

Differential retention and utilization of dissolved organic carbon by bacteria in river sediments

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Abstract

The differential ability of natural sediment biofilms to store and metabolize specific dissolved organic carbon (DOC) fractions was studied with a set of perfusion experiments that combined a chromatographic method for the analysis of several DOC fractions with the measurement of bacterial abundance and activity. High proportions of low-molecular-weight substances and polysaccharides and low but significant proportions of humic substances were retained in sediment cores after perfusion. Bacterial abundance and production in these cores were high ($1.7\text{--}3.0 \times 10^9$ cells cm^{-3} and $0.7\text{--}12.0 \mu\text{g C cm}^{-3} \text{ h}^{-1}$, respectively). Bacterial abundance, production, and turnover times were correlated with the retention of various DOC fractions, especially polysaccharides, indicating a differential microbial utilization of DOC. Temperature as well as the quantity and quality of retained organic matter were the major factors that influenced bacterial production. However, particulate organic carbon represented the main energy source for sediment bacteria. It is concluded that certain fractions of DOC may be readily utilized for bacterial growth, irrespective of molecular size. Instead, the presence and abundance of specific chemical groups might be decisive. Hence, the processing of organic substrates by the microbial biofilm may largely influence the biogeochemistry of DOC in river waters.

Dissolved organic carbon (DOC), whatever its form or origin, either directly or indirectly represents the ultimate source of organic carbon for sustaining the metabolism of heterotrophic bacteria. Processes such as extracellular decomposition of organic macromolecules and selective consumption of specific DOC fractions enable bacteria to exert a considerable influence on quantity and quality of DOC in natural waters (e.g., Fiebig and Marxsen 1992; Findlay et al. 1993; Volk et al. 1997). On the other hand, the metabolic activity of bacteria can be affected by the ambient concentration and composition of DOC (e.g., Kaplan and Bott 1989; Amon and Benner 1996; Baker et al. 1999). Thus, in a number of ways, bacteria may exert an influence on the carbon biogeochemistry of aquatic ecosystems.

In running waters, sediments are the major site of bacterial metabolism (Fischer and Pusch 2001). Their large internal surface area promotes colonization of these sediments by bacterial biofilms (Lock 1993; Brunke and Fischer 1999; Fischer 2002). These biofilms are supplied with nutrients and oxygen by flowing interstitial water, which originates either from the overlying water column being forced into the sediment interstices by physical processes or from groundwater exfiltration (Brunke and Gonser 1997; Pusch et al. 1998). The hyporheic biofilms retain inorganic and organic solutes (e.g., Fischer 2002), and thus they can buffer the supply of organic substrates so that short-term changes in the quality and quantity of DOC need not have an immediate effect on biofilm metabolism (Freeman and Lock 1995; Fiebig 1997).

Unraveling the functioning of the DOC metabolism in the biofilm is difficult because of the methodological problems encountered in measuring microbial activity and in the chemical fractionation of DOC. One particular problem is the difficulty in relating microbial activity to concomitant bacterial DOC utilization. Some recent studies stressed that there are differences in the availability of DOC originating from various sources for bacterial growth or respiration (Battin et al. 1999; Wiegner and Seitzinger 2001). However, it has so far been problematic to make an instantaneous distinction between DOC fractions that may support significant growth of heterotrophic bacteria and the more refractory DOC fractions that do not. Amon and Benner (1996) hypothesized that DOC availability was largely indicated by molecule size, with the larger molecules being more rapidly turned over.

Bacterial production in hyporheic sediments has so far been measured in only very few studies (Findlay and Sobczak 2000). Bacterial productivity in sediments is assumed to be at least a partial function of the amount of sediment organic matter. Whereas a correlation between particulate organic matter and bacterial production has been revealed for river sediments (Brunke and Fischer 1999; Findlay and Sobczak 2000; Fischer et al. 2002), a causal relationship between DOC retention and bacterial production in situ still remains an open question.

In order to reveal relationships between the occurrence and retention of various fractions of DOC in sediments of a lowland river, and concomitant bacterial growth, we characterized DOC in interstitial water and at the same time estimated bacterial abundance and production in the sediments through which this water flowed. We performed these measurements using the natural bacterial community on their indigenous substrate. Thus, we could address the following questions. (1) Does the passage of interstitial water through sediments and the concomitant bacterial activity within these sediments substantially change the quality of DOC? If so,

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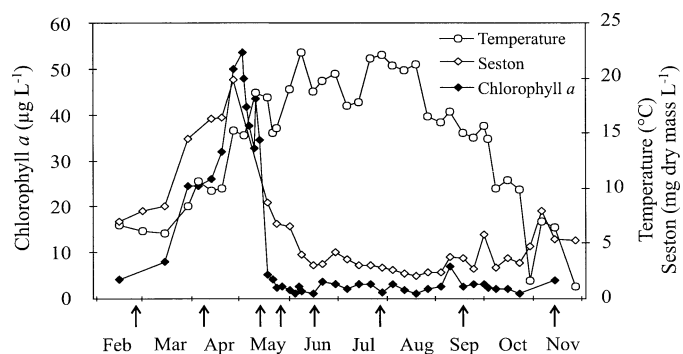


Fig. 1. Temperature, seston concentration, and Chl *a* concentration in the Spree water column in 1998. Arrows indicate the dates when sediment bacterial and DOC variables were measured.

which fractions are preferentially retained within the sediments and utilized by the bacteria? (2) Does quality and quantity of DOC in interstitial water have an immediate effect on bacterial metabolic activity in the sediments? If so, which fractions of the DOC have a specific influence on this activity?

Materials and methods

Study site—Sandy sediments were taken from the Spree, a sixth-order river ~40 km upstream of Berlin, Germany (52°22'N, 13°49'E). In this section, the Spree is a lowland river with sandy sediments in the main channel. Unconfined, shifting sands covered ~20% of the river bottom area (Fischer and Pusch 2001). In 1998, a distinctive vernal phytoplankton bloom developed, followed by an extended clear-water stage. Between February and November 1998, seston concentrations ranged from 2 to 20 mg L⁻¹ (Fig. 1). At a mean discharge of 11.5 m³ s⁻¹, the mean water depth was 1.3 m, mean width was 25 m, and maximum flow velocities in the main channel were 50–60 cm s⁻¹. The sediments had an organic matter content of ~1% ash-free dry mass (loss on ignition), a homogenous particle size distribution with a median particle size of 0.5 mm, high hydraulic conductivity ($k = 0.001\text{--}0.004\text{ m s}^{-1}$), and dark manganese and iron ox-

ide coatings on the individual quartz sand particles. To obtain field replicate samples, we sampled three sites located within a 2-km reach. We took four sediment cores (6 cm in diameter) from each of the three sites on each of eight occasions in 1998 (Fig. 1). From each core, we subsampled smaller cores using 20-ml polyethylene syringes with their tips cut off. These smaller cores had a volume of 24 ml (7.6 cm in length and 2.0 cm in diameter), and they were stored at 2–4°C for 6–9 h until the start of experimentation.

Experimental setup—The sediment cores were perfused with prefiltered river water (8- and 0.45-µm pore size cellulose nitrate filters) freshly taken at each sampling date from the same section of the River Spree. Mean (±SD) perfusion rate was 6.0 ± 0.3 ml cm⁻² h⁻¹, resulting in a mean residence time of 0.5 h. This flow rate was based on estimates of in situ interstitial flow obtained from model calculations (Thibodeaux and Boyle 1987) and dye experiments conducted in laboratory flumes (authors' unpubl. data). The filtered river water was perfused through the sediment cores with a 16-channel peristaltic pump (Ismatec IPC-16 S) in an upward flow and once-through mode, entering the cores from their originally uppermost sediment layer (Fig. 2). Thus, the infiltration of river water into the sediments was simulated. We used Tygon tubing (Norton) and precombusted glassware for pumping and storage of water, respectively. The tubing was rinsed for 2 h with warm (50°C) micropure water and for 2 h with cold micropure water before usage. Vials for collecting DOC samples were washed with NaOH, HCl, and micropure water before use.

The general experimental setup was as follows. During a settling time of 8–10 h, the 12 cores of each sampling date were perfused with prefiltered river water. Nine cores (three from each site) were used for DOC and production measurements, and three (one from each site) served as controls in the production measurements. The perfused water was discarded, and perfusion was continued for 10 h to measure DOC retention in the sediments. Duplicate controls for DOC measurements were run within the same system by use of empty syringes. There were no significant differences between DOC concentrations before and after the passage of

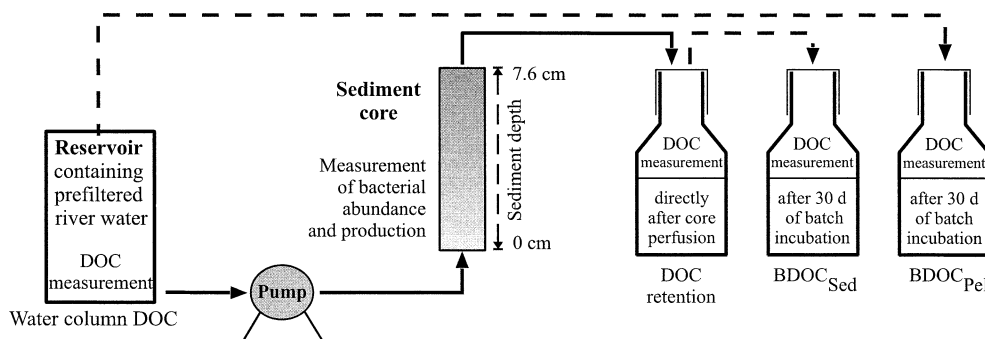


Fig. 2. Schematic view of the experimental setup used for measuring bacterial production and DOC retention in sediment cores and for BDOC. The solid lines indicate the transport of water in core-perfusion experiments. The dashed lines indicate that water before and after perfusion through the sediment cores was subjected to 30-d batch incubation for measurement of BDOC_{Pel} and BDOC_{Sed}, respectively (see also *Materials and methods* section).

river water through the tubing and the empty syringes (paired *t*-test, $p = 0.55$, $n = 11$). Subsequently, bacterial production was measured for 12 h as described below. All incubations were made at in situ temperatures ranging 7–22°C ($\pm 1^\circ\text{C}$), except on 26 May 1998. On that date, the incubation temperature was 18°C, which was 3°C higher than the in situ temperature but was the same temperature as in the previous and following measurements. Thus, bacterial activities during the algal bloom and the clear-water stage could be compared under the same thermal conditions.

DOC analysis—DOC in the river water before and after perfusion through the sediment cores was quantified and characterized by liquid chromatography followed by organic carbon detection (LC-OCD; Huber and Frimmel 1994; Hesse and Frimmel 1999; Sachse et al. 2001). This method combines size-exclusion chromatography via stainless steel columns (250 × 20 mm) packed with TSK HW-50S resin (Toyopearl), ultraviolet (UV) detection of the spectral absorption coefficient at a wavelength of 254 nm, and infrared (IR) detection of the eluting carbon fractions after UV oxidation of DOC at wavelength of 185 nm in a cylindrical UV thin-film reactor (Gränzel; Huber and Frimmel 1991). Phosphate buffer (0.029 mol L⁻¹, pH 6.5) was used as the mobile phase. The flow rate was 1 ml min⁻¹, and the sample injection volume was 2 ml.

Polysaccharides (PS), humic substances (HS), low-molecular-weight acids (LMWA), and two groups of low-molecular-weight substances (LWS, representing amino acids, mono- and disaccharides) were fractionated, oxidized, and subsequently quantified by IR detection. The fraction of PS shows no absorption in the UV range. HS include humic and fulvic polyelectrolytic acids that are UV active because of aromatic groups. LMWA are defined as low-molecular-weight carboxylic acids such as several metabolites in biological and chemical processes. Fractions were identified by use of standards (humic and fulvic acid standards from the IHSS) and simple compounds of different origin, and by characterizing the biological availability of each fraction (Sachse et al. 2001). For molecular-weight calibration, saccharides (raffinose, maltose, glucose, glycerin, and methanol from Merck) and polydextranes ($M_p = 830, 4,400, 9,900, 21,400, \text{ and } 43,500 \text{ g mol}^{-1}$ from Polymer Standards Service) were used. The calibration curve was obtained by plotting the retention times of the standards against the logarithm of their molecular weights by use of the program "Geltreat" (I. Perminova unpubl. data). Molecular weights of HS in the water samples were calculated by comparing their retention times with the calibration curve. Total DOC was quantified by IR detection after UV oxidation, bypassing the chromatographic unit.

DOC retention and biodegradable DOC (BDOC)—We defined DOC retention in sediments and BDOC as indicators of bacterial DOC utilization in sediments and the water column. These variables were calculated from differences in DOC between the river water and the perfused and/or incubated water (Fig. 2). (1) DOC retention in sediment cores was calculated from changes in DOC concentrations before and after perfusion through sediment cores. This fraction

corresponds to BDOC sensu Kaplan and Newbold (1995). (2) BDOC_{Sed} was defined as the amount of DOC consumed during core perfusion and subsequent 30 d of batch incubation of the perfused water. (3) BDOC_{Pel} was defined as the amount of DOC consumed after 30 d of batch incubation of river water (BDOC sensu Servais et al. 1989). Batch incubations for the measurements of BDOC_{Pel} and BDOC_{Sed} were made with 150-ml samples in precombusted glass flasks. Samples were inoculated with 1.5 ml of prefiltered (2- μm pore size) river water and incubated for 30 d at 20°C in the dark. After the incubation, concentration and composition of DOC were measured (Servais et al. 1989) (see Fig. 2).

Bacterial abundance and production—Bacterial production in the perfused sediment cores was estimated from leucine incorporation rates into bacterial protein (Kirchman 1993). After sampling for DOC measurements, we added L-[U-¹⁴C]leucine (Amersham; specific activity 11.3 GBq mmol⁻¹) and unlabeled L-leucine to the perfusion water in order to achieve a final concentration of 50 μM and a final specific activity of 7 Bq nmol⁻¹. The cores were perfused with leucine for 12 h and then fixed by a 4-h perfusion of 5% formaldehyde in prefiltered river water. Leucine uptake in the perfusion water can be neglected in this setup. Only a small amount of pore water was later sampled with the sediments, and biomass and production of bacteria in the sediments exceed those in the river water by factors of 500–1,000 per volume, respectively (Fischer and Pusch 2001). Three control cores—one from each site—were perfused with a mixture of L-[U-¹⁴C]leucine, unlabeled L-leucine, and 5% formaldehyde. The number of controls was later reduced, because activity in controls was low (always <8% of the activity in living sediments). The leucine-incorporation method had previously been tested in a similar experimental setup for linearity of uptake over long time intervals (36 h), for isotope dilution, and for substrate saturation (Marxsen 1996; Fischer and Pusch 1999).

On the next day, the sediment was carefully removed from the cores and divided into depth layers of 0–1.9, 1.9–3.8, 3.9–5.7, and 5.7–7.6 cm. These layers are referred to in the results as 1-, 3-, 5-, and 7-cm depths. The sediment from each layer was mixed, and a 0.5-cm³ aliquot was used to determine bacterial abundance and production, as described elsewhere (Fischer and Pusch 1999, 2001). In brief, subsamples used for bacterial cell counts were taken after a 10-min sonication step and diluted with a sterile filtered aqueous solution of 3.5% formaldehyde, 0.85% NaCl, and 1 mM pyrophosphate. Bacteria were stained with the use of 4',6-diamidino-2-phenylindol at a final concentration of 10 mg L⁻¹. 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 directly counted by epifluorescence microscopy.

Protein was extracted from the remaining samples with the use of hot trichloroacetic acid at 5% final concentration. The precipitate was filtered onto 0.2- μm pore size polyester filters (Oxyphen). Filters were thoroughly rinsed with deionized water and then put into 4-ml scintillation pico-vials, completely dissolved in 0.5-ml solvent (Soluen; Packard Instruments) and mixed with 2.5 ml of scintillation fluid

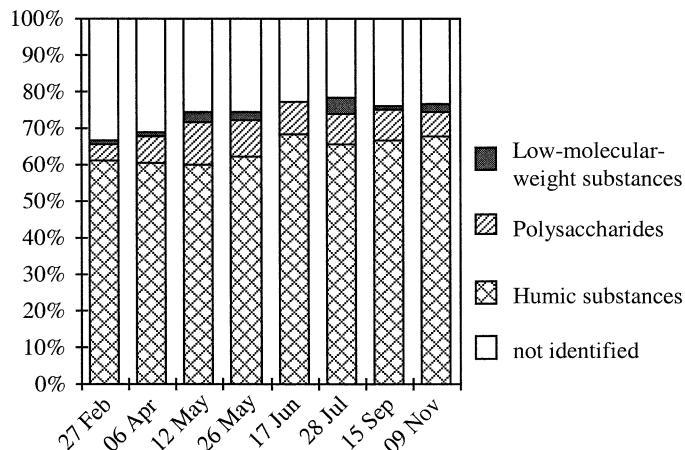


Fig. 3. Composition of DOC in the Spree water column in 1998.

(Hionic Fluor; Packard). Radioactive decays were measured in a Canberra Packard 1900 scintillation counter. Bacterial carbon production was calculated from leucine incorporation rates under the assumption of 7.3 mol % leucine in total protein and a carbon : protein ratio of 0.86 (Simon and Azam 1989). Isotope dilution was low (1.1–1.3; Fischer and Pusch 1999) and was therefore disregarded in the production estimates. Turnover times of bacterial carbon were estimated by dividing bacterial biomass by carbon production assuming a biomass of 22 fg C for a single bacterium (Fischer and Pusch 2001).

Q_{10} values—The metabolic rates of bacteria generally increase with temperature, within certain physiological limits. The magnitude of this increase can be characterized by the ratio of rates measured across a temperature increase of 10°C (Q_{10} ; Lampert 1984). To evaluate the effect of temperature on bacterial production, we calculated (1) the “ecosystem effective Q_{10} ” of bacterial production per unit of sediment volume, which includes the effect of seasonally changing bacterial abundances, and (2) a “community-specific Q_{10} ”

that characterizes temperature dependence as the bacterial production divided by bacterial cell number.

Data analysis—In total, 69 sediment cores were used for the experiments. These divide into eight sampling dates with three sites each and three replicate cores per site. In February 1998, only two sites were sampled, and bacterial abundance was not determined. We performed calculations and statistics on three levels of data aggregation. (1) Bacterial production, abundance, and turnover times were first estimated for single samples (four depth layers from each core, $n = 276$ for production, $n = 252$ for abundance and turnover) to examine their vertical distribution within the sediment cores. (2) Bacterial variables were then aggregated by adding the results of the different depth layers of each core. This was done to compare bacterial variables with DOC retention and $BDOC_{Sed}$, which were also determined for the entire cores ($n = 69$). (3) Temperature, DOC before incubation, and $BDOC_{Pel}$ differed only by sampling date. Therefore, these comparisons were made between sampling dates ($n = 8$), which include measurements from the river water on the respective sampling date as well as data from nine sediment cores each and bacterial data from four depth layers of each core. Data were analyzed with the software SPSS (release 6.0; SPSS) by use of nonparametric tests. Spearman rank correlations were used to reveal the relationships of bacterial data with DOC amount and composition. Kruskal-Wallis tests were performed to test the effects of sediment depth on bacterial abundance and production.

Results

DOC—Total DOC in the studied time period ranged from 5,920 to 7,400 $\mu\text{g L}^{-1}$. DOC composition showed a similar pattern throughout the year with $HS > PS > LWS$ (Fig. 3). Despite the low variability in total DOC concentrations, there were marked seasonal changes in the PS and LWS fractions. Among the fractions detectable by chromatography (Fig. 4A), PS peaked first (mean elution time, 25.6 min)

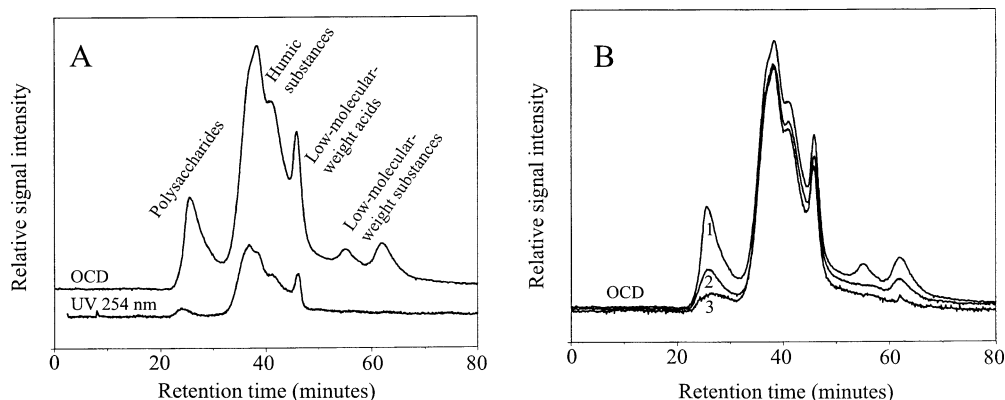


Fig. 4. (A) LC-OCD elution diagram of the DOC composition in the Spree water column, 12 May 1998. Upper line, OCD; lower line, UV detection at 254 nm. (B) LC-OCD elution diagrams of the DOC composition in Spree water, 12 May 1998, before perfusion through river sediments (upper line, 1), after perfusion (central line, 2), and after core perfusion and subsequent 30 d of batch incubation ($BDOC_{Sed}$, lower line, 3).

and made up 4.5%–11.5% of the total DOC. PS concentrations were highest in May, during the peak algal bloom ($854 \mu\text{g L}^{-1}$), and lowest in February ($269 \mu\text{g L}^{-1}$). HS were the major DOC fraction, accounting for 59%–68% of the total DOC (mean elution time, 38.2 min). LMWA only occurred at low concentrations (up to $160 \mu\text{g C L}^{-1}$ in February; mean elution time, 45.5 min) and were not detected during spring or summer. Typically, one or two additional peaks occurred in the river water after mean elution times of 55.1 and 61.9 min, respectively (Fig. 4A). This fraction was not UV active. It is referred to as LWS, is made up of sugars and amino acids (A.S. and I. Perminova unpubl. data), and made up $\leq 4.6\%$ of the total DOC (Fig. 3). The concentration of these substances ranged $22\text{--}289 \mu\text{g L}^{-1}$ and was quantified only when distinct peaks occurred. Other DOC eluting after >50 min and not showing any distinct peaks consisted of other low-molecular-weight aliphatic substances, which could not be assigned to a specific DOC fraction. This mixture contributed 11.7%–16.7% to total DOC. A proportion of 3.8%–17.7% of the total DOC was hydrophobic and was therefore not chromatographable. The mean ($\pm\text{SD}$) spectral absorption coefficient measured at 254 nm was $8.5 \pm 1.0 \text{ m}^{-1}$ for the humic fraction and close to zero for the other fractions identified, indicating a substantial proportion of aromatic structures in the humic fraction of the DOC only.

Vertical distribution of bacterial abundance and activity—Bacteria were relatively homogeneously distributed in space and time within the sandy habitats (Fig. 5A). However, we observed a slight decrease of bacterial abundance with increasing sediment depth (Kruskal-Wallis test, $\chi^2 = 14.5$, $p < 0.01$, $n = 252$). Mean ($\pm\text{SD}$) abundance was $2.66 \pm 0.26 \times 10^9$ bacteria cm^{-3} in 1-cm sediment depth and $2.17 \pm 0.38 \times 10^9$ bacteria cm^{-3} in 7-cm sediment depth.

Bacterial production varied more strongly in space and time than bacterial abundance (Fig. 5B). Mean ($\pm\text{SD}$) production ranged from $0.70 \pm 0.28 \mu\text{g C cm}^{-3} \text{ h}^{-1}$ in February at 7-cm depth to $12.0 \pm 2.3 \mu\text{g C cm}^{-3} \text{ h}^{-1}$ in July at 1-cm depth. The production was highest in the upper sediment layer and decreased significantly with increasing sediment depth (Kruskal-Wallis test, $\chi^2 = 137$, $p < 0.001$, $n = 270$), so that production at 7-cm sediment depth was always less than one-third of the production measured at 1-cm sediment depth. Production did not differ significantly between the sampling sites (Kruskal-Wallis test, $\chi^2 = 0.91$, $p = 0.63$, $n = 270$).

Turnover times of bacterial carbon were in a range of 3.5–220 h, which is equivalent to specific growth rates of 6.9–0.11 d^{-1} . The variation in turnover times of bacterial carbon was primarily driven by bacterial production; therefore, similar statistical relationships with sediment depth applied. We thus found the shortest turnover times of 4.8 ± 0.9 h in the uppermost sediment layer in July at 22°C and the longest turnover times of 76 ± 47 h in the lowermost layer in November at 7°C (Fig. 5C). Mean cell-specific bacterial production was in a range of 0.4–4.4 fg C h^{-1} .

Retention efficiencies for DOC fractions—Retention characteristics differed markedly for the various DOC fractions (Figs. 4B, 6). A total mean ($\pm\text{SD}$) of $898 \pm 429 \mu\text{g DOC}$

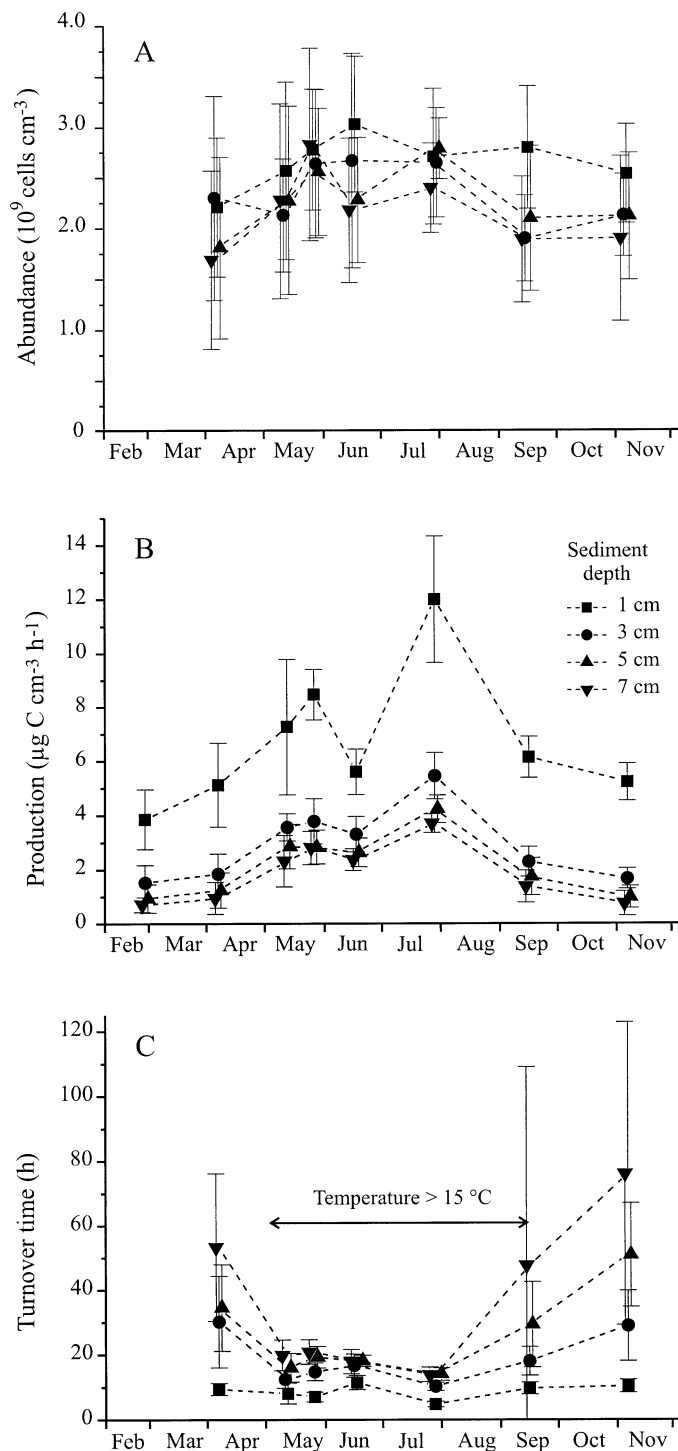


Fig. 5. (A) Bacterial abundance, (B) production, and (C) turnover times of bacterial carbon (C) in four depth layers of sediments of the River Spree, 1998. Means \pm SD, $n = 9$, ($n = 6$ in February). Symbols from each sampling date have been spread to improve clarity.

L^{-1} (14% of the input DOC) was retained in the cores. Retention was most effective in May during the peak algal bloom, with $1,500 \mu\text{g DOC}$ retained per liter of perfused water (20% of the total DOC), when total DOC in the river

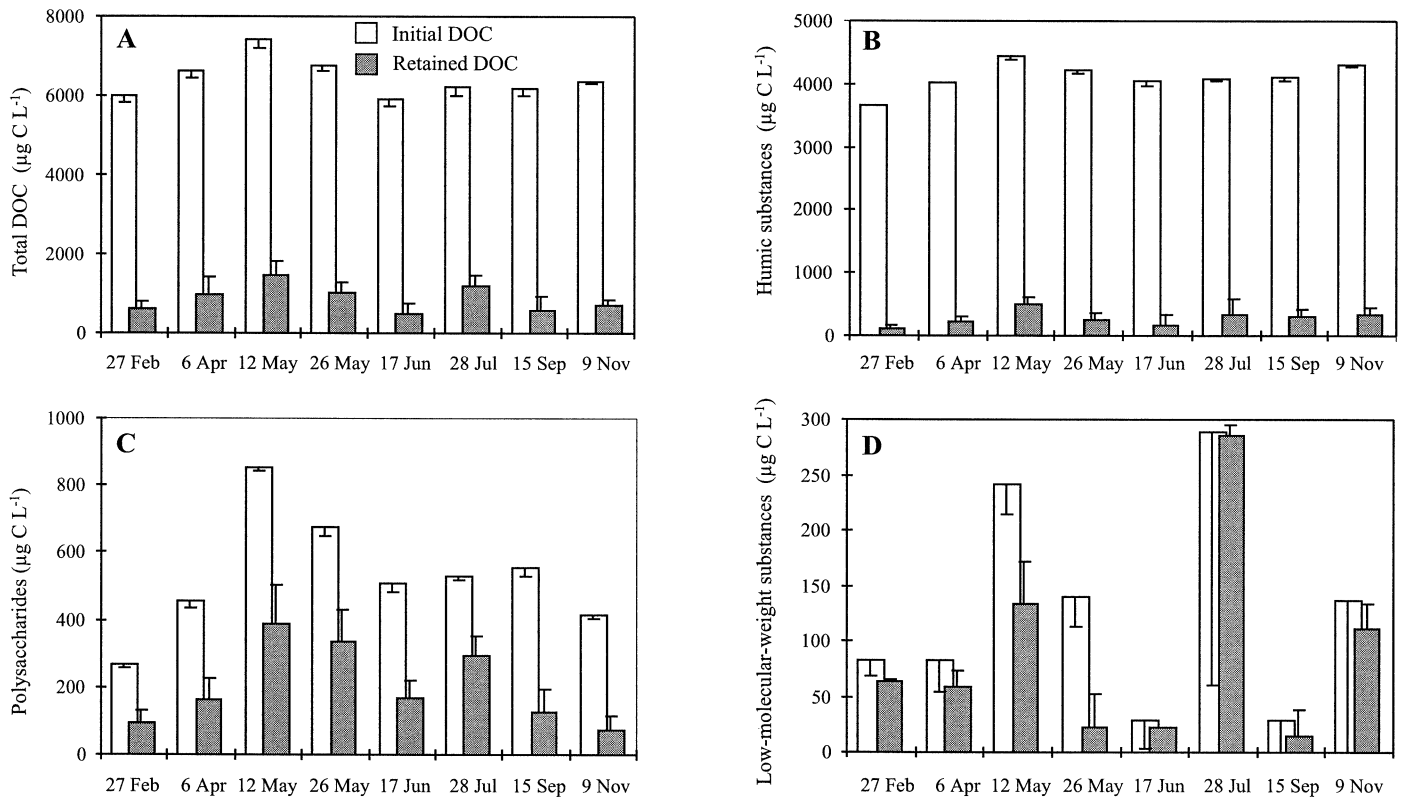


Fig. 6. Initial concentrations of dissolved organic compounds in Spree water and the proportions retained in sediment cores. (A) Total DOC, (B) HS, (C) PS, and (D) LWS. Means \pm SD; $n = 4$ analytical replicates for initial concentrations, $n = 9$ ($n = 6$ in February) experimental replicates for retained organic compounds.

water was highest. PS were retained very effectively by up to 56% on 28 July (mean, 39%). HS were retained less effectively than total DOC (3.2%–11.4% of the input DOC; mean, 7%). The LWS were retained in the sediment cores by up to 98% (mean, 66%) (Fig. 6).

BDOC_{Pel} and BDOC_{Sed} amounted to 10.7%–21.6% and 17.1%–27.7% of the total DOC, respectively (Fig. 7). Both peaked during the vernal algal bloom in May and were low-

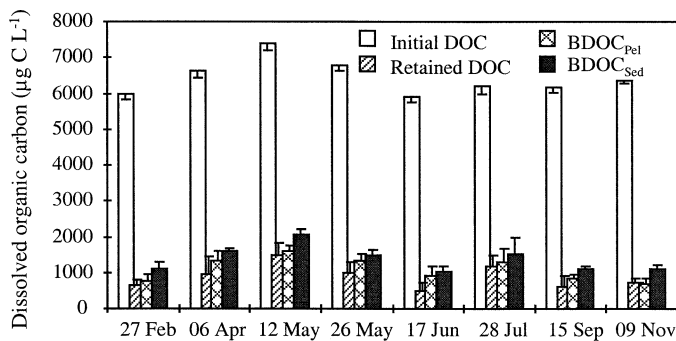


Fig. 7. Initial DOC in Spree water, DOC retained in sediment cores, biodegradable organic carbon after 30 d of batch incubation (BDOC_{Pel}), and biodegradable organic carbon after core perfusion and 30 d of batch incubation (BDOC_{Sed}). Means \pm SD; $n = 4$ analytical replicates for initial concentrations, $n = 4$ experimental replicates for BDOC_{Pel} , and $n = 9$ ($n = 6$ in February) experimental replicates for retained DOC and for BDOC_{Sed} .

est in November. The concentration of BDOC_{Pel} comprised 80% of BDOC_{Sed} and was significantly lower (paired t -test, $p < 0.001$, $n = 8$). However, the composition of the retained DOC was very similar to that of BDOC_{Pel} . Retained DOC and BDOC_{Pel} were mainly made up of PS and LWS. HS were also retained to a certain extent within the sediment cores (Fig. 4B) but remained nearly the same in batch incubations.

Bacterial production within whole sediment cores was most strongly correlated with the incubation temperature ($r_s = 0.9$, $p < 0.001$, $n = 69$). Of interest, total DOC in the river water did not correlate significantly with bacterial variables, whereas BDOC_{Pel} and BDOC_{Sed} both correlated significantly with bacterial production and turnover times (Table 1). We also found significant correlations between bacterial production and DOC retention ($r_s = 0.59$, $p < 0.001$) and with the retention of several fractions of DOC, in particular with the PS fraction ($r_s = 0.84$, $p < 0.001$) in those cores. Correlation coefficients of DOC variables with production or turnover times were higher than with abundance (Table 1), which might reflect the causal relationship between bacterial activity and DOC retention.

Temperature effects—Because there were no significant differences in abundance, production, and turnover times between the sampling sites, we aggregated results from all sites in order to calculate seasonal means for the bacterial vari-

Table 1. Spearman rank-correlation coefficients between bacterial variables in sediment cores and temperature, DOC, and DOC retention in sediment cores. Levels of significance are * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Environmental variable, DOC retention	Bacterial production ($n = 69$)	Bacterial abundance ($n = 63$)	Turnover time of bacterial carbon ($n = 62$)
Temperature ($n = 6$)	0.90***	0.40**	-0.89***
Total DOC ($n = 8$)	0.00	0.17	-0.10
BDOC _{Pel} ($n = 8$)	0.45***	0.08	-0.37**
BDOC _{Sed} ($n = 69$)	0.37**	0.19	-0.34**
<i>Retention of</i>			
DOC ($n = 69$)	0.59***	0.52***	-0.41**
PS ($n = 69$)	0.84***	0.60***	-0.66***
HS ($n = 69$)	0.34**	0.32*	-0.16
LWS ($n = 69$)	0.17	0.14	-0.24

ables. Bacterial variables calculated per sampling date (means for nine cores from April to November and six cores in February) were significantly correlated with temperature: (abundance, $r = 0.80$, $P < 0.05$, $n = 7$; production, $r = 0.91$, $p < 0.01$, $n = 8$; turnover time, $r = -0.96$, $p < 0.01$, $n = 7$). The ecosystem effective Q_{10} value, which was calculated from production per standard volume of sediment in a temperature range 10–20°C, was 2.2 (Fig. 8). The cell-specific Q_{10} value, calculated as production divided by abundance, was 1.8.

Discussion

DOC retention in sediments is the result of abiotic and biotic processes. Extracellular polymers in biofilms provide the primary sorption sites for DOC, which then diffuses into the biofilm. Here, it is stored and subsequently utilized by bacteria (Freeman and Lock 1995; Fiebig 1997). Thus, abiotic and biotic processes are closely connected within the biofilm, where extracellular polymers and bacteria act as a functional entity (Fischer 2002). The correlation between bacterial production and DOC retention in our study supports the hypothesis of a microbially mediated DOC retention. It has long been known that bacterial growth at low substrate concentrations is enhanced by the proximity of solid surfaces (Heukelekian and Heller 1940). ZoBell (1943) argued that solid surfaces may retard the diffusion of extracellular enzymes and hydrolyzates away from attached bacterial cells and thus promote bacterial activity, a hypothesis that was recently corroborated by modeling (Vetter et al. 1998). However, in studies elsewhere it was difficult to find relationships between the activity of sediment bacteria and bulk DOC concentrations (Vervier et al. 1993; Sobczak et al. 1998). Stronger relationships could be shown when particle surfaces were included into models of DOC concentrations and bacterial activity (Brunke and Fischer 1999).

In our study, we were able to directly compare DOC composition with bacterial activity. The composition of DOC in the Spree is typical of many freshwater ecosystems (Thurman 1985; Pusch et al. 1998). However, the chemical com-

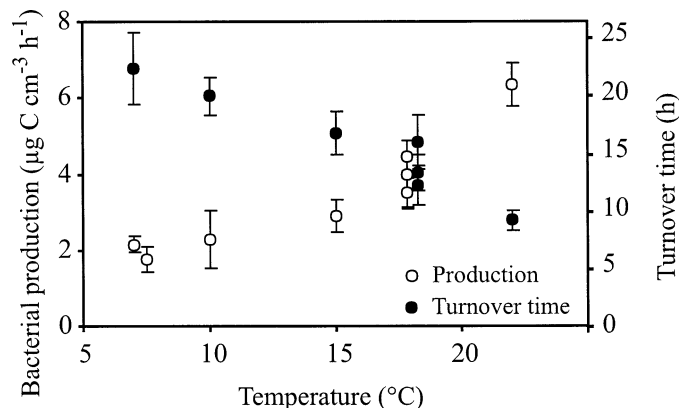


Fig. 8. Effects of temperature on bacterial production and on turnover times of bacterial carbon calculated for the upper 7.6 cm of sediments of the Spree River. Means \pm SD, $n = 9$ ($n = 6$ at 7.5 °C).

position of DOC does not necessarily reflect the availability of DOC fractions for the microbial community because of varying microbial turnover times of these DOC fractions. Their selective retention and the correlations between bacterial activity and retained DOC give a better insight into the significance of DOC for sediment bacteria.

High-molecular-weight PS, possibly originating from algal exudates, were retained and probably decomposed effectively within the sediment cores. Evidence exists from planktonic systems that bacteria utilize high-molecular-weight substances (Tulonen et al. 1992; Amon and Benner 1996), particularly PS (Weiss and Simon 1999), to meet their substrate requirements. In a study that used plug-flow biofilm reactors with stream water, PS were among the substrates that were preferentially utilized by biofilm bacteria (Volk et al. 1997).

The fractions containing mono- and disaccharides and amino acids were only found in low concentrations in the Spree water column, and these fractions were retained very effectively in the sediment cores. In contrast, when leucine was perfused through the sediment cores in 50- μ M concentrations for bacterial production measurements, >80% of this load was recovered at the outflow from the cores. However, the 20% retained from the 50- μ M concentration still are a large increase in retention as compared with the low concentrations of naturally occurring LWS, which are retained by up to 100%. The sediments were thus able to retain larger amounts of amino acids when a pulse with high concentrations became available, as described by Fiebig (1992). Initially, these amino acids are mainly retained abiotically in sediments, and they are then subsequently microbially utilized (Fiebig and Marxsen 1992; Fiebig 1997). Rapid bacterial utilization of amino acids, with gross turnover times of several days for the bulk amino acid pool, has also been reported in pelagic freshwater environments (e.g., Münster 1993; Weiss and Simon 1999).

HS are generally considered to be refractory (e.g., Thurman 1985). They can even have inhibitory effects on metabolic processes in biofilms (Meyer et al. 1987; Freeman et al. 1990), which are possibly caused by the complexation and inactivation of bacterial enzymes (Boavida and Wetzel

1998). In other studies, HS were at least partly bioavailable to the bacterial community (e.g., Tranvik 1990; Volk et al. 1997), but they supported fourfold less bacterial production per unit of carbon than nonhumic substances from the same environment (Moran and Hodson 1990). In the present study, approximately equal amounts (but a much lower percentage) of HS and PS were retained in the sediment cores (Fig. 6B,C), which could potentially be utilized by bacteria. Probably, the varying degree of utilization of HS by bacteria is related not only to the enzymatic properties of bacteria but also to differences in the chemical structures of their substrates. Carbohydrates and amino acids can be bound chemically to HS in significant amounts and thereby enhance the apparent availability of HS. These labile components made up 30% of the utilized "humic substances" in a biofilm reactor study (Volk et al. 1997). Because of their coupling to HS, these labile substances would have been included in the analysis of the HS pool in our study and thus increase the apparent retention of this DOC fraction.

The average amount of biodegradable organic carbon (BDOC_{Pel} and BDOC_{Sed}) in the Spree water column was lower than in Belgian rivers studied by Servais et al. (1989). In particular, BDOC as a proportion of total DOC was lower than in most other studies on BDOC in rivers (Servais et al. 1989), a calcareous stream (Volk et al. 1997), riverine hyporheic interstices (e.g., Claret et al. 1998), and a riverine wetland (Mann and Wetzel 1995). This is probably due to efficient utilization of DOC in the Spree, so that the river water was already depleted in labile DOC at the time of sampling because of intense microbial metabolism *in situ*. Our results from May and June 1998 support this assumption: during spring, algae from the water column may be an important source of labile DOC, especially PS. Correspondingly, BDOC was highest on the sampling date 12 May. In the middle of May, the chlorophyll *a* content, as an indicator of algal biomass, decreased dramatically (Fig. 1). Concomitantly, the amount (Fig. 6C,D) and proportion (Fig. 3) of labile substances decreased strongly in the river water. It is therefore concluded that this change in riverine DOC composition was caused mainly by bacteria, analogous to the bacterial decomposition of DOC in the laboratory incubations.

Despite the good correlation between DOC retention and bacterial production, DOC retained in the sediment cores accounted for only 11% (June) to 33% (February) of the carbon required for bacterial production. This paradox can be explained as follows. Particulate organic matter (POM) was the primary carbon source for sediment bacteria, as was also suggested for interstitial bacteria in the riparian zone of the alpine Enns River (Brugger et al. 2001). The Spree is rich in seston and nutrients, which could fuel a level of high bacterial production when transported into the sediments via infiltrating river water. Including the hyporheic biofilm, POM built up a standing stock within the sediments of ~ 8.2 mg C cm⁻³ sediment volume. If this POM was the sole carbon source for bacteria and if the growth efficiency of bacteria was 30% (Meyer et al. 1987), this carbon would be turned over by bacteria in only 8.6 d in July (at 1-cm sediment depth), but in 147 d in February (at 7-cm sediment depth). Thus, the sedimentary bacterial community was

highly dependent on the continuous input of fresh organic matter, both as POC and DOC. In part, DOC may be leached from POM because of cell lysis and bacterial extracellular enzymes, and bacteria may release DOC during anabolic processes. Thus, certain DOC fractions may be cycled rapidly within the sediments, and their contribution to bacterial metabolism could be higher than calculated via the apparent DOC retention (Fiebig 1992).

Bacterial abundance and production in the Spree sediments were high compared with other running waters, probably because of the high surface area of the shifting sand substratum and a good supply with oxygen and nutrients in the uppermost sediment layer (Fischer et al. 2002). The sediments here act as a fixed-film reactor, providing a "hot spot" of bacterial activity within the river ecosystem. Production in the upper sediment layer was significantly higher than in the lower layers, probably because of the enhanced availability of labile organic matter. Furthermore, the biochemical composition of POM also strongly influences bacterial activity in river sediments (Brunke and Fischer 1999; Fischer et al. 2002). Thus, besides temperature, the supply of high-quality POM and DOC seem to determine bacterial production in river sediments.

Our results show that bacterial activity can be controlled by naturally occurring changes in DOC quality. Although DOC of various size classes was immobilized in sediments, high-molecular-weight PS and, if present, LWS were retained most efficiently. Both PS and LWS were also quickly degraded in batch incubations. This contrasts with the "size-reactivity continuum model" (Amon and Benner 1996), according to which most high-molecular-weight DOC would be more reactive than most low-molecular-weight DOC. In our study, high-molecular-weight DOC was utilized to a greater extent than the LWS on account of the higher concentration of the high-molecular-weight DOC. However, because LWS were present in much lower concentrations, their utilization by bacteria may have resulted in a more rapid turnover. The obvious contradiction of our results with the "size-reactivity continuum model" can thus be explained with the presence of diagenetically young, highly reactive LWS in the River Spree, whereas LWS in oceans are supposed to be the oxidized products of humification processes and are diagenetically extremely old (Amon and Benner 1996). As can be seen by the spectral adsorption coefficient (Fig. 4A), the HS from the River Spree prevailed in the fraction with retention times <40 min, which represents molecular weights >1 kDa. This medium-molecular-weight organic matter clearly was least retained by hyporheic sediments and was most refractory in batch incubations, whereas DOC low and high in molecular weight was retained in the river sediments during a short residence time of 0.5 h. After subsequent 30 d of batch incubation, the quality of the DOC was further changed from a relatively wide molecular-weight spectrum to a narrower spectrum mainly composed of HS. This means that certain compounds of DOC may be readily utilized for bacterial growth irrespective of the molecular weight. We hypothesize that the availability of DOC for bacteria is rather controlled by the presence, abundance, and steric accessibility of specific chemical functional groups than by molecular size.

These findings also have implications for our knowledge of the microbial metabolism of organic matter on the ecosystem level. Most water enters rivers via exfiltrating groundwater, which passes the river sediments. Also, flowing river water temporarily infiltrates into sediments at ripple and dune structures. When the river water passes the sediments, the compounds mentioned above will be stripped off by a combination of physical retention and microbial degradation. About two thirds of the Spree sediments are open-framed, permeable sediments in which bacteria can exhibit high metabolic rates (Fischer and Pusch 2001). This permeable share of the river bottom thus makes up 16,650 m² within 1 river km, with half of it to be assumed to be infiltration and exfiltration areas, respectively. On the basis of the infiltration rate used here, this results in an infiltration rate of 140 L s⁻¹. Hence, the average river discharge of 11.5 m³ s⁻¹ would be infiltrated each 82 km, which means roughly every 2 d at an average flow velocity of 0.4 m s⁻¹. Because we found 900 µg C L⁻¹ to be retained in the sediments, this would result in the retention of 1.3 g DOC m⁻² d⁻¹ in infiltration zones or 10.8 kg DOC km⁻¹ d⁻¹. These values are in a realistic scale compared with DOC sources from primary production and groundwater exfiltration (Fischer and Pusch 2001).

Thus, the riverbed sediments serve as a highly effective sink for organic matter, and the activity of the sedimentary biofilm largely influences the organic carbon biogeochemistry in the river water. The importance of that "liver" function of the river sediments will be greatest in rivers with a diverse channel morphology, including dynamic sediment structures that favor the exchange of river water with the bottom sediments.

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