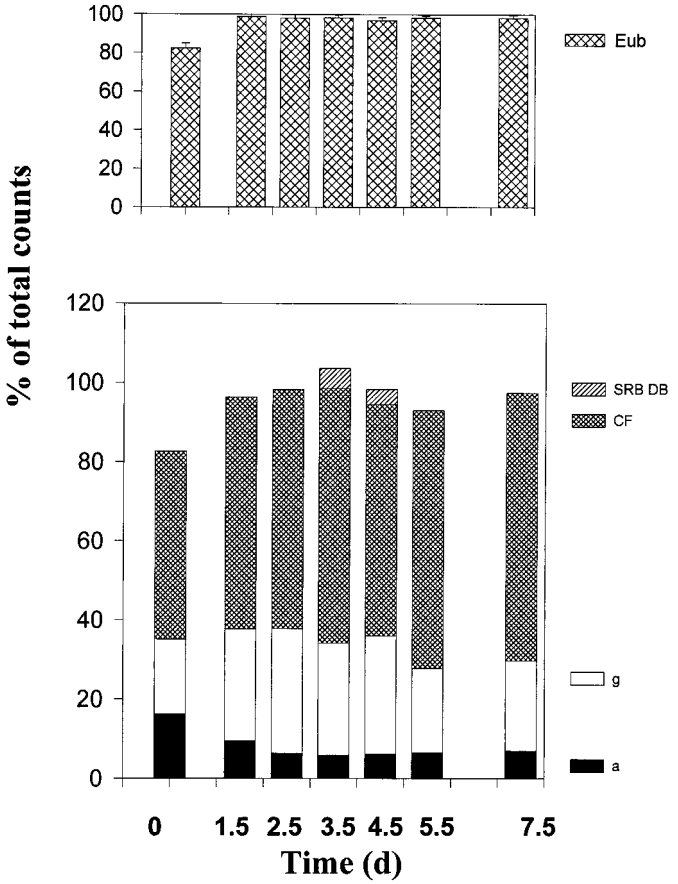

Fig. 2. Changes in bacterial community structure during incubation of diatom aggregates in roller tanks at in situ temperature (15°C) as detected by fluorescence in situ hybridization. For details *see text.*

Bacterial community structure—Fluorescence in situ hybridization revealed that the majority of the aggregate-associated bacteria (81–98%) belonged to *Eubacteria* (Eub) (Fig. 2). Members of the α subclass of *Proteobacteria* (a) comprised 16.3% of total counts on day 0 and decreased to 6% on day 3.5. In contrast, γ *Proteobacteria* (g) and *Cytophaga* (CF) increased from 18.9 and 47.5% on day 0 to 31.4 and 60.4% on day 3.5, respectively. Sulfate-reducing bacteria (SRB DB) only occurred on days 3.5 and 4.5, whereas β*-Proteobacteria* and *Archaea* were not detected at all.

Bacterial production and community respiration on aggregates—To better compare bacterial production on aggregates during time, we have standardized bacterial production to 1 μg of POC, PON, or particulate combined amino acid nitrogen. For the specific nitrogen production we assumed a bacterial C : N (w : w) ratio of 4.5 (Goldman et al. 1987). Specific bacterial production (POC, PON, and particulate combined amino acid nitrogen) significantly decreased over time (Fig. 3a). Carbon-specific bacterial production decreased from 0.082 to 0.020 d^{-1}, i.e., the carbon turnover time due to bacterial production increased from 12 to 50 d. Owing to an almost constant C : N ratio of the aggregates, POC- and PON-specific bacterial production decreased at

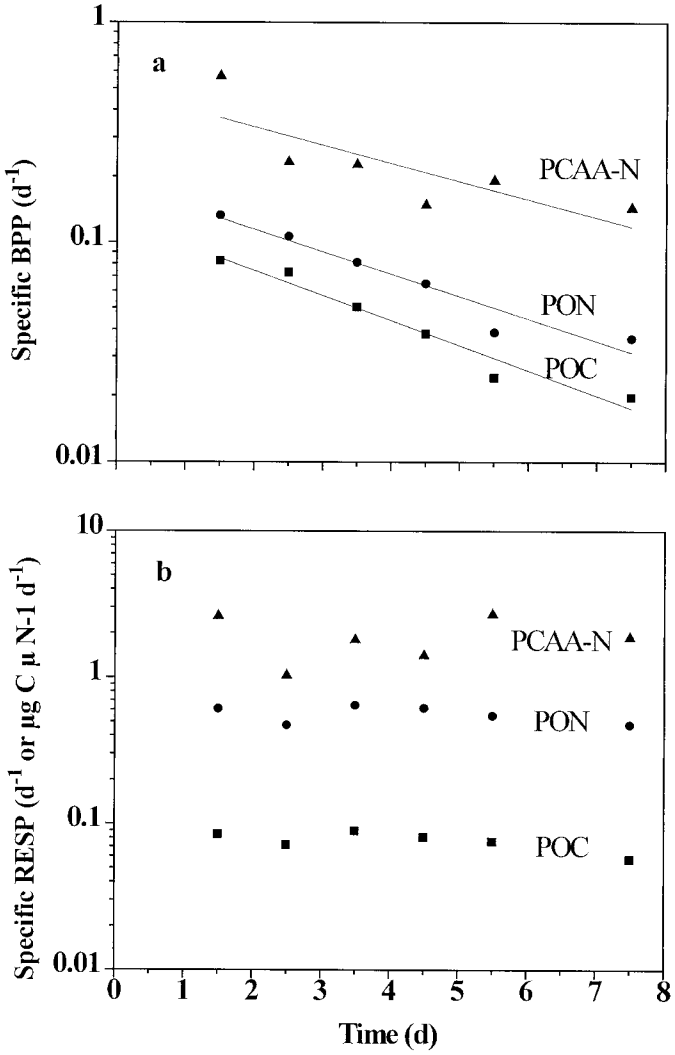

Fig. 3. Time course of specific (a) bacterial production (BPP) and (b) community respiration (RESP) on diatom aggregates that were both standardized to either 1 μg of particulate organic carbon (POC), particulate organic nitrogen (PON), or nitrogen of particulate combined amino acids (PCAA-N). Each data point represents the average value measured on 10–20 aggregates.

almost the same rate but faster than particulate combined amino acid nitrogen-specific bacterial production, which suggests that the quality of POC and PON changed more than that of particulate combined amino acids. In contrast, POC, PON, or particulate combined amino acid nitrogen-specific community respiration did not change much over time (Fig. 3b), which suggests that aggregate-associated respiration was dependent on quantity and less on quality of the organic substrates. Carbon-specific community respiration ranged between 0.057 and 0.089 d^{-1}. Thus, carbon turnover time due to respiration varied between 11.2 and 17.5 d.

Apparent growth efficiency and growth rate—Bacterial growth efficiency on aggregates decreased with time (Fig. 4). Growth efficiency was highest (~0.5) on days 1.5 and 2.5 when numbers of protozoa were still low, which indicates a high and efficient transfer of organic matter into bacterial biomass on aggregates. The bacterial growth rate was measured by uptake of ^{3}H-labeled thymidine and, thus, independently of bacterial production. It was highest on day 1.5 (1.78 $\pm$ 0.02 d^{-1}) and lowest on day 7.5 (0.39 $\pm$ 0.04 d^{-1}), but growth efficiency and growth rate on individual aggregates did not show any correlation. However, mean values of apparent growth efficiency (BGE) and growth rate (μ) on different dates correlated well (BGE = 0.199μ + 0.1227, r^2 = 0.94) when excluding day 2.5. At this time growth rate was very low as compared to apparent growth efficiency, which suggests higher increase in bacterial biomass than in number. Hence, growth rate decreased before the onset of bacterial grazing between days 2.5 and 3.5, which indicates that bacterial production became substrate limited during day 2.5.

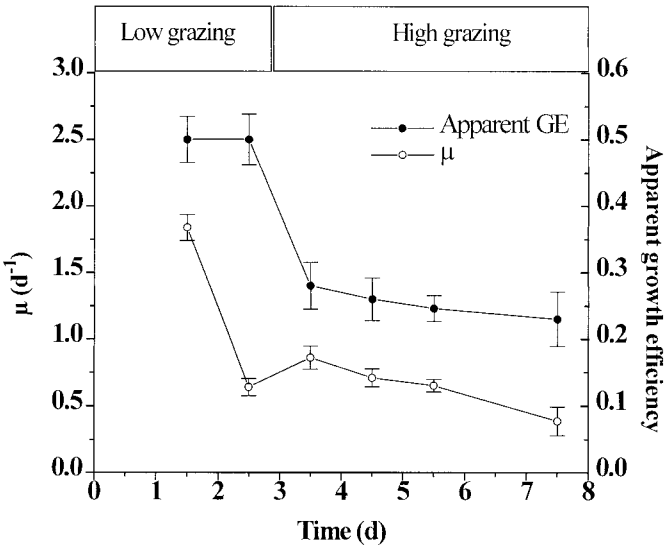

Fig. 4. Time course of apparent growth efficiency (GE) and bacterial growth constant (μ) on diatom aggregates. Thymidine and leucine incorporation, as well as respiration, were measured on the same aggregates. Each data point represents the average value with the standard error of the mean (n = 10).

Bacterial abundance, ecto-enzymatic activities, and microbial activities—Potential protease activity (Fig. 5a), bacterial abundance (Fig. 5b), and total bacterial production (Fig. 5c) varied proportionately to particulate combined amino acid nitrogen over time. Cell-specific protease activity did not change with decreasing particulate combined amino acid content, and, therefore, we assume a more direct link between potential protease activity and bacterial biomass than with particulate combined amino acid content. Total bacterial production showed a strong correlation to particulate combined amino acids. In contrast, bacterial abundance did not show any correlation to POC (Fig. 6a), whereas potential β-glucosidase activity was weakly correlated to POC (Fig. 6b) and community respiration was strongly correlated to POC (Fig. 6c).

Since we were not able to measure dissolved amino acids in the pore water of aggregates, in situ uptake and turnover rates of amino acids and glucose could not be determined.

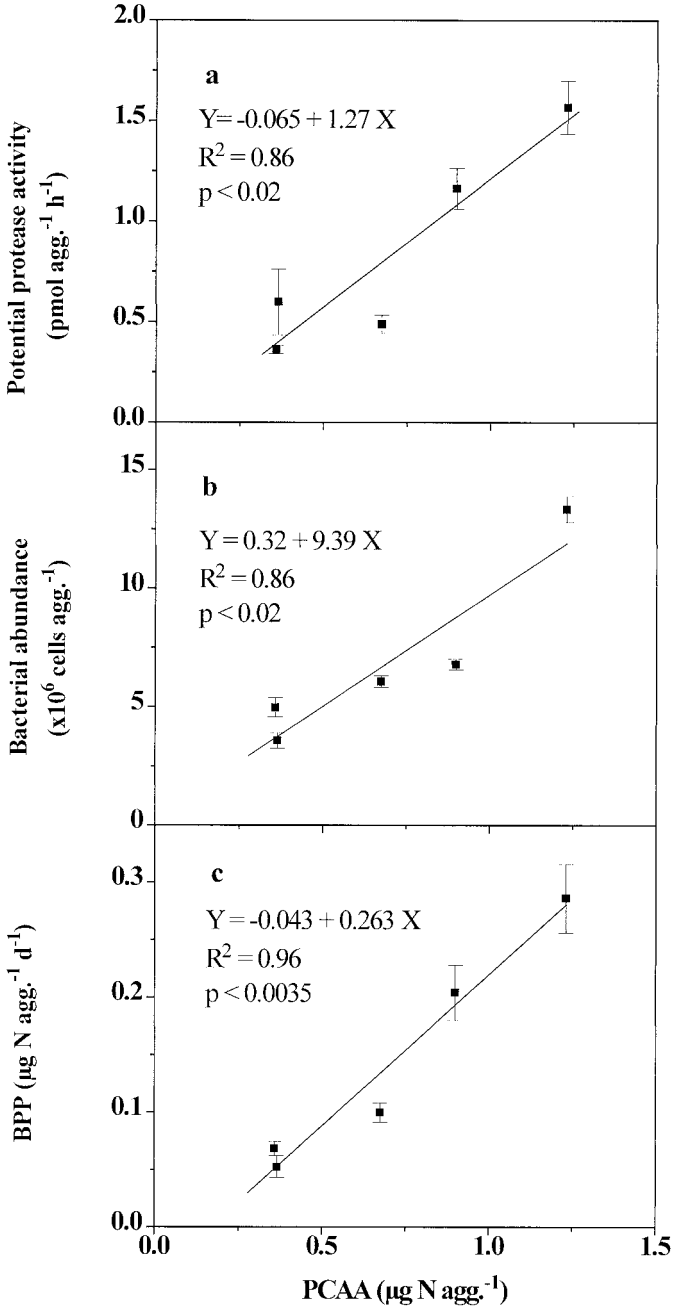

Fig. 5. Correlation between (a) potential protease activity, (b) bacterial abundance, or (c) total bacterial production (BPP) and nitrogen of particulate combined amino acids (PCAA-N) throughout the incubation of diatom aggregates. Each data point represents the average value with the standard error of the mean (n = 10–20).

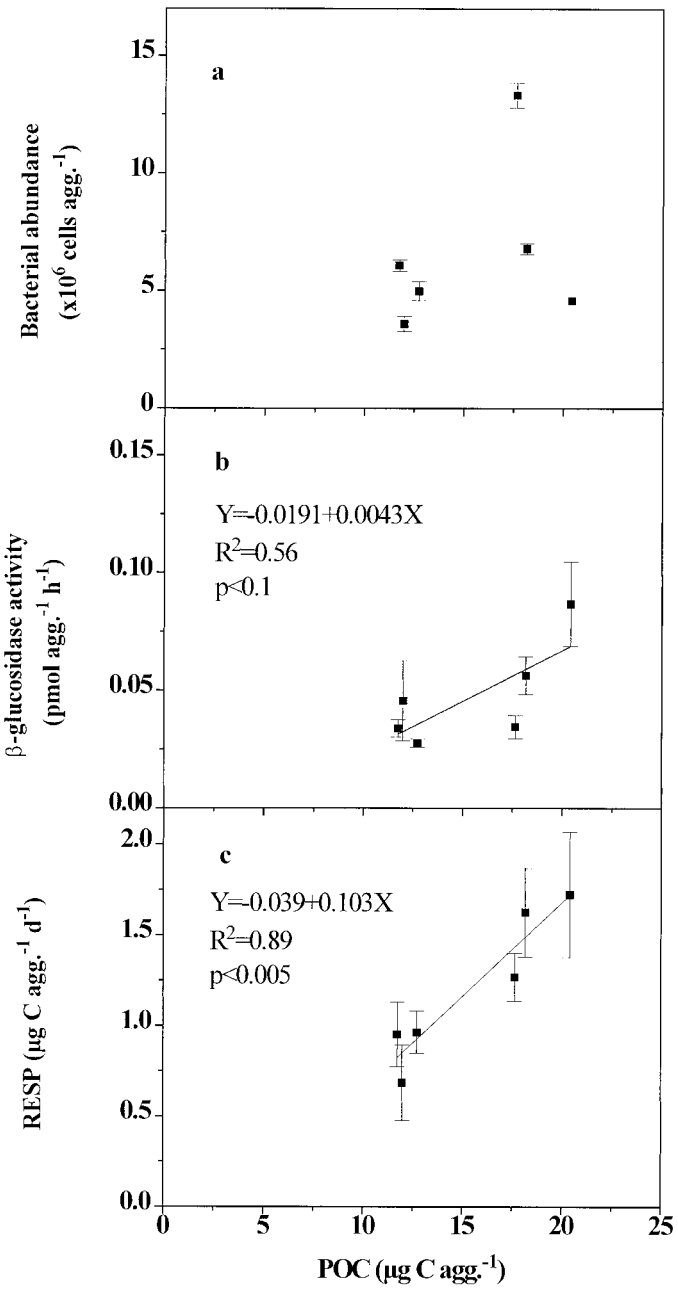

Fig. 6. Correlation between (a) bacterial abundance, (b) β-glucosidase activity, or (c) community respiration and particulate organic carbon (POC) throughout the incubation of diatom aggregates. Each data point represents the average value with the standard error of the mean (n = 10–20).

However, measured turnover rates of radiolabeled amino acids and glucose added to the surrounding water are indicative for changes in bacterial uptake rates and/or variations of isotope dilution. Turnover time of amino acids was significantly correlated to particulate combined amino acid content between days 2.5 and 7.5 (Fig. 7a) and to POC (Fig. 7b), and it increased during incubation, which indicates increased isotope dilution of the radiolabeled amino acids in the pore water. Turnover time of glucose also increased with decreasing particulate combined amino acids (Fig. 7c) and POC (Fig. 7d) over time except at day 7.5. The C : N (w : w) ratio increased from 7.09 to 9.37 (molar ratio: 8.24 to 10.89) from day 5.5 to day 7.5, which indicates an increasing fraction of refractory organic matter during this time period.

Although potential protease activity was high on our model aggregates, the measured release of dissolved amino acids was relatively low (data not shown). The highest net release of total dissolved amino acids was 5.5 ng N agg^{-1} d^{-1} be-

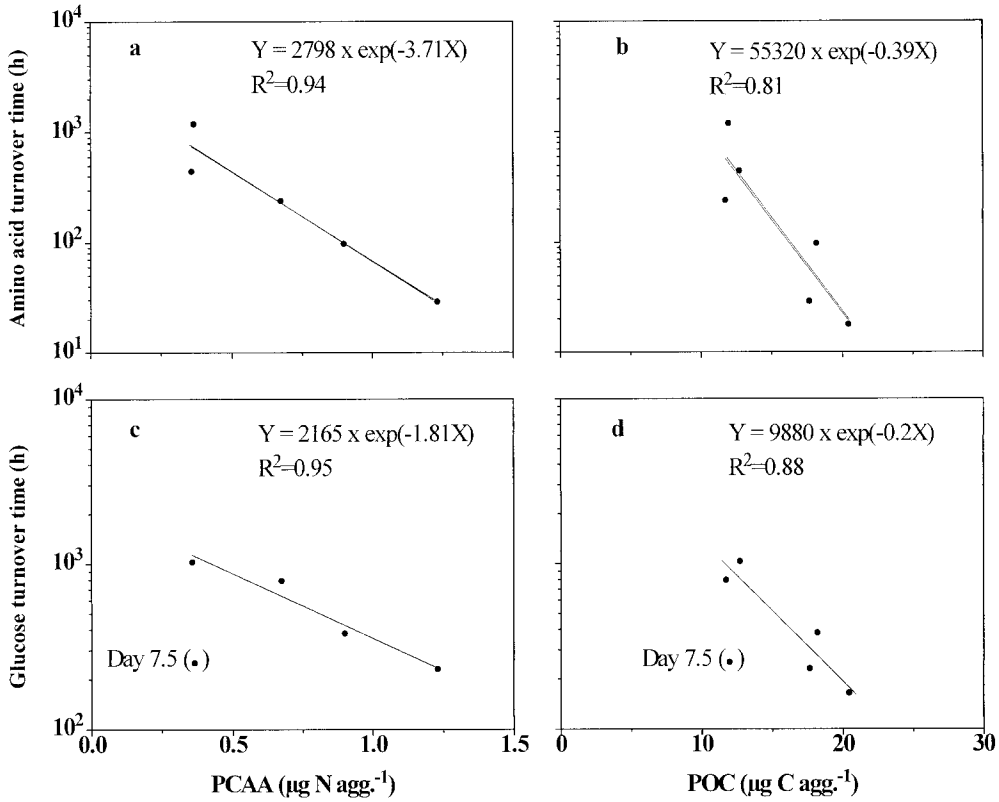

Fig. 7. Calculated turnover time of radiolabeled amino acids and glucose in relation to nitrogen of particulate combined amino acids (PCAA-N) (left panel) and particulate organic carbon (POC) (right panel) during the incubation of diatom aggregates.

tween days 2 and 5, accompanied by an increased signal of NH_4^+. Unfortunately, the sensitivity of our analytical method was too low to allow for quantitative measurements of NH_4^+ uptake and release. Total dissolved amino acids in the surrounding water of two aggregates per sample disappeared at a rate of 400–600 ng N d^{-1} during the first day of incubation. A decrease of 331 ng N d^{-1} in the control suggested high adsorption of amino acids to the glass syringes. Alanine increased from 14 to 18% and γ ABA from almost 0 to 3% during the incubation. The composition of released amino acids was comparable to that of particulate combined amino acids, only glycine/threonine and alanine were slightly enhanced. It was also similar to that of the prefiltered control, which indicates that the source of dissolved amino acids in situ was the same. We did not determine amino acid uptake by free-living bacteria. The bacterial abundance in the surrounding water reached $>10^5$ bacteria ml^{-1}. However, carbon lost as CO_2 by respiration explained 78% of the total decrease in POC, and the average POC-specific release of carbon due to amino acids accounted for 22% of the total POC loss, giving a POC-specific release of 0.023 d^{-1} (Ploug and Grossart 2000). POC-specific bacterial production decreased from 0.082 d^{-1} to 0.020 d^{-1} at the end of the experiment (Fig. 3a). Thus, the loss of organic substrates due to inefficient coupling between bacterial hydrolysis and uptake was relatively low during the initial degradation process but increased with increasing aggregate age and grazing by protozoa.

Carbon and nitrogen budgets—The carbon turnover was calculated from the POC-specific bacterial production and respiration rates shown in Fig. 3. Bacterial production was equal to community respiration until day 3.5, when community respiration became 2.9-fold higher than bacterial production. The bacteria were grazed at approximately the same rate as they divided from day 3.5 to day 7.5, thus, the pathways of bacterial production and respiration overlap (Ploug and Grossart 2000). Cumulative bacterial production and community respiration accounted for 26% and 40%, respectively, of the initial POC content. PON decreased by 1.24 μg N agg^{-1} during 5 d (i.e., 48.7% of the PON were lost from aggregates) (Fig. 1). Cumulated bacterial production consumed 0.8-μg particulate combined amino acid nitrogen, assuming a constant C : N ratio of 4.5 for bacterial biomass from day 2.5 to day 7.5. Thus, more than 50% of the particulate combined amino acid nitrogen was channeled through bacterial production and presumably partly remineralized to NH_4^+ by bacteria and protozoa in the food web. Additional 0.4-μg particulate combined amino acid nitrogen from the protein fraction disappeared during the last 4 d of the incubation (Fig. 8). The total decrease in particulate combined amino acid nitrogen accounted for 87% of the decrease in PON during 5 d.

Discussion

This is the first study in which community respiration, bacterial production, net release of amino acids, ecto-enzy-

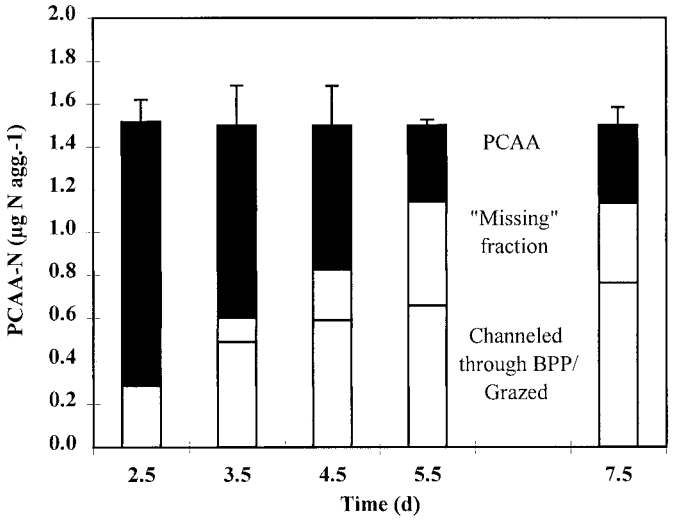

Fig. 8. Nitrogen pool of particulate combined amino acids (PCAA-N), which was measured on aggregates (filled bar); nitrogen of particulate combined amino acids channeled through bacterial production (BPP) and remineralized by protozoa (hatched bars); and nitrogen of particulate combined amino acids, which in addition disappeared from the aggregates (open bars) during the incubation of diatom aggregates. A constant C : N ratio of 4.5 for bacterial biomass was assumed for calculations.

matic activity, and uptake of amino acids, as well as of glucose, have been quantified on the same aggregates to directly determine apparent growth efficiency and substrate turnover by attached bacteria. Bacterial production and respiration were the dominant pathways of both carbon and nitrogen during early colonization of fresh diatom detritus when protozoan biomass was low. Bacterial production in the present study was much higher than that previously measured on natural marine (Alldredge et al. 1986; Simon et al. 1990; Smith et al. 1992) and limnetic aggregates (Grossart and Simon 1993, 1998) with similar bacterial numbers. Our particulate combined amino acid–specific bacterial production rates were >47-fold higher than those measured by Smith et al. (1992). We partly attribute this to the fact that all aggregates were incubated individually and in suspension with the radiolabel (Ploug and Grossart 1999). Bacterial production measured on sedimented aggregates was up to tenfold lower than that on suspended aggregates (Ploug and Grossart 2000). Note also that we have used artificially made aggregates of fresh detritus highly enriched in organic substrates. POC-specific community respiration rates, however, were in the same range as those on field-sampled marine snow from the Southern California Bight (Ploug et al. 1999). But when comparing POC-specific bacterial production and community respiration rates on aggregates of the present study with those of the river Weser, which were measured by exactly the same methods (Grossart and Ploug 2000), it becomes obvious that the quality of available substrates mainly controls bacterial production. POC-specific bacterial production and community respiration rates on the fresh diatom aggregates were by a factor of ~10 higher than those on the riverine aggregates, which were mainly composed of refractory organic substrates with a much higher C : N ratio. In the present study we have used a well-defined source of fresh diatom detritus, which allowed us to follow microbial transformation processes in relation to substrate quality in much greater detail.

Rates of potential protease and β-glucosidase activities were similar to those found on marine (Smith et al. 1992) and lake snow (Grossart and Simon 1998). Protease activity and organic matter content, as well as bacterial biomass, are frequently positively correlated (Münster 1991). Carbon turnover times measured on the fresh diatom detritus were much shorter than those calculated on natural diatom aggregates in the sea (Smith et al. 1992). The short half-time of POC determined by $^{14}CO_2$ release (Lee and Fisher 1994) was attributed to physicochemical leaching, as well as to activities of free enzymes that can temporarily exceed microbial transformation. A substantial release of DOM from ^{14}C-labeled seston by solubilization was also found by Berman et al. (1999) in Lakes Kinneret and Constance. However, high proportions of abiotic solubilization can be excluded in our experiments since community respiration accounted for most of the POC loss from the aggregates. The rapid disappearance of particulate combined amino acid nitrogen in relation to that of PON and POC indicates a predominant loss of particulate combined amino acid nitrogen from aggregates due to microbial activity, such as protease activity, bacterial production, and subsequent grazing by protozoa. The preferential degradation of particulate combined amino acids compared to other nitrogen sources is consistent with the idea that the C : N ratio of a substrate must be lower than that of the bacteria in order to support high growth efficiencies and regeneration of NH_4^+ (Fenchel and Blackburn 1979; Goldman et al. 1987). Release of ammonium was detected by high-pressure liquid chromatography after 2.5 d of incubation, and a significant fraction of the attached bacterial carbon and nitrogen biomass was presumably remineralized by protozoa (Ploug and Grossart 2000).

Rapid microbial turnover of labile fractions of POM, e.g., proteins and sugars, by free-living bacteria has been measured in the ocean. Preferential degradation of proteins and high uptake of amino acids by attached bacteria were also found on marine snow (Shanks and Trent 1980), as well as on sediment trap material (Lee and Wakeham 1988). Gries (1995) examined the decomposition of PON in sediment traps in Lake Constance and measured a loss of 40% of total PON during a 3-d deployment. In comparison, our aggregates had lost 50% of the initial PON content during 5.4 d. The rapid decomposition of PON is consistent with the preferential turnover of PON, e.g., of particulate combined amino acids, on our model aggregates. Preferential losses of PON and particulate combined amino acids in sediment traps in Lake Constance (Gries 1995; Grossart and Simon 1998) and in various marine systems (Wakeham et al. 1984; Cowie and Hedges 1992) have been mainly attributed to high ectoenzymatic activities (Karner and Herndl 1992; Smith et al. 1992; Grossart and Simon 1998). High enzymatic hydrolysis but relatively low hydrolysate uptake by attached bacteria implies that a large fraction of the particulate organic matter in aquatic systems serves to supply free-living bacteria with dissolved organic matter and nutrients (Smith et al. 1992, 1995; Cho and Azam 1988; Grossart and Simon 1998). In

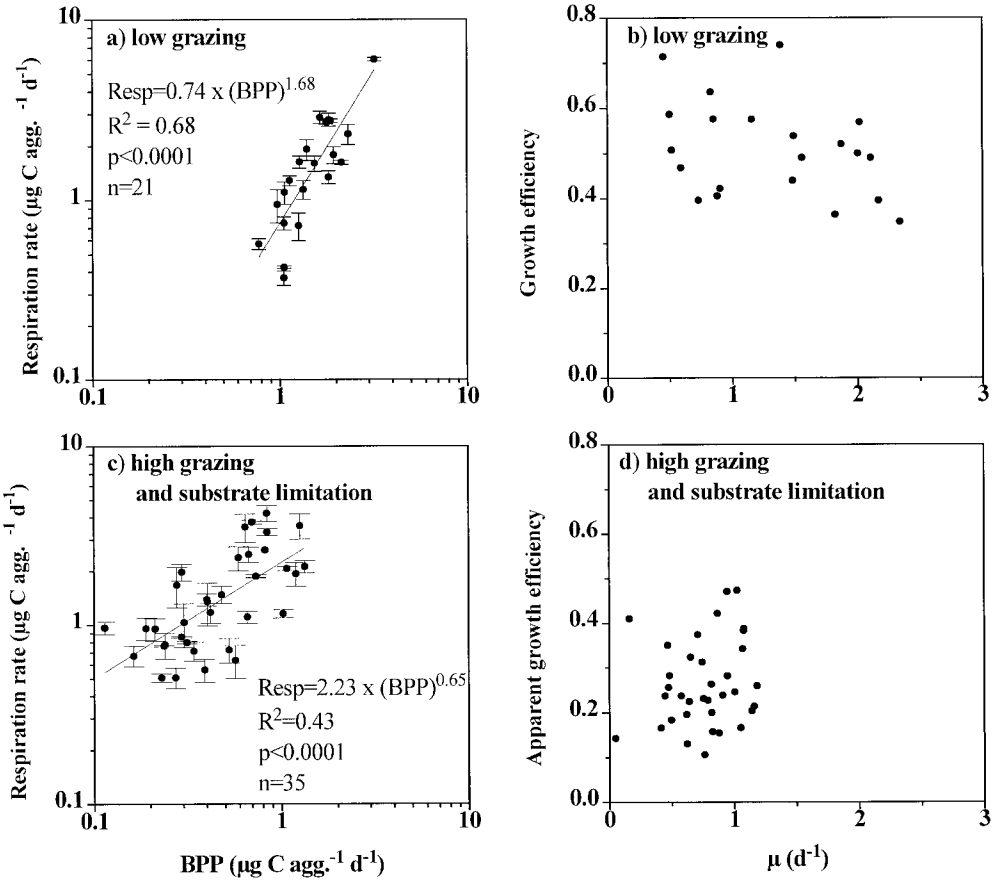

Fig. 9. Bacterial production and community respiration (left panel) and bacterial growth efficiency and growth rate (μ) (right panel) during periods of (a + b) low grazing and no substrate limitation as well as (c + d) high grazing and substrate limitation. For further explanations *see text.*

our study, the uncoupling between hydrolysis and bacterial production increased during time, concurrent with decreasing bacterial production and bacterial grazing by protozoa. Hence, aggregates leaking amino acids in the ocean and in lakes are presumably not newly formed, and protozoa may likely be responsible for release of amino acids during grazing.

From days 1.5 to 2.5 the bacterial community structure had significantly changed but remained relatively constant thereafter. This is hardly indicated by fluorescence in situ hybridization using only group-specific 16S rRNA probes. However, changes in bacterial community structure became more obvious when using PCR-amplified 16S rRNA genes, separated by denaturating gel electrophoresis (DGGE, Grossart unpubl. data). Large changes in bacterial community structure occurred simultaneously with greatly increased cell numbers during day 1.5 and reflect high cell multiplication rates of specific bacteria. The cell multiplication rates were significantly lower at day 2.5, and flagellates and ciliates became highly abundant during day 3.5. Grazing-resistant filamentous bacteria became more abundant throughout the incubation, which suggests a ''bacterial response'' to the grazing pressure by protozoa (Pernthaler et al. 1997; Jürgens et al. 1999). Unfortunately, it is not possible to distinguish between bacterial and protozoan respiration by using microelectrodes, and hence bacterial growth efficiency is always underestimated when numbers of protozoa are high (Ploug and Grossart 2000).

Relatively high respiration rates at low bacterial production rates and a slow increase of respiration with increasing bacterial production for free-living bacteria are predicted by two models of Del Giorgio and Cole (1998). On 1–5 d old riverine aggregates with high growth efficiency, we found that bacterial production (BPP) and respiration (RESP) were correlated as $\text{RESP} = 1.57 \times \text{BPP}^{0.86}$ ($R^2 = 0.58$; $p < 0.001$; $n = 47$; Grossart and Ploug 2000). In the present study $\text{RESP} = 0.74 \times \text{BPP}^{1.67}$ ($R^2 = 0.68$; $p < 0.0001$; $n = 21$) when measured on single aggregates during the initial 2.5 d with a low abundance of protozoa and $\text{RESP} = 2.25 \times \text{BPP}^{0.65}$ ($R^2 = 0.43$; $p < 0.0001$; $n = 35$) when the bacteria were grazed by protozoa and bacterial production was substrate limited (Fig. 9). The relationship between respiration and bacterial production found by Del Giorgio and Cole (1998) is similar to the latter one. Hence, growth efficiency is relatively high even at low bacterial production, when grazing is low and the bacteria are not substrate limited. In addition, community respiration then increases faster in proportion to increasing bacterial production on aggregates than it does for free-living bacteria. On our model, aggregates apparent growth efficiency was highest in parallel to high

bacterial production and efficient uptake of labile organic substrates, such as particulate combined amino acids, by attached bacteria. With decreasing quality of substrates over time, bacterial production decreased 2.9-fold relative to community respiration. Our study shows that aggregate-associated bacterial production is mainly controlled by labile organic substrates, such as proteins, and that carbon and nitrogen turnover on these relatively fresh diatom aggregates result in a rapid transformation of particulate organic matter through bacterial growth and grazing. These pathways have significant implications for transformation and remineralization of particulate organic matter and the overall flux of carbon and nitrogen in aquatic systems.

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