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Bacterial abundance and production in river sediments as related to the biochemical composition of particulate organic matter (POM)

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Abstract. The major proportion of heterotrophic activity in running waters is localized on the solid surfaces of sediments in the benthic and hyporheic zone and is dominated by microorganisms. However, this assertion is based on the studies of small streams, and little is known about the microbial metabolism of organic matter in river ecosystems. We therefore explored the relationships between bacterial abundance and production and the gradients of organic matter quality and quantity in sediments of a sixth-order lowland river (Spree, Germany). We found vertical gradients of detrital variables (particulate organic matter (POM), particulate organic carbon (POC), nitrogen (PN), and protein) and of bacterial variables (abundance, production, turnover time, and proportion of bacterial carbon in total POC) in two different sediment types. These gradients were steeper in stratified sediments than in the shifting sediments. Detrital variables correlated strongly with bacterial abundance and production. The best correlation was found for detrital variables indicating substrate quantity and quality (r_S = 0.90 for PN with abundance). Although bacterial biomass comprised only 0.7% of the POC (1.9% of PN, 3.4% of the protein) in sediments, the turnover of sedimentary organic carbon was fast (median = 62 d), especially in the shifting sediments. Our findings demonstrate that sediment dynamics significantly foster organic carbon metabolism in river systems. Thus, these sediments, which are typical for lowland rivers, strongly influence the metabolism of the whole ecosystem.

Introduction

The role of heterotrophic microbial processes is now central to current concepts in river ecology (Wetzel 1992; Meyer 1994). In small streams, most of the heterotrophic activity is localized on the solid surfaces of sediments of the benthic and hyporheic zone, where it is dominated by microorganisms (Pusch and Schwoerbel 1994; Naegeli and Uehlinger 1997; Pusch et al. 1998). However, the level of these processes may vary by several orders of magnitude within and between streams, and their contribution to whole system metabolism has rarely been examined in larger rivers (Edwards et al. 1990; Schwoerbel 1994a; Fischer and Pusch 2001).

The content of organic matter has been proposed as a major determinant of heterotrophic activities in sediments (Lock 1993; Pusch and Schwoerbel 1994; Fuss

and Smock 1996; Findlay and Sobczak 2000). Sediment organic matter content thus can be the limiting factor and an important indicator for bacterial abundance and production in both aerobic and anaerobic sediments (Sander and Kalff 1993; Schallenberg and Kalff 1993; Pusch et al. 1998; Brunke and Fischer 1999). The degradability of particulate organic carbon for heterotrophic bacteria is influenced by several factors. First, bacteria need to synthesize extracellular enzymes specific for the available substrates (Chróst 1991). Second, they have to be in close contact with those substrates in order to efficiently apply these enzymes. Thus, it has been shown that the feeding efficiency using extracellular enzymes decreases with increasing distance from the substrate (Vetter et al. 1998), and that bacteria growing directly on an organic substrate were more active than those growing in an epilithic biofilm (Sinsabaugh et al. 1991; Brunke and Fischer 1999). Third, the nutritional quality of the available substrates can strongly influence bacterial activity (Goldman et al. 1987; Enriquez et al. 1993; Bonin et al. 2000), although it has also been found that bacterial growth rates can be largely independent of the substrate C/N ratio (Tezuka 1990). Protein provides a direct measure of substrate quality (Mayer et al. 1986; Cowie and Hedges 1994; Pusch and Schwoerbel 1994). The content of chlorophyll, which is rapidly lost during the diagenesis of plant detritus, can serve as a measure for fresh autochthonous material (Boon and Duineveld 1996).

In our present study, we related small and medium scale distribution of the quantity and quality of particulate organic matter (POM) with bacterial abundance and production. We hypothesized that in river sediments a direct relationship exists between the quality of POM and the bacterial variables. Since POM is transported into the sediments from the pelagic zone (Pusch et al. 1998), gradients of organic matter composition caused by the degradational activity of bacteria should exist within the sediments. These gradients should be paralleled by lower bacterial abundance and production in deeper sediment layers due to a decrease in total amount and nutritious quality of the organic compounds, and of terminal electron acceptors (Hendricks 1993; Findlay and Sobczak 2000). In a comparison of two different sediment types, the gradient should be steeper in stratified sediments than in shifting sediments which are constantly moving under in situ conditions and are well perfused by river water entraining fresh organic material. In places densely colonized by bacteria, these are supposed to recycle efficiently the metabolic wastes produced within the ecosystem, and thus may contribute a substantial amount to the energy supply of organisms of higher trophic levels (Kemp 1990; Meyer 1994; Hall and Meyer 1998). In order to quantify this contribution, we examined which proportion of total carbon, nitrogen, and protein in organic matter can be attributed to bacterial biomass.

Methods

Characterization of sediments and sedimentary particulate organic matter (POM)

On each of four sampling dates (August 1997, October 1997, March 1998, and June 1998), five sediment cores were taken from each of two different sediment types found in a sixth-order lowland river (Spree, Germany): The sediments were shifting sands, low in organic matter, and with a relatively homogenous particle size distribution and coatings of iron and manganese oxides, and stratified sediments from closer to the banks of the river where higher amounts of organic matter accumulate. The cores were cut into depth layers of 0–2 cm, 8–10 cm, and 18–20 cm, later referred to as 1, 9, and 19 cm layers. Subsamples were taken from each layer in order to estimate bacterial production, abundance, and biomass, and to measure the content of particulate organic matter, carbon, nitrogen, protein, and chlorophyll a and phaeopigments. The remainders of the samples were pooled for each layer, dried, and sieved through a standard set of sieves comprising mesh sizes from 63 μm through 6.3 mm in order to examine the particle size distribution and the sorting coefficient $(d25/d75)^{0.5}$ of the sediments (Schwoerbel 1994b).

Total particulate organic matter (POM) was determined as loss on ignition. Samples of 15–25 g wet weight were dried to constant weight at 105 °C, and subsequently burnt for 6 h at 550 °C in order to determine POM as ash free dry mass. Particulate organic carbon and nitrogen content were determined using a CHN Analyzer NA 1500 Series 2 (Carlo Erba/Fisons Instruments). About 50 g of the dried sediment samples were homogenized with an analytical mill (IKA A 10) for 30 seconds to achieve grain sizes of <1 mm. Triplicate subsamples were placed into cylindrical silver capsules (9 mm height, 5 mm diameter; Lüdi AG, Flawil, Switzerland) for analysis. Inorganic carbon was removed with 1 M HCl. The calibration curve was established using acetanilide.

Samples for the determination of protein and chlorophyll a content were stored at − 28 °C and freeze dried (Lyovac GT 2, Amsco/Finn-Aqua) for 24 h at a pressure of 3 mbar and a temperature of − 12 °C prior to further analysis. Protein was extracted according to Rausch (1981) and the protein content was then estimated using the micro-biuret method of Itzhaki and Gill (1964) based on the UV absorption of the complex formed between protein and copper in strongly alkaline copper sulfate solution. In previous tests, this method proved to be less sensitive to humic substances than the method of Lowry et al. (1951). The calibration curve was established using bovine serum albumin.

For the extraction of phytopigments, 1 cm^3 of glass beads (1 mm in diameter) and 10 ml of hot ethanol (90%, 78 °C) were added to 1–5 g of the sample. The mixture was strongly vortexed for 2 min in a Vibrogen cell mill (Eduard Bühler). Extraction was continued for 12 h at room temperature in the dark. Samples were then centrifuged for 10 min. at 2720 g. The clear ethanol-pigment-mixture was used to determine chlorophyll a and phaeopigments with a UV-2401 PC Spectrophotometer (Shimadzu) according to DIN 38412-L16 (Deutsche Einheitsverfahren 1985).

Bacterial production, abundance, and biomass

Subsamples of 1 ml sediment were taken from each depth and weighed into precombusted 10 ml centrifuge vials containing 4 ml of fresh, sterile-filtered (0.2 μm pore size) river water. The vials were then kept at 4 °C until the experiment, which was started within 5 h after sampling. Bacterial production was measured using the leucine-incorporation method (Kirchman 1993) modified according to Fischer and Pusch (1999). We used L-[U-^{14}C]-leucine (Amersham Ltd., specific activity 11.6 GBq/mmol) diluted with cold L-leucine to a specific activity of 148 Bq/nmol leucine and incubated the samples in vials at in situ temperatures with gentle shaking. Two controls for each set of five samples were fixed with 3.2% formaldehyde (final concentration) immediately at the start of the incubation. The detection limit for bacterial production was defined as the level where control values reached 50% of the measured values. Bacterial carbon production was calculated from leucine incorporation assuming 7.3 mol % leucine in total protein, and a carbon/protein ratio of 0.86 (Simon and Azam 1989).

Subsamples for bacterial cell counts were taken from the supernatant of the incubated samples, after a 10 min sonication step and rigorous vortexing (Brunke and Fischer 1999; Fischer and Pusch 1999). They were diluted with a sterile filtered aqueous solution of 3.5% formaldehyde, 0.85% NaCl, and 1 mM pyrophosphate. Bacteria were stained using 4′,6-diamidino-2-phenylindol (DAPI) (Porter and Feig 1980) at a final concentration of 10 mg/l. After 40 min of dark incubation, bacteria were filtered onto black polycarbonate filters (Nuclepore, pore size 0.2 μm). At least 200 bacteria within at least 10 microscopic fields were counted by epifluorescence microscopy (Nikon FXA Microscope, HBO 100 W, Ex 330–380, DM 400, BA 400, immersion objective CF N DIC Plan Achromate 100 ×).

Pictures of DAPI stained bacteria from the 1 cm and 19 cm depth layers were taken and analyzed with a Nikon SMZ-U stereomicroscope and an image analyzing system. Length and width of 100 bacteria per sample, taken from at least two pictures, were recorded and their biovolumes were calculated according to Fry (1988). The bacteria in this model are considered to be straight-sided rods with hemispherical ends. The formula is

$$V = (d^2\pi/4)(L - d) + \pi d^3/6$$

where the cell dimensions are V = volume, d = width, L = length. Bacterial volume (V in μm^3) was converted into carbon (C in fg) by using the allometric model C = 104.5 $V^{0.59}$ × 0.86, where 104.5 is the protein (in fg)/biovolume (in μm^3) conversion factor and 0.86 the carbon/protein conversion factor (Simon and Azam 1989; Simon et al. 1990). The measurements were calibrated using fluorescent latex beads with a nominal diameter of 0.50 μm (fluoresbrite carboxylate microspheres, Polysciences Inc.). The mean diameter of the fluorescent latex beads was measured accurately, but the standard deviation of the diameter was 4 times higher in our measurements than given by the manufacturer. The small error in diameter measure-

ments lead to an overestimation of the volumes of the latex beads of 2.9% so that the bacterial biomass would be overestimated by 1.6%.

Turnover times of bacterial carbon were calculated as biomass/production. Turnover of POC was calculated as total POC/bacterial respiration, assuming a bacterial growth efficiency of 30% (Benner et al. 1988). For further calculations, bacteria were assumed to contain 10% nitrogen in the dry weight (Fenchel et al. 1998).

Data analyses

Spearman rank correlations were used to show the relationships of bacterial data with POM variables. Data were log-transformed prior to further statistical analysis. The principal component analysis (PCA) of the standardized variables was conducted for an ordination of sampling sites, sediment depths and seasons, and to explore the correspondence between the gradient of POM amount and quality and the bacterial data. Regression analysis and analysis of variance (ANOVA) were applied to test the effects of sediment depth and POM amount and quality on bacterial variables. Analyses were performed with the software SPSS (Release 6.0, SPSS Inc.).

Results

Spatial distribution of particulate organic matter (POM) and bacteria

Vertical gradients were found for all bacterial and detrital variables in the sediments of the Spree (Figure 1). The effect of sediment depth on bacterial and detrital variables was stronger in the stable (stratified) sediments than in the shifting sediments, as shown by higher F-values in the analysis of variance (Table 1). The post hoc test (Tukey's HSD) revealed that in stratified sediments the gradation began within the upper 10 cm. In the shifting sediments, however, no significant decrease was found within this layer for most variables, and significant gradation could only be found in the deepest sediment layer (19 cm) (Table 1). Absolute values of most bacterial and detrital variables in the uppermost sediment layer were greater in the stratified sediments than in the corresponding layer of shifting sediments (Figure 1). In contrast, absolute values of bacterial variables in the deeper sediment layers were greater in the shifting sediments than in the stratified sediments (Figure 1).

Loss on ignition as a measure of total POM was strongly correlated with the particulate organic carbon content ($r_S = 0.80$; $n = 117$, $p < 0.001$). Particulate organic carbon (POC) contributed 42% to POM. Both POC and particulate nitrogen (PN) decreased with sediment depth. The vertical decrease in nitrogen was stronger than that of carbon resulting in increasing C/N ratios (Table 1). A mean (± SD) proportion of 9.3 ± 4.4% of the POM was recovered as protein, with decreasing values in lower sediment layers (Table 1). The mean (± SD) protein/nitrogen ratio was 3.5 ± 1.7 (wt/wt). The decrease in protein with sediment depth equaled that of

Table 1. Results of 1-way analysis of variance testing the effect of sediment depth (df = 2) on bacterial and detrital variables in two sediment types (n = 60). Symbols (o; +; −) indicate differences between the sediment-depth layers (Tukey's HSD, $p < 0.05$, n = 20). From upper to lower sediment layers, (+) indicates a significant increase, (−) indicates a significant decrease, and (o) indicates no significant difference.

	Stratified sediments					Shifting sediments				
			depth layer					depth layer		
	F-value	p-value	1 vs 9	1 vs 19	9 vs 19	F-value	p-value	1 vs 9	1 vs 19	9 vs 19
Abundance	372	<0.0001	−	−	−	40.3	<0.0001	o	−	−
Production	80.3	<0.0001	−	−	−	23.5	<0.0001	o	−	−
POM (ign.)	86.0	<0.0001	−	−	−	0.733	n.s.	o	o	o
Carbon	65.1	<0.0001	−	−	o	2.56	n.s.	o	o	o
Nitrogen	105	<0.0001	−	−	−	10.3	<0.001	o	−	−
Protein	73.9	<0.0001	−	−	o	10.0	<0.001	o	−	−
C/N ratio	9.52	<0.001	+	+	o	4.24	<0.05	o	+	o
Particle diameter (d50)	176	<0.0001	+	+	+	3.06	n.s.	o	o	o
Particle sorting coeff.	158	<0.0001	+	+	o	46.0	<0.0001	−	−	−
Protein/nitrogen	0.727	n.s.	o	o	o	1.62	n.s.	o	o	o
Protein/POM	4.55	<0.05	−	o	o	14.6	<0.0001	o	−	−
Bacterial carbon/POC	24.1	<0.0001	−	−	−	16.7	<0.0001	o	−	−
Turnover bacteria	0.528	n.s.	o	o	o	5.42	<0.01	o	+	+
Turnover POC	8.44	<0.001	o	+	+	18.1	<0.0001	o	+	+

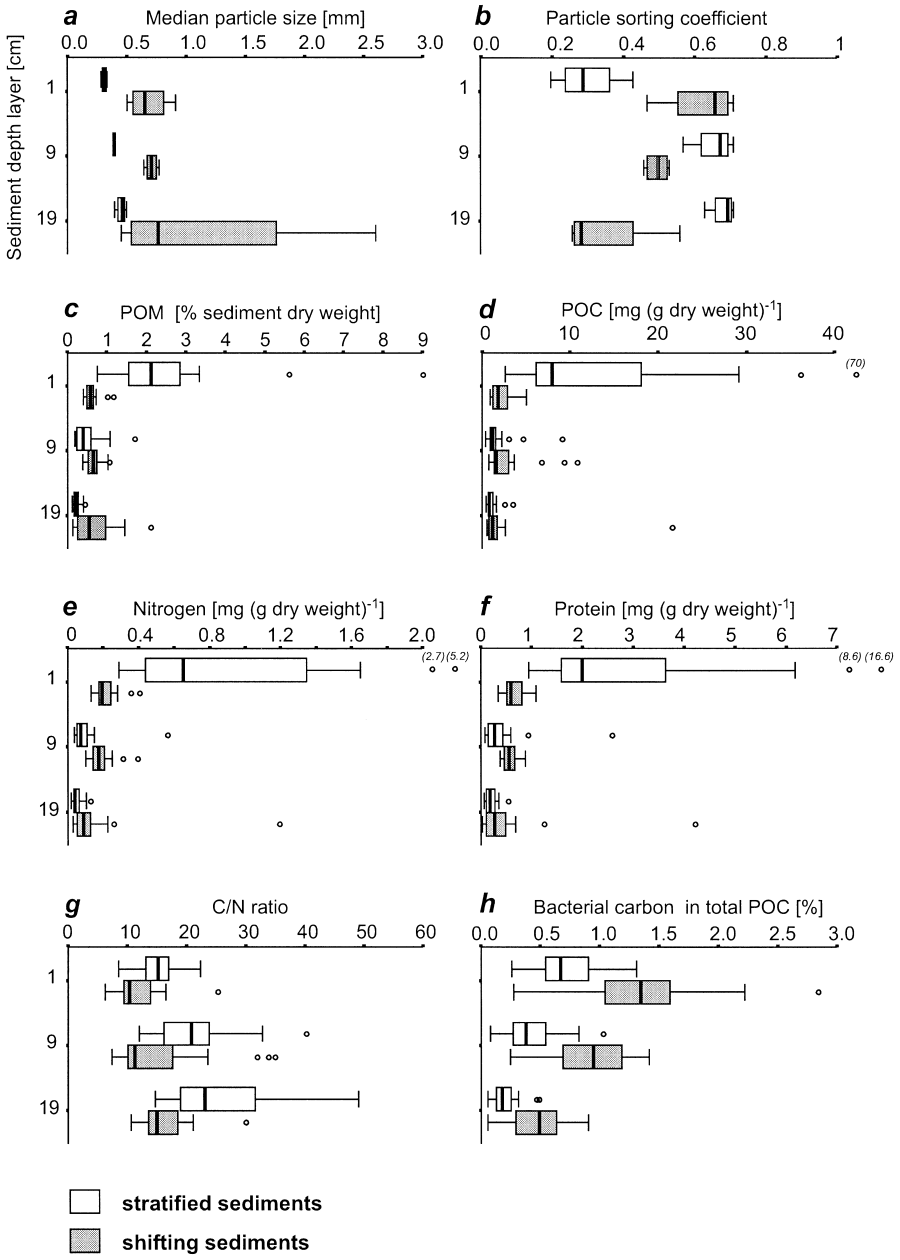

Figure 1. Depth distribution of sedimentary variables from all four sampling occasions in stratified sediments (white boxes) and shifting sediments (grey boxes). Boxes mark the 25th percentile, the 50th percentile (median) and the 75th percentile. The left and the right margins of the horizontal lines mark the minimum and the maximum values excluding outliers. Outliers are defined as values lying more than 1.5 box sizes from the box margins and are shown separately as circles.

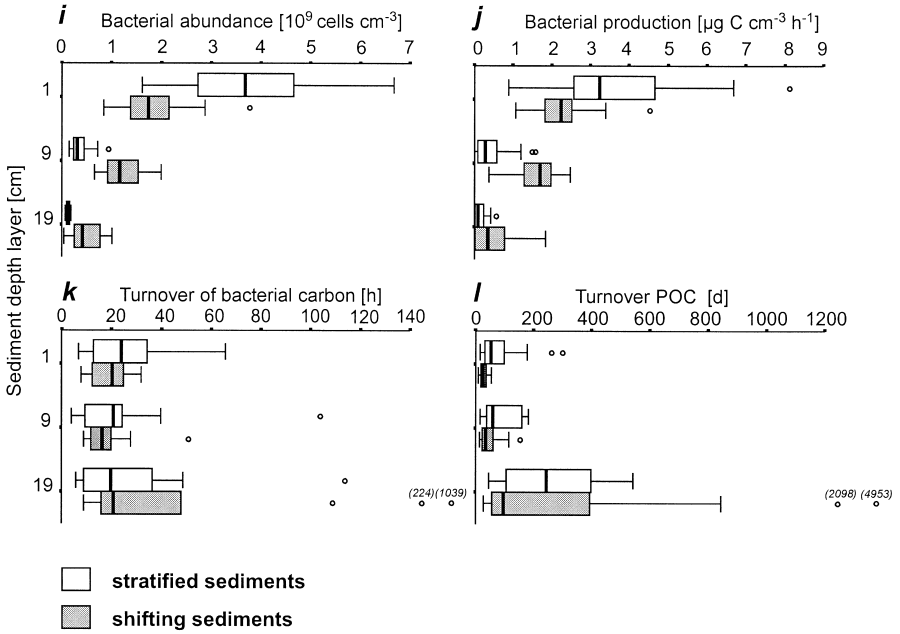

Figure 1. Continued.

nitrogen, so that the protein/nitrogen ratio did not vary significantly with sediment depth (analysis of variance (ANOVA); df = 115, F = 0.15, p = 0.86).

As with the detrital variables, bacterial abundance and production peaked in the upper layer of the stratified sediments (median abundance = 3.69×10^9 cells cm^{-3}, median production = 3.23 μg C cm^{-3} h^{-1}) (Figures 1 i, j). Bacterial abundance and production were at a minimum in the lower layers of the stratified sediments, where production was below the detection limit in some samples (median abundance = 1.3×10^8 cells cm^{-3}, median production = 0.11 μg C cm^{-3} h^{-1}). The turnover times of bacterial carbon as well as that of POC were shortest in the upper layers of the shifting sediments (median turnover of bacterial carbon = 21 h, of POC = 27 d). The turnover times of POC were longer in the stratified sediments with their higher content of POC (median turnover of POC, upper sediment layer = 53 d). The POC turnover times increased in lower layers in both sediment types. Turnover times of bacterial carbon showed that pattern only in shifting sediments. Median turnover times over all types and layers of sediment were 20 h for bacterial carbon and 62 d for POC (Figures 1 k, l; Table 1).

Proportion of bacterial biomass in total POM

Mean bacterial volume was 0.061 μm^3 (median, 25 percentile, 75 percentile were 0.044, 0.028 and 0.072 μm^3, respectively). This corresponds to a mean bacterial biomass of 21.5 fg C (18.2, 13.4, 25.5 fg C, respectively) and a mean protein con-

tent of 25.0 fg calculated with the model of Simon et al. (1990). Mean (± SD) bacterial biomass calculated from other models for volume/biomass conversion factors derived from natural bacterial populations give the following values: 61.9 ± 34.4 fg C with the model of Theil-Nielsen and Søndergaard (1998), 32.1 ± 26.3 fg C with that of Loferer-Kröβbacher et al. (1998), 23.2 ± 1.8 fg C with that of Lee and Fuhrmann (1987), and only 12.5 ± 10.6 fg C with that of Norland et al. (1987). This variation, by a factor of 5, has to be considered when the relation of bacterial biomass with other sedimentary variables is calculated and discussed. As the models are allometric, differences between them vary in dependence of the bacterial size distribution (Psenner 1990). Using 21.5 fg C for mean bacterial biomass and 25 fg for mean protein content, bacteria contributed (±SD) 0.69 ± 0.52% to the total POC, 1.93 ± 0.94% to total organic nitrogen, and 3.43 ± 1.88% to the total protein content in Spree sediments.

Relationships between bacterial and detrital variables

The factorial map of the principal component analysis (PCA) based on the bacterial and detrital variables is shown in Figure 2a, the ordination plot in Figure 2b. The first two factors of the principal component analysis (PCA) account for 70.5% of the variation of detrital, bacterial and combined variables (Figure 2b). Factor 1 is strongly correlated with the detrital variables ($r > 0.93$) and also correlates with bacterial abundance ($r = 0.82$) and production ($r = 0.62$). Factor 2 is more determined by bacterial activity and substrate quality. It correlates with the turnover of POC ($r = -0.96$), the proportion of bacterial biomass in total POC ($r = 0.82$), C/N ratio ($r = -0.71$) and bacterial production ($r = 0.70$). The factorial map reveals three different habitat types (Figure 2a): (i) the upper layer of the stratified sediments; these sampling positions are located on the right part of the factorial map. The total POM content and the contents of its various components (protein, nitrogen, carbon) were high as shown by their location on the far right side of the ordination plot (Figure 2b). These detrital variables also correspond with high bacterial abundance and production. (ii) Most samples from the shifting sediments are found in the middle of the factorial map. As revealed by the variables on the ordination plot, they are characterized by lower contents of organic matter, protein and nitrogen than those of the stratified sediments, paralleled by low C/N ratios. They exhibited a high bacterial production and short turnover times of bacterial biomass. (iii) Samples from the middle and lower layer of the stratified sediments and some samples of the lowermost layer of the shifting sediments are found on the lower left side of the factorial map. They mostly had low organic matter contents, with high C/N ratios. In these samples, bacterial abundance and production were low, and turnover times of bacterial and total carbon were high.

The detrital variables were closely correlated with bacterial variables (Table 2). These correlations were highest for variables indicative of both organic matter quality and quantity (nitrogen, protein), and were still high for variables indicative of organic matter quantity only (carbon, loss on ignition). The proportion of bacterial biomass in total POC also correlated with the detrital variables. Algal pig-

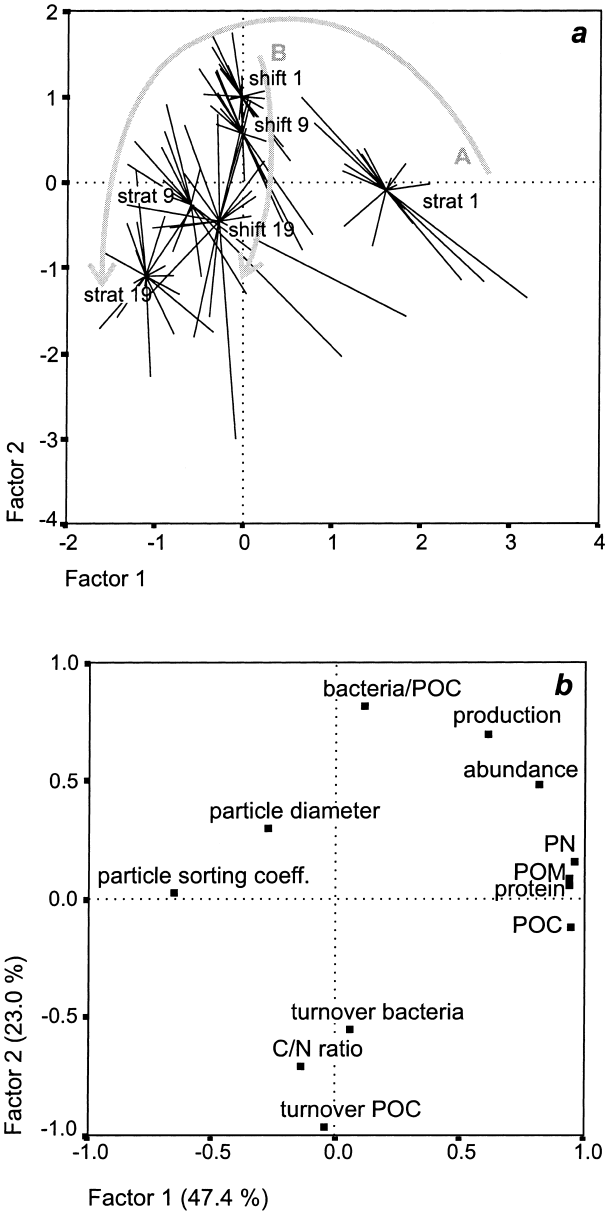

Figure 2. PCA ordination plot of sedimentary variables. (a) Factorial scores of sampling positions. Numbers indicate sediment depth, shift = shifting sand, strat = stratified sand. Arrow "A" indicates a sequence of overall metabolic activity, arrow "B" indicates a gradient of specific metabolic activity. (b) Ordination of the sedimentary, detrital and bacterial variables. Correlation arrows are removed to improve clarity. Values in parentheses along axes are the amounts of variation explained by the principal component.

Table 2. Table 2. Spearman rank correlation coefficients between bacterial variables and particulate organic matter (POM) characteristics. Levels of significance are $^{***}p < 0.001$, $^{**}p < 0.01$, n.s = not significant at a 0.05% level. Correlation values marked with asterices hold a level of significance of $p < 0.01$ after sequential Bonferroni correction (Holm 1979; Peres-Neto 1999).

POM	n	Bacterial abundance	Bacterial production	Turnover time bacteria	Bacteria/POC
Loss on ignition	119	0.77***	0.66***	n. s.	0.30**
POC	117	0.75***	0.68***	n. s.	0.07 n.s.
Protein	119	0.81***	0.73***	n. s.	0.32***
PN	116	0.90***	0.82***	n. s.	0.34***
POC:PN	116	–0.46***	–0.43***	n. s.	–0.81***
Chlorophyll a	21	0.82***	0.20 n. s.	n. s.	–0.42 n. s.
Turnover POC	106	–0.41***	–0.63***	0.56***	–0.77***
Bacteria/POC	117	0.64***	0.55***	n. s.	

ments (chlorophyll a and phaeopigments) were only detected in the upper layer of the stratified sediments and in one additional sample from the 9 cm layer, and were therefore not included in figures or ANOVA. However, algal pigments also correlated well with bacterial abundance (Table 2). The ratio chlorophyll/phaeopigments was relatively constant in all samples (0.43 ± 0.09) from the upper sediment layer, but much lower in the only sample where pigments could be detected in the 9 cm sediment depth.

Discussion

Gradients and variability of particulate organic matter (POM)

Bacterial abundance and production in the Spree sediments were strongly correlated with the amount and quality of POM. Also, carbon, nitrogen, and protein contents markedly decreased with increasing sediment depths as shown in Figure 1 and Table 1. The differences found between the stratified and the shifting sediments are probably due to the different modes of POM supply: Sedimentation processes prevail in the stratified sediments, such that fresh organic material sinking out from the water column accumulates on the sediment surface only. However, in permeable and shifting sediments, organic material, nutrients and oxygen are advectively transported into lower sediment layers (Thibodeaux and Boyle 1987; Huettel et al. 1996). The principal component analysis of all data supported this differentiation between the sediment types and revealed a sequence of overall metabolism (arrow A, Figure 2a), which was highest in the upper layer of the stratified sediments, due to high amounts of POM. This habitat was hydrologically disconnected from the underlying sediments, as shown by the opposite location of the deeper sediment

layers on the factorial map. Bacterial and POC turnover times were shortest in the upper layer of the shifting sediments, indicating the highest activity "per bacterium" in this habitat. The depth layers of the shifting sediments were hydrologically connected to each other, as shown by the close connection of all variables in the upper 9 cm of these sediments. There were little variations in variables describing factor 1 (POM, particle sorting), but a gradient of bacterial activity and C/N ratio with sediment depth was present, indicating a hydrological gradient within these homogenous sediments (arrow B, Figure 2a).

High scattering within parallel sampling locations as well as high standard deviations of detrital variables can be attributed to their high spatial variability. As an example, a single piece of wood of 0.2 g dry weight (assumed to consist of 100% lignin (phenylpropan) for simplicity) in a 50 g dry weight sample may increase the mean organic matter content of this sample from 0.6% to 1.0%, the carbon content from 0.24% to 0.51%, and the C/N ratio from 8 to 17. Additional variation may be caused during sample processing by inhomogenous grinding and mixing of the sediment samples. In spite of this variability, the mean of the calculated proportion of POC in total POM of 0.42 exactly meets the published values (e.g. Sutherland (1998)).

C/N ratios, origin and diagenesis of organic matter

The increasing C/N ratios in lower sediment layers indicated that a depletion of nitrogen took place in the diagenesis of organic matter. Although the C/N ratio is an appropriate indicator for the nutritive quality of organic matter it is difficult to conclude whether a high C/N ratio results from diagenetic alterations or from a high proportion of allochthonous sources of POM (Cowie and Hedges 1994). This problem can be partially resolved if the hydrologic flow paths are considered: Assuming an infiltration of river water and POM into the sediments, vertical gradients of C/N ratio, nitrogen content, and protein would indicate diagenetic alterations of the POM. Horizontal variation of these variables within the upper sediment layer would indicate different sources of POM. In stratified sediments, settling seston is the dominant source of carbon and nitrogen. Even so, the C/N ratio of 13–17 in shifting sediments is higher than that of the organic fluff overlying the sediments (10.7; Wanner and Pusch (2001)) and that of seston in the Spree (9 ± 2 (mean ± SD); n = 24; unpubl. data). The abundant algal species which are mostly diatoms had a C/N ratio of 8 (Behrendt 1990). Thus, the flux paths and diagenesis of pelagic POM can be traced onto the sediment surface. The contribution of algal N to total particulate N (PN) can be estimated by assuming a C/Chl ratio of 50 and the C/N ratio of the algae of 8. Then, fresh algal material would only contribute 6.3 ± 1.7% to the total PN in this sediment type. The C/N ratio increases in lower sediment layers, probably due to further diagenetic alterations. As bacterial biomass made up less than 1% of total detrital carbon and less than 2% of detrital nitrogen, the nitrogen content of the bacteria counted did not have a major effect on the carbon and nitrogen content of total POM.

In shifting sediments, no phytopigments were detected so that the carbon and nitrogen there probably originated from other sources such as the bacterial biofilm that covers the sediment particles and particulate organic matter of mostly allochthonous origin, that was entrained into or buried in these sediments (Pusch et al. 1998). The biofilm here mainly consists of adsorbed dissolved organic substances (Fiebig and Lock 1991; Fischer et al. 2002), bacterial extracellular polymeric substances (Wingender et al. 1999), and bacterial biomass which contributed 1.16 ± 0.57% to total POC in this sediment type.

Protein and nitrogen

The average proportion of nitrogen in protein is 16%, which equals a protein/nitrogen ratio (wt/wt) of 6.25 (Pirie 1955). A lower ratio of 3.5, as found in the present study, may indicate a higher proportion of "non-protein nitrogen". It has been proposed that this "non-protein nitrogen" originates from amino acids and proteins that were subsequently bound to carbohydrates and phenolics of the detritus, via condensation reactions, and then incorporated into heterocyclic rings of humic substances (e.g. Melillo et al. (1984)). In this case we should expect a downcore increase of "non-protein nitrogen" in our samples, similar to that found by Mayer and Rice (1992) (who, however, determined a much higher proportion of "non-protein nitrogen" than we found). However, this was not the case. Other authors found that the transformation of amino nitrogen into heterocyclic rings did not occur. Refractory fractions of nitrogen remained bound to detritus, e.g. to lignocellulose (Buchsbaum et al. 1991). Lignocellulose from vascular plants, however, would be efficiently degradable for bacteria (Benner et al. 1988). Also, recent models of humic substances propose high proportions of amino acids, polypeptides and proteins bound to dissolved humic substances (Volk et al. 1997). This humified proteinaceous material may be efficiently retained and subsequently decomposed by the bacterial biofilm (Volk et al. 1997; Fischer et al. 2002). Thus, although the extraction method chosen for our study proved to be most effective in a comparison of various methods (Rausch 1981), it seems that incomplete extraction of amino nitrogen was the reason for the low protein/nitrogen ratios found rather than inefficient degradation by bacteria. This would explain the uniform protein/nitrogen ratio in various depths and sediment types.

Turnover of POC

Theoretical turnover times vary widely for different POC fractions. Detritus of macroalgal origin for example is turned over more rapidly than that of vascular plants (Buchsbaum et al. 1991). In our study, protein was used in preference compared to bulk organic matter, as indicated by the decrease of protein in relation to POC with increasing sediment depth. However, theoretical turnover times of bulk POC are useful indicators of ecosystem metabolism.

Turnover times ranging from 2 weeks to 425 years were found in reviews covering 22 intensely studied streams (Sinsabaugh 1997; Webster and Meyer 1997).

Relying on measurements of bacterial respiration, Pusch (1996) found a mean carbon turnover time of 4 years for bulk POC, and 4 months for the fraction loosely associated with sediment surfaces. Hedin (1990) calculated a long turnover time of 10 years for benthic POC, whereas Proft (1998) calculated carbon turnover times of only 20–400 days in sediments of a polluted upland river. These latter data are very similar to our measurements, where 90% of the cases ranged from 10 to 400 days with a median of 62 days. However, it has to be kept in mind that for methodological reasons production rates in the lower sediment layers are overestimated in our study. By incubating these sediment samples in vials, bacteria were not exposed to the same gradients of dissolved organic matter and electron acceptors as in the streambed sediments. When these gradients were simulated in sediment cores perfused with river water, production was significantly higher in the uppermost sediment layer, and lower in the lower sediment layers (Fischer and Pusch 1999). Additionally, we have excluded those samples from calculations, where bacterial production was below the detection limit (see methods section). Therefore, the real turnover times in these deep layers are probably longer than showed in Figure 1, but even so, they would not essentially change the median turnover time of 62 d. Thus, the carbon turnover times found in the present study are shorter than most of those reviewed by Sinsabaugh (1997)andWebster and Meyer (1997) and from the studies by Hedin (1990) and Pusch (1996).

One reason for the shorter turnover times found by us is that by using bacterial production as a measure for carbon turnover, we are able to include anaerobic production into calculations of carbon turnover. Additionally, many of the former studies were performed in low order woodland streams where allochthonous, scarcely processed carbon dominates. Thus, in the reviewed streams, high turnover times were generally related to a high content of wood, or at least to a high content of coarse organic matter (CPOM) which may include small pieces of wood (Webster and Meyer 1997). In contrast, higher quality organic matter prevails in the Spree, especially in the shifting sand habitat which is additionally supplied with dissolved organic matter by advective flow and sediment movement. We therefore propose that in rivers including extended lowland sections, POM may be easily degradable and that these systems are important sites of organic matter transformation. Our system thus also shows a very high ratio of bacterial productivity:organic matter content in comparison to other streams examined (Sobczak et al. 1998; Findlay and Sobczak 2000; Fischer and Pusch 2001).

Bacteria in the sediment food web

The proportion of bacterial biomass in total POC was relatively high in Spree sediments as compared to sediments of the Hudson River estuary (Sinsabaugh and Findlay 1995). However, because bacterial protein still contributes only 3.2% to total protein (4.7% in the uppermost sediment layer of shifting sands), bacteria cannot be the major food source for detritivorous organisms. These organisms, mainly chironomids and oligochaetes in the Spree, would instead have to rely on bulk organic matter. Indirectly, however, bacteria have an important function in the

sediment food web for two reasons. First, they are able to retain dissolved organic matter and convert it into the particulate fraction, thus making it available for higher trophic levels (Meyer 1994). Second, biofilm bacteria produce exopolymeric substances (e.g. Wingender et al. (1999)) which develop a biomass five times higher than that of bacteria themselves and which are readily consumed by detritivorous organisms (Hall and Meyer 1998). Some organisms, however, are able to selectively graze on single bacteria and thus profit from the high nutritious value of bacteria as compared to bulk POM, for example particle feeding heterotrophic nanoflagellates with a cytopharynx (e.g. *Rhynchomonas nasuta* and other bodonids) (Caron 1987; Fenchel 1991), some ciliate species (Eisenmann et al. 1998), and some nematode species (Giere 1993). However, the few published studies on the sedimentary food web show that micro- and meiofaunal grazing did not exert a strong regulatory function on bacterial abundance (Kemp 1990; Borchardt and Bott 1995).

A high loss of bacterial biomass due to viral lysis can be postulated, although viruses were not examined in this study. Like bacteria, viruses are not adversely affected by the harsh physical conditions in the shifting sand habitat. In addition, viral abundance was found to be high in lake (Maranger and Bird 1996) and stream (Lemke et al. 1997) sediments. Lysis by viruses is a major sink for bacterial biomass production in pelagic environments (Weinbauer and Höfle 1998) and can be significantly correlated with bacterial abundance and production (Wommack and Colwell 2000). This may also be true in river sediments, which have so far never been examined for virus-induced losses of bacterial biomass. Another important fate of the growing biofilm in shifting sediments is probably to be abraded by physical forces and resuspended as seston rather than directly grazed.

Conclusions

In the Spree, the biochemical properties of the organic matter varied widely depending upon sediment depth and type. Diagenesis of organic matter could be traced from the water column via the upper sediment layer to deeper layers. Substrate quality and quantity strongly influenced bacterial abundance and activity. The exploitation of the substrates by bacteria was enhanced by a permeable sediment structure such as that of the shifting sediments, where higher quality POM could reach the deeper sediment layers via advective flow and sediment movement. High bacterial productivity and relatively high quality POM in these sediments lead to short turnover times of the sedimentary organic matter and to a high overall metabolic activity. Our findings demonstrate that in areas of the river bottom with shifting sediments the bacterial metabolism of organic carbon is much higher than in stratified sediments due to deeper hydraulic exchange with the water column. Thus, these sediments which are typical for lowland rivers, strongly influence the metabolism of the whole ecosystem. As the presence of shifting sediments is typically reduced by river regulation, the microbial degradation of organic matter will be reduced in highly altered river channels. Since transport in river systems represents a

carbon flux of global importance, large-scale alterations in river dynamics are supposed to influence the global carbon balance.

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