

# Microbial Extracellular Polymeric Substances (EPS) in Fresh Water Sediments

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**Abstract** Microbially produced extracellular polymeric substances (EPS) have been linked with many important ecological functions in natural sediments; yet, most information has been derived from marine systems. The present paper is the first comprehensive study on EPS (i.e., carbohydrates and proteins) dynamics in riverine sediments addressing spatial (six reservoirs and four groyne fields across three European rivers), temporal (all seasons in 2003–2005), and vertical (over a 50-cm sediment depth transect) pattern. The variation in hydrodynamic regime found in the reservoirs and groyne fields was reflected in the biomass and composition of the benthic microorganisms that produce EPS. The microphytobenthic communities consisted mainly of diatoms and a higher algal biomass (up to 248  $\mu\text{g g}^{-1}$  dry weight, DW) seemed to be indicative for higher amounts of secreted colloidal carbohydrates. Consequently, the model proposed by Underwood and Smith (1998) for the relation chlorophyll–colloidal carbohydrates was also applicable for upper riverine sediment layers. The close relation between algal biomass and bacterial cell counts ( $10^8$ – $10^9$  cells  $\text{g}^{-1}$  DW) supports the idea of bacterial use of the secreted EPS. However, the data also suggest a contribution to the EPS pool through bacterial secretion of

proteins/extracellular enzymes and possibly carbohydrates. Over depth, the relationships between microorganisms and EPS became increasingly decoupled along with increasing ratios of bound (refractory) to colloidal (labile) EPS. These data suggest fresh production of polymeric substances in upper sediment layers and mainly accumulation of refractory, biodegraded material in deeper layers. The high contents of EPS colloidal and bound carbohydrates (0.1–1.8 and 1.3–6.7  $\text{mg g}^{-1}$  DW, respectively) and EPS proteins (0.4–12.9  $\text{mg g}^{-1}$  DW) at the freshwater study sites might indicate an important role in sediment ecology.

## Introduction

Extracellular polymeric substances (EPS) are high-molecular-weight carbon-rich molecules released by bacteria, microalgae, and macrozoobenthos [38, 43, 63]. These organic compounds represent a high proportion of the total metabolic production of microbes: on average 10–20% of net primary production in phytoplankton [1] and at least 30–40% in benthic algal communities [63]. The composition of the EPS matrix varies widely depending on its provenance and local environment. EPS can be broken down into different classes of macromolecules that contribute to the total EPS content including polysaccharides (often 40–95%), proteins (up to 60%), and minor traces of nucleic acids, lipids, and other biopolymers [20]. Polymeric substances perform important functions for the secreting organisms including cell attachment or locomotion, protection from desiccation, resistance to toxins, and enhancement of their ability to sequester nutrients [16]. The ecosystem function of EPS in aggregation, increasing sediment stability, altering contaminant adsorption/modification, or providing nutritional food sources for inverte-

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brates has been widely acknowledged in marine coastal systems in recent years [3, 16, 44, 45]. Studies on EPS in freshwater have focused on aspects of applied biotechnology such as biofouling, biocorrosion (binding metal ions), and bioweathering (dissolving minerals) [21]. The impact of EPS on flocculation and removal of pollutants and nutrients from wastewaters have been described in over 200 publications [40]. Some environmental studies have been performed concerning EPS in freshwater, but the data basis lags the depth of knowledge from the marine habitats. This applies to EPS or transparent exopolymer particles (TEP) in the pelagic zone (reviewed in, e.g. [44]), but even more for benthic habitats where almost nothing is known about EPS concentration, composition, and possible ecosystem function in natural sediments [7, 34], in contrast to the intensively studied marine benthic habitats [58, 63].

In the pelagic zone, a close correlation between phytoplankton growth and TEP has been observed [44]. For intertidal mudflats, the microalgae—mainly diatoms—have been identified as the main producers of EPS carbohydrates, and this has been verified both in the field [48] and in laboratory cultures [14]. Microalgal EPS secretion and composition has been investigated through varying physiological stages, with varied species composition, nutrient regimes, over day/night and immersion/emersion periods [22, 32, 56, 65]. It has become evident that EPS secretion by benthic diatoms is central to their locomotive abilities and thus coupled to the tidal cycles and light availability/photosynthetic activity [11, 15, 57]. Thus, light availability and the functional importance of EPS are related for intertidal systems. While it is intuitive to suggest that these models of microalgal EPS production [62] and the role of EPS are transferable to freshwater habitats, it may not be correct. Where the sediment is permanently immersed and epipelagic diatoms less common, EPS may have a less important functional role. In addition, the microalgae may experience light limitation due to the turbidity of the overlying water column, resulting in less consistent primary production and EPS secretion. To date, there is limited information on the importance of microalgae for EPS production in freshwater sediments [2, 7].

Bacterial EPS excretion is a well-studied phenomenon in medicine, biofilm, and wastewater research [30, 36, 50]. Although benthic assemblages invariably include a significant biomass of heterotrophic bacteria capable of secreting copious amounts of polymeric substances [8], the importance of bacterial EPS in sediments has been largely overlooked in both marine and freshwater habitats. Pioneering studies indicated the stabilization of sand and clay beds by bacterial EPS [9, 10]. Presumably, in aquatic systems with low light availability, e.g. in turbid rivers, bacterial

EPS production may dominate within the sediments, especially in layers below the sediment photic zone [16, 35]. Therefore, research into this field has the potential to change the conceptual view of the role of bacteria within the EPS cycle in sediments, which was historically focused on degradation and modification of microalgal EPS moieties [29, 66]. While microalgae secrete mainly carbohydrates as EPS, bacteria are known to release a higher proportion of proteins [20, 35, 65]. Proteins represent an important nutritional source for benthic invertebrates, but may also enhance the adhesion of polysaccharide fibres or strengthen polysaccharide complexes [6, 47]. Thus, research on EPS production in natural sediments appears to be incomplete without considering the two major EPS components, carbohydrates and proteins.

Most of the studies concentrating on EPS production by microalgae consider only the upper sediment layers ranging from the centimeter to micrometer scale [7, 12, 13, 46, 59, 65]. However, buried microalgae may also contribute to the pool of polymeric substances in aphotic zones via EPS secreted during migration [33, 55]. Moreover, understanding the role of EPS production by the ubiquitous heterotrophic bacterial component in deeper layers may give valuable new insights into EPS distribution and possible functions.

The present paper aims to address the following questions:

- Is the concentration of EPS (carbohydrates and proteins) in riverine sediments comparable to marine habitats?
- Are microalgae of similar importance for EPS production in riverine sediments compared to the intertidal mudflats, and is the relation between colloidal carbohydrates and microalgae for the marine habitat [62] applicable to freshwater sites?
- Can bacteria contribute to the overall EPS signal beyond their degradation activity in freshwater sediments?

These questions have been addressed by a comprehensive sampling strategy covering vertical (surface versus deeper sediment layers down to 50 cm), temporal (all seasons from 2003 to 2005), and spatial (ten study sites: six reservoirs and four groyne fields across three European rivers) aspects of the varying importance of microbial produced EPS (carbohydrates and proteins) in freshwater sediments. Simultaneously, microalgal species composition/biomass, bacterial cell numbers, water content, particle size, total organic content (TOC), cation exchange capacity (CEC), and bulk density were determined in order to highlight the interactions between sedimentology and biology, and thus, EPS secretion.

## Materials and Methods

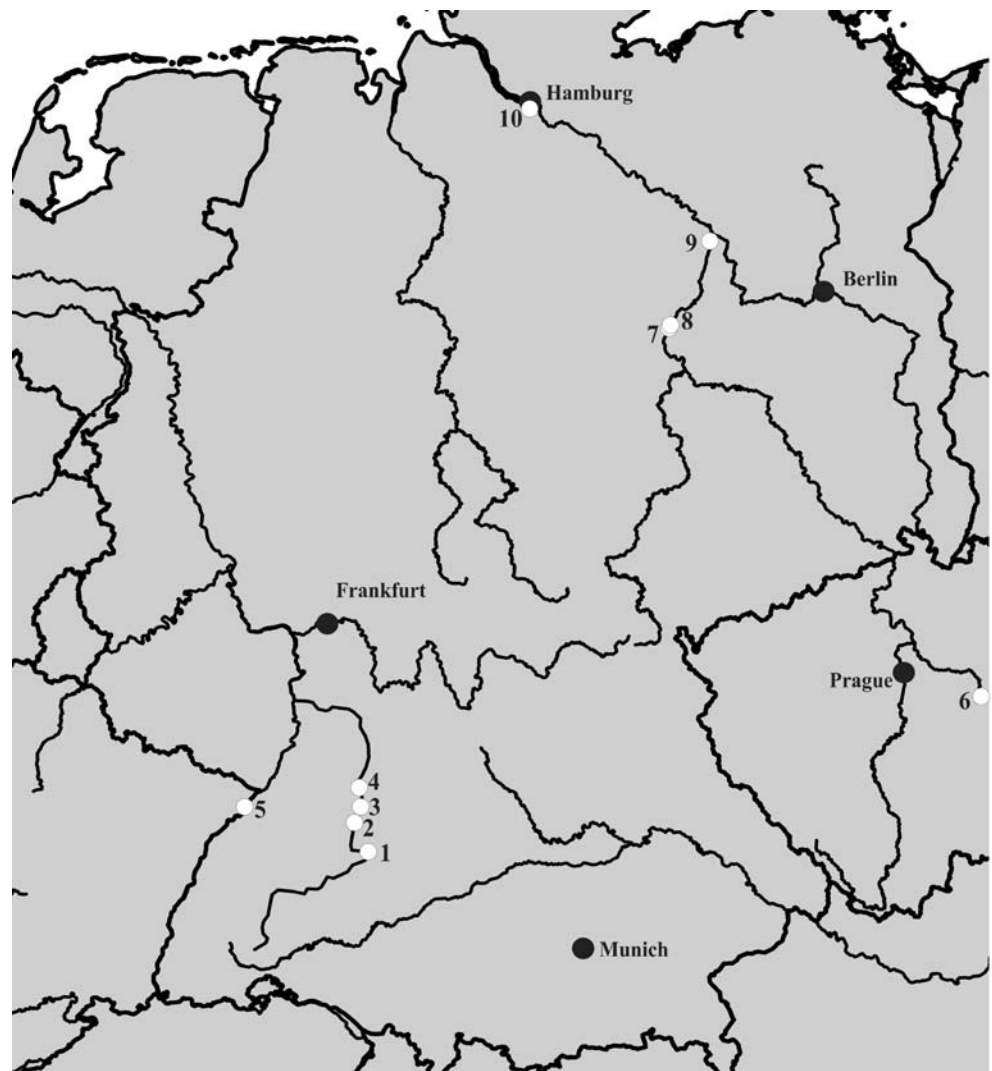
### Site Description

The lock-regulated River Neckar is a major tributary of the River Rhine and is located in the southwest of Germany. It drains a mixture of industrial and agricultural catchments with a total surface area of 14,000 km<sup>2</sup> (Fig. 1). Sampling took place upstream of weir sections of the Lauffen (river km 137), Poppenweiler (km 165), Hofen (km 176), and Deizisau (km 200) reservoirs. The River Rhine is the largest river in Western Europe with a catchment area of about 165,000 km<sup>2</sup> (Fig. 1). Sediment samples were collected upstream of the Iffezheim barrage (river km 334). The River Elbe is 1,100 km long (372 km in Czech Republic, 728 km in Germany) and has a catchment area of 148,268 km<sup>2</sup> (Fig. 1). Sampling took place upstream of the weir at Prelouc (Czech Republic, lock-regulated part, km 223) and from the center of

groyne fields close to Magdeburg (km 318), Fahlberg-List (km 319), Havelberg (km 419), and Hamburg (km 607; Germany).

The hydrodynamic regime within the sampled reservoirs (upstream of the river weirs or barrages built to regulate the water level for shipping and to generate electricity or as a flood control) and the groyne fields (areas between barriers constructed perpendicular to the river banks to maintain a desired navigation channel) is very different. In the reservoirs, the hydrodynamic forces are mostly moderate except for episodic events such as severely enhanced discharges or floods [31]. In the groyne fields, the current recirculation pattern and resulting eddies are more variable on a day-to-day basis depending on water levels, geometry, or length-to-width ratio within the field [49, 54]. During a hydrologic year, reservoirs and groyne fields represent depositional sites with a positive net balance. This was reflected in the grain size distribution with a clear dominance of silt and clay. The cohesive sediments were

**Figure 1** Main waterways in Germany and Czech Republic. The *circles* indicate the study sites at the lock-regulated River Neckar (reservoirs: 1 Deizisau, 2 Hofen, 3 Poppenweiler, 4 Lauffen), the River Rhine (5 Iffezheim barrage), and River Elbe (6 Přelouc reservoir; groyne fields: 7 Magdeburg, 8 Fahlberg-List, 9 Havelberg, 10 Hamburg)



all taken from areas that were close to the banks and in less than 2-m water depth.

### Sampling

Sediment cores were collected over all seasons over a 3-year period (2003–2005) at ten sampling sites (six reservoirs/weirs and four groyne fields) across three European rivers (Table 1). At least six sediment cores were collected from each site using Perspex core tubes [150 cm long by 13.5-cm internal diameter (ID) or 100 and 11 cm ID, respectively]. At first, two of the intact sediment cores, with overlying water, were analyzed for bulk density [18]. Then, the overlying water of the remaining four sediment cores was carefully removed by a suction tube. Subsequently, in each core, the flocculent sediment surface layer was obtained by suctioning the upper 0.5–1 cm layer, while the deeper layers were sectioned at intervals of 0.5–5 cm. The corresponding sediment layers of the four cores were pooled and mixed thoroughly to reduce the effects of small-scale heterogeneity in samples. In each of these pooled sediment layers, physicochemical and biological sediment properties were repeatedly (three to ten) determined, except for a single determination of grain size.

In total, 324 of these pooled sediment layers have been examined, of which 256 were sampled in the reservoirs (upper layers 0–5 cm, 134; lower layers 5–50 cm, 122) and

68 in the groyne fields (upper layers 0–5 cm, 38; lower layers 5–50 cm, 30).

### Sediment Properties

The determination of sediment properties has been described in detail [26–28]. Briefly, bulk density (by gamma ray densitometer [18]), water content (DIN 18121-1), particle size classes (by hydrometers after removing organic material and carbonates [53]), TOC (TOC Analyzer Shimadzu TOC-5000/5050), and CEC [61] have been determined.

The species composition of the microalgal community [37, 39] was assessed (a) directly in fixed samples (Lugol's solution) by light microscopy and (b) after mounting the cleaned samples in Naphrax (refractive index  $n_D=1.710$ ). Chlorophyll *a* and pheophytin were extracted from 0.5 cm<sup>3</sup> wet sediment in 96% ethanol and determined spectrophotometrically before and after acidification with HCl [5]. Chlorophyll *a* and pheophytin data were given as concentration (per volume,  $\mu\text{g cm}^{-3}$ ) and as content (per sediment dry weight, DW,  $\mu\text{g g}^{-1}$  DW).

Bacterial cell numbers were determined within 0.5 cm<sup>3</sup> subsamples of wet sediment that were fixed in 4% formaldehyde (1.5 h) followed by three washing steps in 1.5 ml phosphate buffered saline. After resuspension, 3×5  $\mu\text{l}$  of the supernatant was transferred to 12 well

**Table 1** Overview of spatial, temporal, and vertical aspects of the sampling strategy: the study sites, type of study site, their location, the period of sampling, the sediment layers sampled, and the sample numbers

| Study site   | River  | Site         | GPS—coordinates<br>deg, min, s   | Sampling dates<br>(year: months) | Sampling<br>depth (cm) | Samples numbers<br>(per campaign/total) |
|--------------|--------|--------------|----------------------------------|----------------------------------|------------------------|---|
| Lauffen      | Neckar | Reservoir    | 9°09'52.32" E<br>49°04'10.99" N  | 2005: 1–12                       | 57                     | 11–13/127                               |
| Poppenweiler | Neckar | Reservoir    | 9°15'10.18" E<br>48°54'42.14" N  | 2004: 4, 6, 9                    | 30                     | 11–14/38                                |
| Hofen        | Neckar | Reservoir    | 9°12'50.90" E<br>48°50'12.60" N  | 2004: 4, 6, 11                   | 28                     | 8–10/26                                 |
| Deizisau     | Neckar | Reservoir    | 9°23'19.19" E<br>48°43'01.69" N  | 2004: 4, 6, 11                   | 30                     | 9–11/29                                 |
| Iffezheim    | Rhine  | Reservoir    | 8°06'9.8" E<br>48°49'47.7" N     | 2004: 3                          | 35                     | 6–8/20                                  |
| Preloc       | Elbe   | Reservoir    | 15°34'28.4" E<br>50°02'36.4" N   | 2005: 4                          | 35                     | 16/16                                   |
| Magdeburg    | Elbe   | Groyne field | 11°43'15.04" E<br>52°01'54.66" N | 2003: 8                          | 38                     | 11/11                                   |
| Fahlberg     | Elbe   | Groyne field | 11°40'28.3" E<br>52°4'44.4" N    | 2005: 6, 8, 11                   | 29                     | 12–14/38                                |
| Havelberg    | Elbe   | Groyne field | 12°01'47.4" E<br>52°49'36.9" N   | 2003: 8                          | 51                     | 12/12                                   |
| Hamburg      | Elbe   | Groyne field | 10°05'03.8" E<br>53°26'53.9" N   | 2003: 8                          | 10                     | 7/7                                     |
| Total        | 3      | 10           | –                                | Jan 03–Nov 05                    | 0–57                   | 324                                     |



microscope slides where the samples were dehydrated (increasing ethanol series) and stained with DAPI (4',6-diamidino-2-phenylindole; final concentration of  $1 \mu\text{g ml}^{-1}$ ). For each of the subsamples, bacteria were enumerated in at least ten randomly chosen grids ( $7,752 \mu\text{m}^2$ ) counting a minimum of 1,000 cells per sample using epifluorescence microscopy and Meta Vue Software. Bacterial cell numbers were given per volume ( $0.5 \text{ cm}^{-3}$ ) and per sediment DW ( $\mu\text{g g}^{-1}$  DW).

EPS were extracted from 0.3 g dried and homogenized sediment subsamples in 400  $\mu\text{l}$  of distilled water (for 1 h at  $30^\circ\text{C}$ ) followed by a secondary extraction in 3 ml distilled water with 100 g/g volatile solids cation exchange resin (CER, Dowex, APA-1 16-45 mesh, Fluka 44445) for 18 h at room temperature [11, 24]. After the first water extraction, the samples were centrifuged for 15 min at  $13,414 \text{ g}^{-1}$  at  $4^\circ\text{C}$  (Sigma 202MK Centrifuge, Sigma GmbH, Osterode, Germany) and the supernatants were removed to be analyzed spectrophotometrically for colloidal (water-extractable) carbohydrates [19]. Subsequently, new water and CER was added to the pellet and the sample was resuspended again and kept well mixed by rotation during the second incubation (see [26, 27] for details). After centrifugation, the supernatants were analyzed spectrophotometrically for bound (CER-extractable) carbohydrates [19] and proteins [51], the latter corrected for humic acids [23]. Both extraction procedures were optimized in terms of sediment weight, extractant volume, and extraction time with regards to the yield and ratio of the different EPS fractions in avoidance of intracellular contamination (data not shown). Underwood et al. [64] recommended precipitation of the extracted fraction in order to distinguish between low and high molecular fraction, of which only the latter is defined as EPS. However, De Brouwer and Stal [11] have demonstrated that precipitation yields only polymers greater than 100 kD (about 25%), while most of the material extracted is polymeric ( $>1$  kD). We define EPS measured here as the oligomeric to mainly polymeric fraction gained without further precipitation step.

## Statistics

Prior to statistical analysis, the assumptions of normality and homogeneity of variance were tested via visual assessment of the frequency histogram and normal plot and with the Shapiro–Wilk  $W$  test (“Analyse-it” for Microsoft Excel). The significance level in rejecting equal distribution of data in different sets was given for  $p < 0.05$  and  $p < 0.001$ . Differences of microalgal biomass/bacterial cell number and EPS components (colloidal/bound carbohydrates and proteins) between study sites, seasons, and depths were assessed by one-way analysis of variance (ANOVA). All measured variables were analyzed by

principal component analysis (PCA; SPSS 14.0 for Windows and STATBOX software, Grimmer Logiciels, 1997, Paris, France) in order to reduce the large data set by constructing PC principle components (vectors) with eigenvalues  $> 1$ . The first PC accounts for the major part of variance within the data set, and each subsequent component progressively explains smaller portions of the variance in the total sample. The loading of the single variables within one of the PC indicates their association and is equivalent to Pearson’s correlation coefficient as visualized in their position in the circle of correlation. The individual plots are shown in a MDS multidimensional scaling matrix to indicate potential clusters of data and explain their separation by the axes (PC).

## Results

### Microalgal Community

Assessment of the microalgal community revealed the dominance of benthic diatoms (Bacillariophyceae, Pennales) at all sites (a total of 80 spp. were observed) including the genera *Achnanthes*, *Amphora*, *Cocconeis*, *Cymatopleura*, *Cymbella*, *Diatoma*, *Fragilaria*, *Gomphonema*, *Gyrosigma*, *Navicula*, *Nitzschia*, *Pinnularia*, *Rhoicosphenia* (*abbreviata*), and *Surirella*. The majority of species were from the genus *Navicula* (24) and *Nitzschia* (21). Deposited phytoplankton species were commonly observed at all sites but were more abundant in the sediments of the groyne fields sites (located solely in the River Elbe) relative to the reservoirs. The occurrence of pelagic diatoms of the genus *Aulacoseira*, *Cyclostephanos* (*dubios*), *Cyclotella*, *Melosira* (*varians*), *Stephanodiscus* (Bacillariophyceae, Centrales), as well as green algae of the genus *Chlorella*, *Oocystis*, *Pediastrum*, *Scenedesmus*, and *Tetraedron* (Chlorophyta, Chlorococcales/Chlorellales) in the sediments of the groyne fields reflected the main components of phytoplankton in the River Elbe. Benthic cyanobacteria (e.g. Oscillatoriales) were rarely found in any of the sites (*Oscillatoria* spec., Deizisau reservoir/River Neckar; *Merismopedia* spec., *Lyngbya* spec., Hamburg groyne field/River Elbe).

### Microalgal Biomass and Bacterial Cell Numbers

The sediment chlorophyll *a* concentrations/contents were higher than expected for turbid environments (Table 2). Chlorophyll maximum values of  $11\text{--}47 \mu\text{g cm}^{-3}/35\text{--}198 \mu\text{g g}^{-1}$  DW were observed within the surface sediments of the reservoirs (example shown in Fig. 2a) and were significantly (one-way ANOVA,  $p \leq 0.001$ ) lower than the maximum levels of the groyne fields of  $46\text{--}83 \mu\text{g cm}^{-3}/136\text{--}248 \mu\text{g g}^{-1}$  DW (example shown in Fig. 3a). Within

**Table 2** Range of chlorophyll *a* (proxy for microalgal biomass) and pheophytin (proxy for microalgal breakdown products) concentrations ( $\mu\text{g cm}^{-3}$ ) as well as contents ( $\mu\text{g g}^{-1}$  DW) within the sediment surface layer (0–0.5 cm) given for different study sites by integrating replicates and seasons

| Study site   | River  | Chlorophyll <i>a</i>      |                            | Pheophytin                |                            |
|--------------|--------|---------------------------|----------------------------|---------------------------|----------------------------|
|              |        | ( $\mu\text{g cm}^{-3}$ ) | [ $\mu\text{g g}^{-1}$ DW] | ( $\mu\text{g cm}^{-3}$ ) | ( $\mu\text{g g}^{-1}$ DW) |
| Deizisau     | Neckar | 15–47                     | 99–198                     | 12–21                     | 68–169                     |
| Hofen        | Neckar | 3–18                      | 10–38                      | 7–21                      | 16–69                      |
| Poppenweiler | Neckar | 10–13                     | 29–35                      | 7–14                      | 23–38                      |
| Lauffen      | Neckar | 6–28                      | 12–70                      | 4–14                      | 13–54                      |
| Iffezheim    | Rhine  | 4–19                      | 13–138                     | 6–18                      | 19–126                     |
| Prelouc      | Elbe   | 11                        | 41                         | 14                        | 54                         |
| Magdeburg    | Elbe   | 36–46                     | 106–136                    | 36–42                     | 106–129                    |
| Fahlberg     | Elbe   | 6–52                      | 15–215                     | 11–31                     | 26–128                     |
| Havelberg    | Elbe   | 75–83                     | 221–248                    | 61                        | 179                        |
| Hamburg      | Elbe   | 60                        | 176                        | 88                        | 259                        |

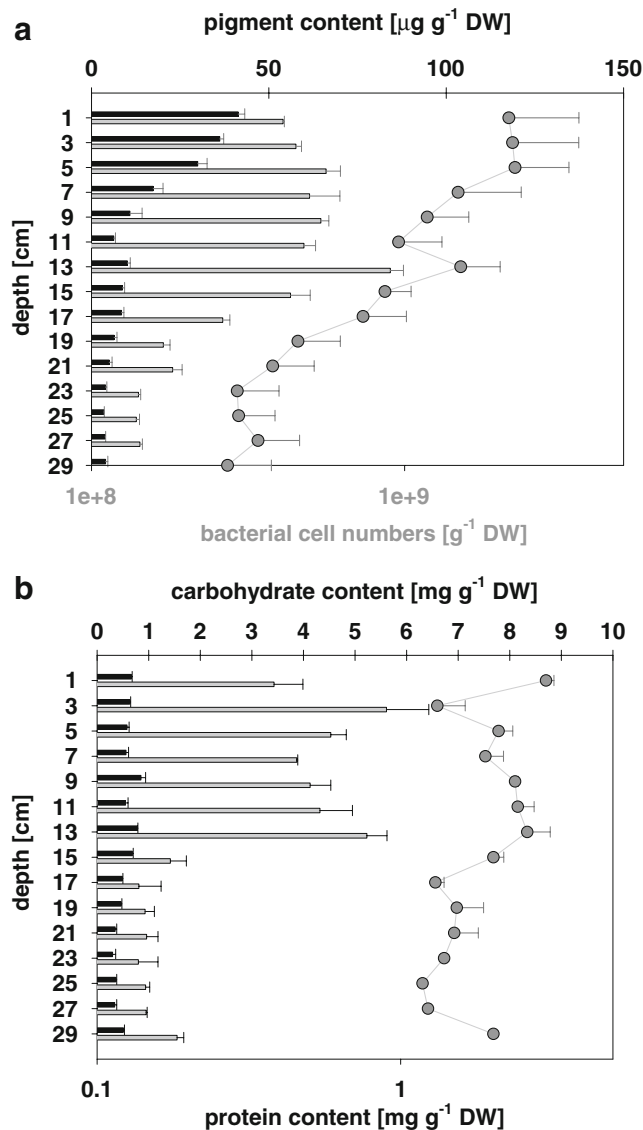
this range, the sediments of Deizisau showed the highest chlorophyll values compared to the other reservoirs, while in Fahlberg, the lowest values of groyne fields were observed. Sediment bacterial abundances within the surface layers were in a similar range in reservoirs and groyne fields ( $2.8 \times 10^8 - 8.4 \times 10^8$  and  $1.8 \times 10^8 - 8.6 \times 10^8$  cells  $\text{cm}^{-3}$ , respectively, as well as  $1.4 \times 10^9 - 8.7 \times 10^9$  and  $8.6 \times 10^8 - 3.2 \times 10^9$  cells  $\text{g}^{-1}$  DW, respectively; Figs. 2a and 3a). However, bacterial cell numbers were significantly lower in the groyne fields sediments as compared to the reservoirs ( $p \leq 0.001$ ). Over the seasons, the microalgal biomass in the upper sediment layers was usually highest in late spring and early summer, along with the highest bacterial cell numbers. Biomass of microphytobenthos (MPB) and bacterial cell numbers showed highest values in the sediment surface layer and decreased with sediment depth (Figs. 2a and 3a).

#### Composition of EPS (Carbohydrates and Proteins)

The colloidal carbohydrates in the sediment surface of the reservoirs were in the range of 0.1–0.5  $\text{mg g}^{-1}$  DW (mean value of all seasons and all depths  $0.32 \pm 0.28$   $\text{mg g}^{-1}$  DW), with significantly higher values of up to 3.4  $\text{mg g}^{-1}$  DW (mean value  $0.54 \pm 0.30$   $\text{mg g}^{-1}$  DW) observed in the sediment surface of the groyne fields (one-way ANOVA,  $p \leq 0.001$ ; Table 3). The bound carbohydrates and proteins were both comparable between the reservoirs and the groyne fields (mean value reservoir,  $2.72 \pm 1.0$  and  $4.31 \pm 3.4$   $\text{mg g}^{-1}$  DW, respectively; groyne field,  $2.66 \pm 1.1$  and  $3.73 \pm 1.8$   $\text{mg g}^{-1}$  DW, respectively; Table 3). The EPS contents in the top sediment layer were highest in late spring and early summer along with the higher biomass of microalgae and bacterial cell numbers, with some variations observed to be site-specific. The highest EPS concentrations were determined in the sediment surface, usually decreasing with sediment depth (Figs. 2b and 3b).

The bound carbohydrate content was significantly higher than the colloidal carbohydrate content (one-way ANOVA,  $p \leq 0.001$ ), the latter represented about 10% of the measured carbohydrate pool in the reservoir sediments but up to 30 % in the groyne field sediments over the seasons (Table 3, Figs. 2b and 3b). The contribution of the colloidal and bound carbohydrate to EPS (=the ratios of bound to colloidal carbohydrate  $\text{EPS}_{\text{Cb}}/\text{EPS}_{\text{Cc}}$  within EPS) varied among the ten study sites (spatial variation), the season (temporal variation), and the sediment depth (vertical variation). In general, considerable secretion of colloidal carbohydrate due to high microbial metabolic activity resulted in lower  $\text{EPS}_{\text{Cb}}/\text{EPS}_{\text{Cc}}$  ratios in the sediment surface layers; for example, in the sediments of Fahlberg in June (2.0) and August (2.7) as compared to November (4.8; 0–2 cm, *t* test,  $p \leq 0.05$ ). Over depth,  $\text{EPS}_{\text{Cb}}/\text{EPS}_{\text{Cc}}$  ratios usually increased due to the increasing contribution of bound carbohydrate to EPS in deeper layers (all seasons and sites; Fig. 4). Despite this observation, a positive exponential inter-correlation between bound and colloidal carbohydrates was observed in the reservoir sediments that was notably stronger in the upper sediment layers (0–5 cm,  $R^2=0.77$ ,  $p \leq 0.001$ ) compared to the deeper layers (5–50 cm,  $R^2=0.47$ ,  $p \leq 0.001$ ). This correlation did not hold in the groyne field sediments (e.g. upper layers  $r=0.36$ ,  $p=0.08$ ).

The bound proteins were significantly higher in concentration than the bound carbohydrates (one-way ANOVA,  $p \leq 0.001$ ), resulting in ratios of EPS proteins to the sum of the carbohydrates measured ( $\text{EPS}_{\text{p}}/\text{EPS}_{\text{c}}$ ) above 1 (Table 3, Figs. 2b and 3b). The  $\text{EPS}_{\text{p}}/\text{EPS}_{\text{c}}$  ratios were similar across the study sites (between 0.8 and 2.0, except for some distinctive higher values, e.g. 3.0 in Poppenweiler reservoir), but highest in spring and early summer along with the highest microalgal biomass, bacterial cell number, and EPS content and varied little with depths.



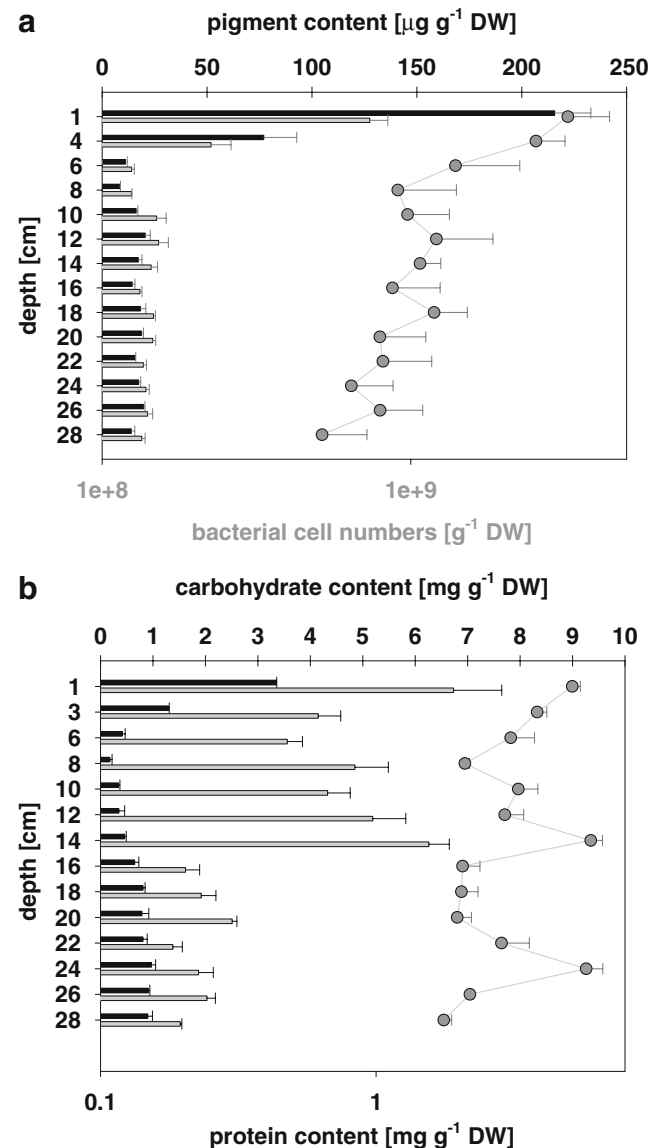
**Figure 2** Vertical sediment profiles (0 to 30 cm depth) of the microorganisms (a) and EPS (b), example shown for the reservoir Prelouc/River Elbe, April 2005. Mean values ( $n=3$ ) with standard deviations are given for a chlorophyll *a* (black bars), pheophytin (gray bars), bacterial cell numbers (gray circles) and b carbohydrates (black bars/colloidal, gray bars/bound), proteins (gray circles/bound)

Interactions Between Microorganisms and Their Habitat

MPB biomass and bacterial cell numbers were significantly correlated in the sediments of all study sites at all seasons (Table 4). The absolute numbers and the ratios of MPB/bacteria, and hence the empirical relation between both variables, were different between study sites and seasons; however, the degree of correlation held and was comparable. When combining all data for the top centimeter of sediment surface, the correlation between MPB and bacteria was more pronounced for the groyne fields than for the reservoirs (Table 5). Over sediment depth, the correlation

between microalgae and bacteria was stronger in the upper layers as compared to the deeper layers (e.g. 0–10 cm Prelouc  $R^2=0.86$ , Fahlberg  $R^2=0.81$ ; 10–35 cm Prelouc  $R^2=0.56$ , Fahlberg  $R^2=0.70$ , all  $p \leq 0.05$ ).

In the reservoir sediments, the vertical profiles of microbial biomass and EPS showed a uniform decrease with depth (Fig. 2). Positive inter-correlations were observed between the biological and the sedimentological variables, particularly in the upper layers (Table 5). In the 5 to 50 cm sediment depth, microbial biomass and EPS were less correlated to physicochemical variables, while the



**Figure 3** Vertical sediment profiles (0 to 30 cm depth) of the microorganisms (a) and EPS (b), example shown for the groyne field Fahlberg/River Elbe, June 2005. Mean values ( $n=3$ ) with standard deviations are given for a chlorophyll *a* (black bars), pheophytin (gray bars), bacterial cell numbers (gray circles) and b carbohydrates (black bars/colloidal, gray bars/bound), proteins (gray circles/bound)

**Table 3** Range of colloidal and bound carbohydrates (=CH) as well as bound proteins (mg g<sup>-1</sup> DW) within the sediment surface layer (0–0.5 cm) given for different study sites by integrating replicates and seasons

| Study site   | River  | colloidal CH (mg g <sup>-1</sup> DW) | Bound CH (mg g <sup>-1</sup> DW) | Bound protein (mg g <sup>-1</sup> DW) |
|--------------|--------|--------------------------------------|----------------------------------|---------------------------------------|
| Deizisau     | Neckar | 0.5–2.4                              | 2.0–6.0                          | 3.2–12.9                              |
| Hofen        | Neckar | 0.2–0.4                              | 2.4–3.1                          | 3.3–9.6                               |
| Poppenweiler | Neckar | 0.2–0.4                              | 1.6–2.7                          | 2.8–10.2                              |
| Lauffen      | Neckar | 0.1–0.5                              | 1.9–3.6                          | 0.9–5.1                               |
| Iffezheim    | Rhine  | 0.3–0.5                              | 2.0–2.4                          | 2.0–3.1                               |
| Prelouc      | Elbe   | 0.7                                  | 3.4                              | 3.0                                   |
| Magdeburg    | Elbe   | 0.6                                  | 2.3–3.5                          | 3.9–7.4                               |
| Fahlberg     | Elbe   | 0.4–3.4                              | 1.3–6.7                          | 2.0–5.2                               |
| Havelberg    | Elbe   | 1.3–1.8                              | 3.1–3.2                          | 4.3–4.6                               |
| Hamburg      | Elbe   | 1.5                                  | 3.8                              | 5.6                                   |

inter-relation among the sedimentological variables became much stronger than for the upper layers of sediment (data not shown). Figure 5 visualizes a general increase in microbial biomass (e.g. chlorophyll as a proxy for microalgal biomass) and microbial produced EPS (e.g. colloidal carbohydrates) with increasing water content, decreasing grain size (parallel to increasing total organic carbon TOC and cation exchange capacity CEC), as well as decreasing bulk density over 0 to 50 cm depth in the reservoir sediments.

In the groyne field sediments, the steep gradients of declining microbial biomass and EPS in the top 0 to 5 cm layers were followed by a uniform distribution of microorganisms beneath (Fig. 3). The microorganisms showed no significant positive correlations to the sedimentological variables in the upper layers (0–5 cm, Table 5) and weak correlations in the deeper layers (5–50 cm) of groyne field sediments. Physicochemical variables showed no relation-

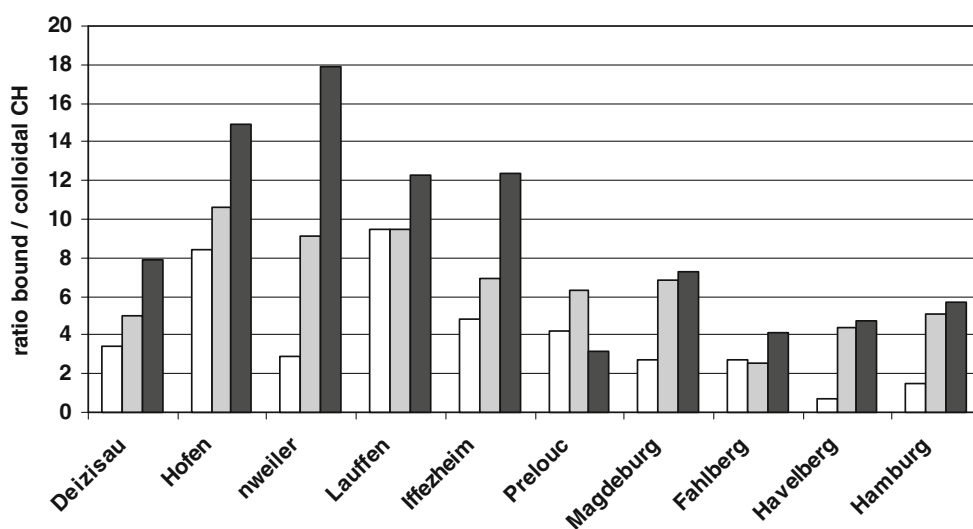
ships in the upper layers, but only in the 5 to 50 cm depth of the groyne field sediments (data not shown).

Common to all study sites was the correlation of microorganisms and EPS to water content (WC, positive) and to bulk density ( $\rho$ , negative) with depth (Fig. 5).

#### Microalgae and Bacteria as Potential EPS Producers

Significant positive inter-correlations were observed between the microalgal biomass, bacterial cell numbers, and EPS proteins/carbohydrates (Table 5). High MPB biomass was related to both high EPS proteins and carbohydrate contents (Table 4). However, the strongest correlation was between MPB and colloidal carbohydrate in the top centimeter sediment surface layer (Table 5) with some variations in temporal, spatial, or vertical terms. In seasonal terms, the relation between MPB and colloidal carbohydrate in the upper sediment layers was closest in the more productive

**Figure 4** Ratios of carbohydrates “CH” bound/colloidal within the sediments at the ten study sites (reservoirs: Deizisau, Hofen, Poppenweiler, Lauffen/River Neckar, Iffezheim/River Rhine, Prelouc/River Elbe and groyne fields: Magdeburg, Fahlberg, Havelberg, Hamburg/River Elbe), integrated over the seasons and given for three sediment depths (white bars, 0–0.5 cm; gray bars, 0–5 cm; dark bars, 5–50 cm)





**Table 4** Results of one-way ANOVA to test for the relations between high and low values/ different location of variables on microorganisms (MPB, bacteria) and EPS (protein, carbohydrates = CH)

|          |              | MPB          | Bacteria           | Bound protein | Colloidal CH | Bound CH     |
|----------|--------------|--------------|--------------------|---------------|--------------|--------------|
| MPB      | High         | 32.361       | $5.73 \times 10^8$ | 5.54          | 0.64         | 3.25         |
|          | Low          | 7.597        | $3.88 \times 10^8$ | 3.87          | 0.33         | 2.55         |
|          |              | $p < 0.0001$ | $p < 0.0001$       | $p = 0.0040$  | $p < 0.0001$ | $p < 0.001$  |
| Bacteria | High         | 11.93        | $5.03 \times 10^8$ | 4.44          | 0.34         | 2.70         |
|          | Low          | 5.64         | $1.56 \times 10^8$ | 2.40          | 0.45         | 2.26         |
|          |              | $p < 0.0001$ | $p < 0.0001$       | $p < 0.0001$  | $P = 0.0137$ | $p = 0.0065$ |
| CH total | High         | 19.895       | $4.53 \times 10^8$ | 5.451         | 0.488        | 3.570        |
|          | Low          | 10.983       | $3.62 \times 10^8$ | 3.359         | 0.276        | 2.131        |
|          |              | $p < 0.0001$ | $p = 0.0011$       | $p < 0.0001$  | $p < 0.0001$ | $p < 0.0001$ |
| Location | reservoir    | 8.85         | $4.59 \times 10^8$ | 4.15          | 0.32         | 2.62         |
|          | Groyne field | 16.93        | $2.24 \times 10^8$ | 3.73          | 0.54         | 2.66         |
|          |              | $p < 0.0001$ | $p < 0.0001$       | $p = 0.3413$  | $p = 0.0137$ | $p = 0.8100$ |
| Depth    | High         | 16.99        | $5.24 \times 10^8$ | 3.753         | 0.406        | 2.735        |
|          | Low          | 12.95        | $3.29 \times 10^8$ | 4.256         | 0.328        | 2.541        |
|          |              | $p = 0.0540$ | $p < 0.0001$       | $p = 0.1808$  | $p = 0.0312$ | $P = 0.1395$ |

months (e.g.  $R^2 = 0.99$  and  $0.95$  in June and August, respectively, as opposed to  $R^2 = 0.04$  in November, all for 0–10 cm, Fahlberg, all  $p \leq 0.05$ ). MPB and colloidal carbohydrate were also more closely related in the groyne fields where the MPB biomass was significantly higher as compared to the reservoirs (Tables 4 and 5). In contrast to the upper layers, MPB was less strongly (reservoirs:  $R^2 = 0.23$ ,  $p < 0.0001$ ) or not (groyne fields:  $R^2 = 0.01$ ,  $p = 0.66$ ) correlated to colloidal carbohydrate over the depth of 5–50 cm; the same applied for the relation between MPB and bound carbohydrate as well as protein. Colloidal carbohydrates predicted from chlorophyll *a* content [62] showed good correlations to the contents of measured colloidal carbohydrates, especially in the upper sediment layers ( $R^2 = 0.74$ , 0–0.5 cm and  $R^2 = 0.59$ , 0–5 cm, both  $p < 0.0001$ ; Fig. 6).

High bacterial cell numbers coincided with high EPS protein and bound carbohydrate contents, but were reciprocal to colloidal carbohydrate when considering the complete data set of all study sites, seasons, and depths

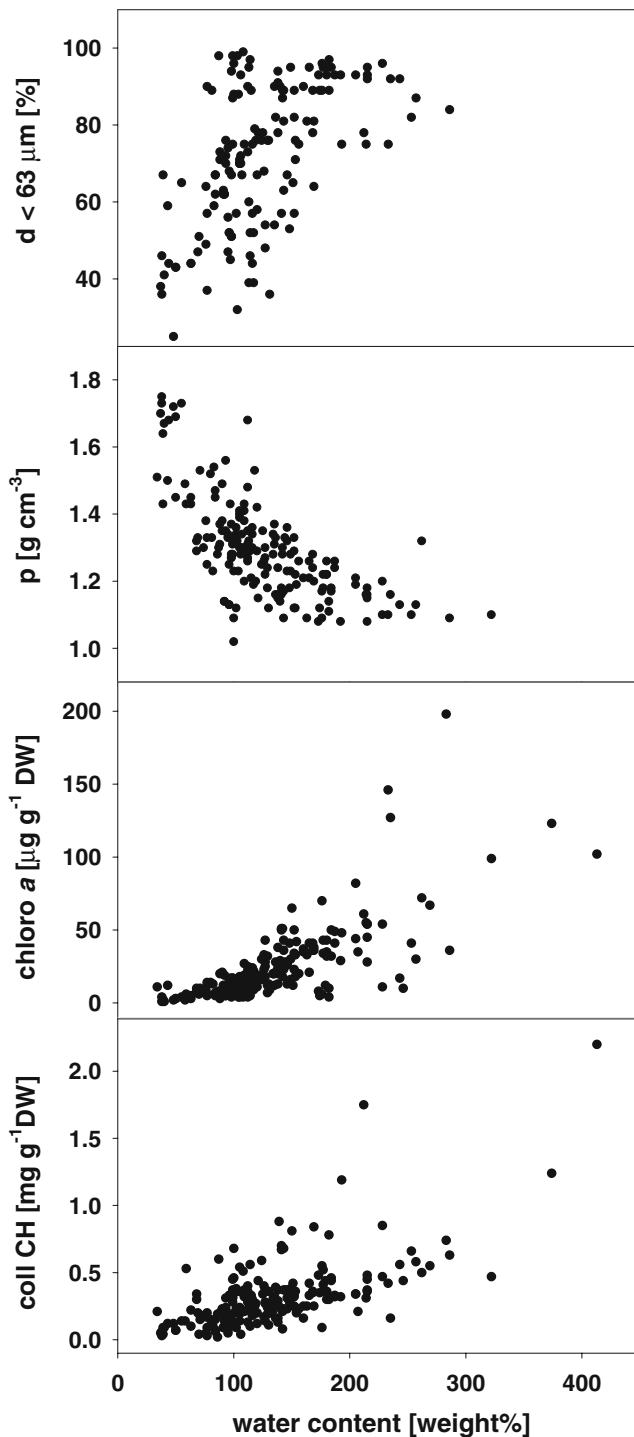
(Table 4). However, in the upper sediment layers only, the bacterial cell numbers were positively correlated to the colloidal carbohydrate (Table 5). In deeper sediment layers (5–50 cm), the bacteria were only related to EPS bound carbohydrate [ $R^2 = 0.34$  and  $0.16$ , reservoirs ( $p < 0.0001$ ) and groyne fields ( $p = 0.008$ ), respectively]. The correlation between bacteria and EPS was much closer in the groyne field sediments compared to the reservoirs where the bacterial cell numbers were significantly higher (Table 5). The correlation between bacteria and EPS varied as a function of study site and season, but generally, the correlation to the colloidal carbohydrate was strongest in the productive months, while no such pattern could be observed for proteins and bound carbohydrate.

The ratios between the different EPS fractions and chlorophyll *a* (EPS/Chl) as well as bacterial cell numbers (EPS/Bac) showed a similar increase with depth up to a factor of 2 in the groyne field sediments (data not shown). In the reservoirs, the ratios “EPS/Chl” and “EPS/Bac”

**Table 5** Correlation coefficients (Pearson’s *r*) are given for the inter-relations between microorganisms, EPS, and sedimentological factors considering the surface (0–1 cm) and the upper sediment layers (0–5 cm)

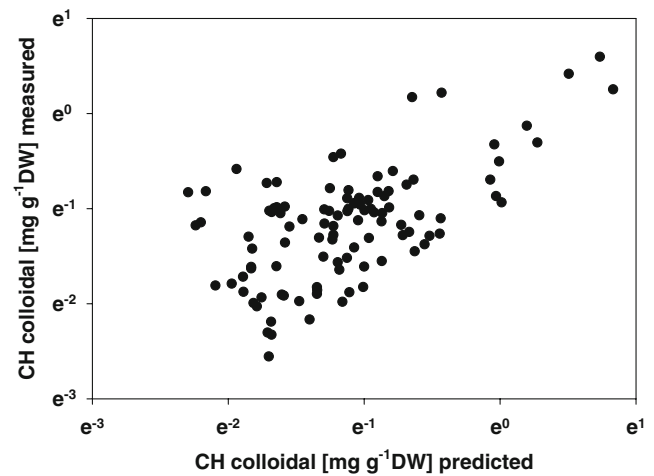
|                              | Chlorophyll a |        | Bacteria |        | Chlorophyll a  |         | Bacteria            |        |
|------------------------------|---------------|--------|----------|--------|----------------|---------|---------------------|--------|
|                              | R             | G      | R        | G      | R              | G       | R                   | G      |
| Bacteria                     | 0.58**        | 0.78*  |          |        | 0.68**         | 0.83**  |                     |        |
| Pheophytin                   | 0.78**        | 0.41   | 0.55**   | 0.83** | 0.78**         | 0.48*   | 0.55**              | 0.79** |
| Water content                | 0.70**        | 0.20   | 0.80**   | 0.99*  | 0.75**         | 0.32    | 0.72**              | 0.77*  |
| Grain size <63 $\mu\text{m}$ | 0.66**        | -0.73  | 0.63**   | 0.44   | 0.55**         | -0.81** | 0.43**              | -0.19  |
| TOC                          | 0.60**        | -0.68  | 0.40     | -0.42  | 0.54**         | -0.76*  | 0.25                | -0.40  |
| Colloidal CH                 | 0.56**        | 0.90** | 0.39*    | 0.81*  | 0.58**         | 0.86**  | 0.35**              | 0.63*  |
| Bound CH                     | 0.51**        | 0.71** | 0.27     | 0.98** | 0.51**         | 0.37    | 0.14                | 0.98** |
| Bound protein                | 0.29*         | 0.44   | -0.06    | 0.77*  | 0.23*          | 0.25    | 0.02                | 0.85** |
|                              |               |        |          |        | Surface 0–1 cm |         | Upper layers 0–5 cm |        |

The data sets were combined for reservoir sites (R) and groyne field sites (G), both integrating different replicates and seasons  
\* $p < 0.05$ , \*\* $p < 0.01$



**Figure 5** Correlations between the water content and grain size ( $d < 63 \mu\text{m}$ ,  $n=152$ , linear:  $R^2=0.36$ ,  $p \leq 0.001$ ), bulk density ( $p$ ,  $n=193$ , linear:  $R^2=0.45$ ,  $p \leq 0.001$ ), chlorophyll  $a$  contents ( $\text{chloro } a$ ,  $n=222$ , linear:  $R^2=0.57$ ,  $p \leq 0.001$ ), and colloidal carbohydrates ( $\text{coll CH}$ ,  $n=222$ , linear:  $R^2=0.55$ ,  $p \leq 0.001$ ). The data set originates from the reservoirs (six study sites, all seasons, all depths 0–50 cm)

increased moderately with depth for the colloidal carbohydrates (factor 2.5 and 1.5, respectively), but were more pronounced for the bound carbohydrates (factor 5.2 and 3.5, respectively) and proteins (factor 7.0 and 3.9, respec-



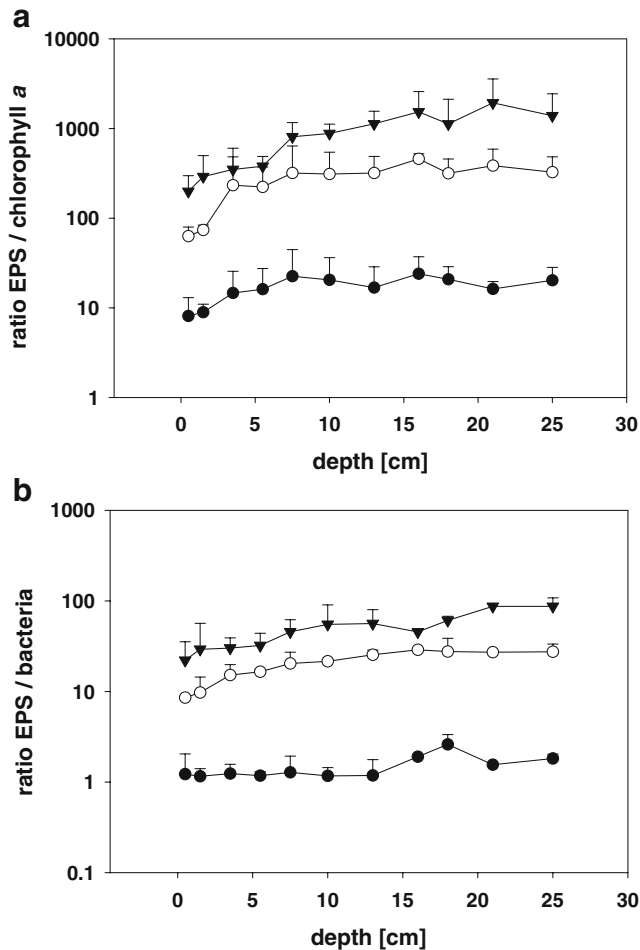
**Figure 6** Correlation between measured and predicted (model Underwood and Smith [62]) colloidal carbohydrates for the sediment surface layer (0–5 cm,  $R^2=0.59$ ,  $p < 0.0001$ )

tively; example shown for Poppenweiler, Fig. 7a, b). “EPS/Chl” ratios increased with depth immediately below the sediment surface, while the “EPS/Bac” ratios were quite stable over the first 15 cm for colloidal carbohydrates.

#### Covariance Pattern by PCA

The individuals (=number of elements in each vector/variable of the data set, here representing the different study sites, seasons, and depths over 5–50 cm) were projected in the plane of the factorial components (F1–F2). This indicated a clear separation between the study sites in River Rhine/Neckar (only reservoirs) and the study sites in River Elbe (mainly groyne fields) by the first axis (Fig. 8). The first axis (F1 or PC1 = principle component) explained about 45% of the variance mainly through sedimentological variables. The separation was driven by the high TOC values in the River Elbe sites as compared to the other sites. However, in the projection of the individuals for all depths including the surface (0–50 cm), it became clear that reservoirs and groyne fields were separated by both axes: the first axis represented granulometry, while the second axis was dominated by biological factors such as microorganisms and EPS (Fig. 9). Biology (axis 2) has driven the distinct clusters of reservoir and groyne field individuals originating from the upper sediment layers (0–5 cm) only (plot not shown). The reservoir Prelouc in the River Elbe overlapped with the two clusters reservoirs and groyne fields, but was considered to be closer to the reservoirs in all variables except TOC. Following this clear separation by MDS, the PCA was conducted independently for the two data sets, reservoirs and groyne fields.

PCA on the complete data set for the reservoirs showed that microbial biomass (microalgae and bacteria), EPS



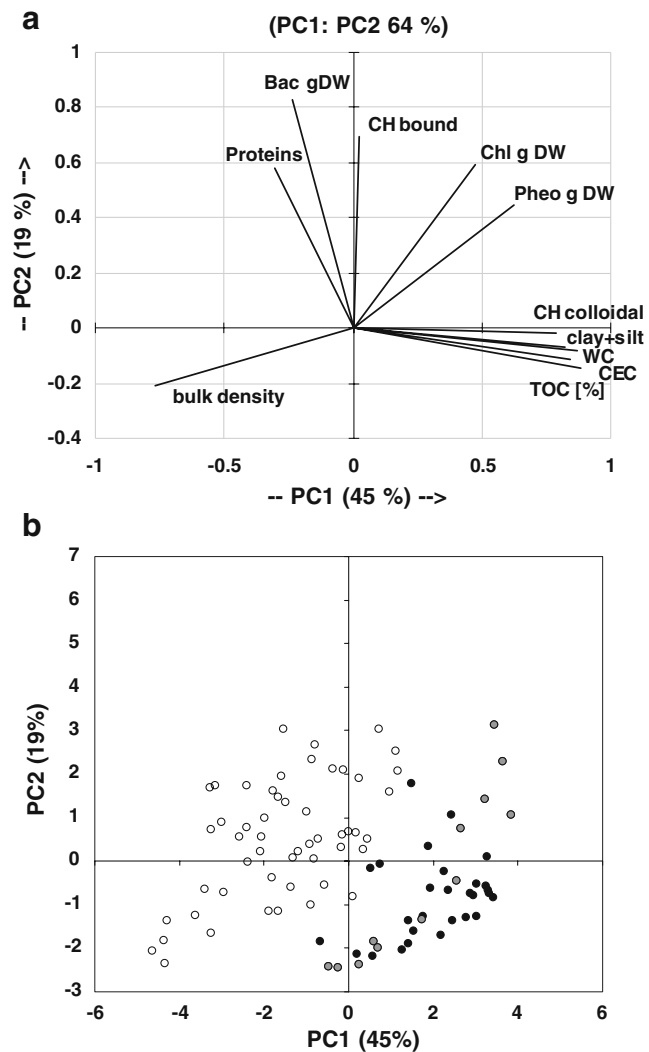
**Figure 7** Ratio of EPS/chlorophyll *a* (a) and EPS/bacterial cell numbers (b) ( $\text{g}^{-1} \text{DW}/\text{g}^{-1} \text{DW}$ ) with depth (0–30 cm), example shown for the reservoir Poppenweiler (River Neckar, April 2004). The *circles* indicate the ratio EPS carbohydrates/microorganisms (*dark*: colloidal, *white*: bound) and the *black triangles* refer to the ratio EPS proteins/microorganisms. Mean values ( $n=3$ ) are given for every sediment layer

carbohydrates, and sedimentological variables accounted for the variance explained by the first principal component (Table 6a). The variations explained by the second PC were mainly due to colloidal carbohydrates and proteins that may have reflected bacterial enzyme activity. Thus, most variation was given by the first two PCs (in total 62%), while the third PC which pointed to the inter-relation of the EPS fractions explained only little of the variance (12%). Performing PCA on the complete data set for the groyne fields indicated the strong inter-relation between microorganisms and EPS fractions in PC 1 with higher loadings as compared to the reservoirs. For the groyne fields, no relation could be established between biology and sedimentology (except for water content; Table 6). Instead, the second principal component was driven solely by granulometry. Again, the first two PCs accounted for the main part of the variance (in total 68%).

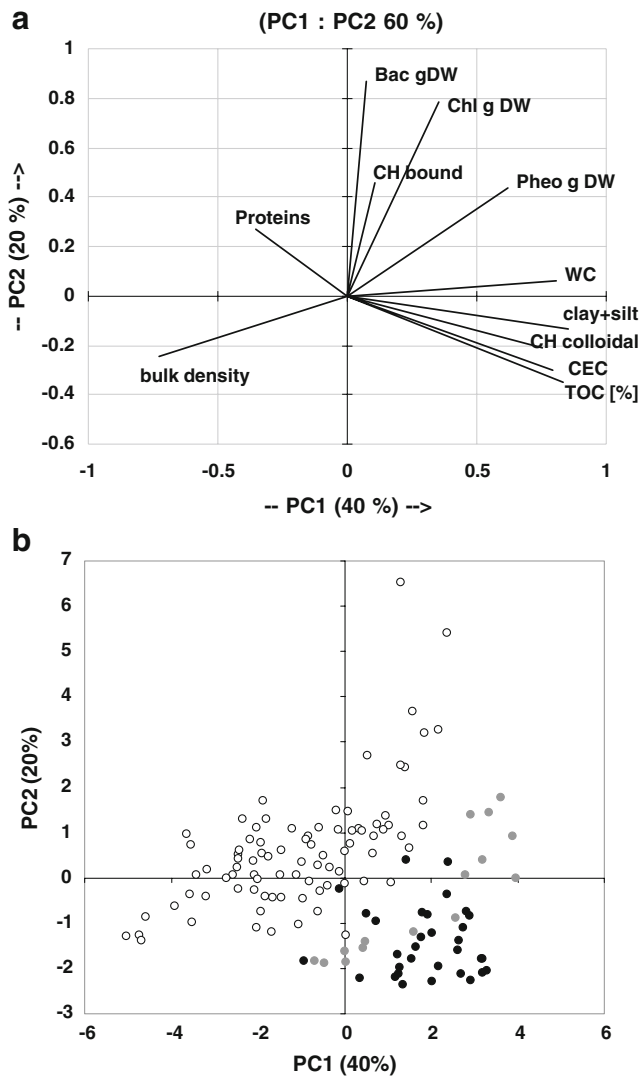
## Discussion

### Interplay Between Biology and Sedimentology

In the reservoir sites, the hydrodynamic forces are moderate throughout most of the year, with episodic local stresses caused by extreme events such as floods [31]. Hence, the reduced flow regime allows for elevated deposition of fine sediment material and promotes a long-term development of biota–non-biota relations in the benthic habitat. Settlement of microorganisms has been mainly observed on smaller particles with high surface/volume ratio that are



**Figure 8** PCA—Circle of correlation for variables (a) and projection of the individuals in the factorial plane PC1–PC2 (b) for combined data set (all study sites, all seasons, lower sediment layers 5–50 cm). **a** *PC* principle component, *Bac* bacteria, *Chl* chlorophyll, *Pheo* pheopigment, *CH* carbohydrate, *WC* water content, *TOC* total organic carbon, *CEC* cation exchange capacity, *g DW* per gram sediment/dry weight. **b** *White circles*, reservoir data; *gray circles*, Prelocou data (reservoir in River Elbe); *black circles*, groyne field data



**Figure 9** PCA—Circle of correlation for variables (a) and projection of the individuals in the factorial plane PC1–PC2 (b) for combined data set (all study sites, all seasons, all depths 0–50 cm). **a** PC principle component, *Bac* bacteria, *Chl* chlorophyll, *Pheo* pheopigment, *CH* carbohydrate, *WC* water content, *TOC* total organic carbon, *CEC* cation exchange capacity, *g DW* per gram sediment/dry weight. **b** White circles, reservoir data; gray circles, Prelouc data (reservoir in River Elbe); black circles, groyne field data

characterized by a high content of organic material and water [42]. In turn, microorganisms actively altered the sediment properties by binding fine sediment particles via the secretion of EPS [13]. The microorganisms have been shown mutual dependency, for example, bacteria profited from the exudates of microalgae and vice versa [68]. Accordingly, strong inter-correlation between microalgal biomass, bacterial cell numbers, water content, grain size, TOC, bulk density, and CEC were determined in the reservoirs over seasons and depth. In the groyne fields, the hydrodynamic forces show a distinctive circulation pattern with lowest flow velocities in the center [54]. Thus,

the groyne fields also represent a depositional site with a positive net balance during a hydrologic year, but on a day-to-day basis, the hydrodynamic forces are more significant and variable than in the reservoirs [54]. Consequently, interactions between biology and sedimentology were only established in deeper layers (below 5 cm depth) that were less prone to regular disturbance events or depositional flux. This was also evident in the microalgal benthic communities of the different habitat types where a more developed and typically benthic community was observed in the top layer of the reservoir sites as opposed to the groyne fields with stronger occurrence of habitat disturbance. Instead, deposited phytoplankton contributed more to the total benthic chlorophyll *a* signal in the groyne field sites than in the reservoir sites.

#### Microbial Community and EPS Components in Riverine Sediments

Although high light attenuation was observed at all sites, the resulting sediment light regime ( $10\text{--}100 \mu\text{mol m}^{-2} \text{s}^{-1}$  for the days measured) allowed the establishment of a benthic diatom community. Most of the observed benthic and deposited pelagic species were ubiquitous and known to be tolerant of a wide range of abiotic variables [37, 39]. Only a few species were specific to certain habitats or abiotic conditions such as *Didymosphenia geminata* (alpine-boreal areas), *Ellerbeckia arenaria* (small mountain rivers), and *Actinocyclus normanii* (high salt conditions) [37]. Chlorophyll *a* concentrations were higher than expected for relatively turbid habitats ( $35\text{--}198$  and  $136\text{--}248 \mu\text{g g}^{-1} \text{DW}$  in the sediment surface of reservoirs and groyne fields, respectively), comparing well to values commonly observed in densely colonized tidal flats [12]. Since both study types are net depositional areas with reduced flow regime, these high chlorophyll values might be due to an accumulation of drifted benthic algae as well as deposited pelagic algae. The bacterial cell numbers were in a range that is typical for muddy, organic-rich sediments (cell numbers  $10^8\text{--}10^9$  [42]).

The 1 to 2 m-deep sediments at the study sites in the Rivers Elbe, Neckar, and Rhine have shown remarkably high contents of EPS carbohydrates and proteins. The colloidal carbohydrates were well within the range of values reported from the upper sediment layers in highly productive intertidal flats (Table 7). In contrast to the marine environment, few studies are available on EPS contents and distribution in freshwater sediments. Hirst et al. [34] and Cyr and Morton [7] reported similar values to our present study for colloidal carbohydrates from shallow littoral sediments of several Canadian Shield lakes (Table 7). The data on colloidal carbohydrates from different laboratories are probably best for inter-comparison since water

**Table 6** PCA Loading matrix showing three extracted components (=vectors) for reservoirs and groyne fields (both data sets 0–50 cm, all seasons), explaining 48%, 14%, 12% (reservoirs) as well as 48%, 20%, 10% (groyne fields) of the variance in the data set

Extraction method: principal component analysis a, three components extracted. Loadings correspond to Pearson's  $r$  and are significant  $>0.35$  ( $n < 200$  for groyne fields)  
*CH* carbohydrates, *TOC* total organic carbon, *CEC* cation exchange capacity

|                         | Component matrix(a)  |       |       |                         |       |       |
|-------------------------|----------------------|-------|-------|-------------------------|-------|-------|
|                         | Reservoirs component |       |       | Groyne fields component |       |       |
|                         | 1                    | 2     | 3     | 1                       | 2     | 3     |
| Chl g <sup>-1</sup> DW  | 0.59                 | 0.67  | -0.23 | 0.96                    | -0.13 | -0.13 |
| Pheo g <sup>-1</sup> DW | 0.79                 | 0.02  | 0.21  | 0.93                    | 0.01  | -0.14 |
| Bac g <sup>-1</sup> DW  | 0.51                 | 0.71  | -0.29 | 0.91                    | -0.22 | 0.00  |
| Proteins                | -0.16                | 0.42  | 0.77  | 0.52                    | 0.18  | 0.63  |
| CH colloidal            | 0.73                 | -0.37 | 0.36  | 0.87                    | -0.05 | -0.15 |
| CH bound                | 0.45                 | 0.21  | 0.56  | 0.58                    | -0.37 | 0.58  |
| Bulk density            | -0.77                | 0.01  | -0.01 | -0.60                   | -0.55 | 0.17  |
| Water content           | 0.92                 | 0.15  | -0.14 | 0.93                    | 0.21  | -0.10 |
| Clay+Silt               | 0.75                 | -0.32 | -0.27 | -0.14                   | 0.69  | 0.23  |
| TOC (%)                 | 0.86                 | -0.30 | 0.09  | -0.05                   | 0.80  | 0.29  |
| CEC                     | 0.70                 | -0.21 | -0.05 | 11                      | 0.71  | -0.28 |

extraction is commonly used for this fragile EPS component. In contrast, a variety of extraction protocols have been employed for the tightly bound EPS components, making the direct comparison of EPS composition and concentration problematic (e.g. EDTA—ethylenediaminetetraacetic acid [11]; formaldehyde-NaOH [40], CER [24]). Keeping the variety of extraction methods in mind, the bound carbohydrates extracted by CER in the present study were in a comparable range to intertidal areas but higher than for other freshwater sites (Table 7).

There are few data with which to contrast the EPS protein contents (EPS<sub>p</sub>), since the focus in intertidal flats was on microalgae that secrete only a small fraction of proteins as extracellular material [57]. Cyr and Morton [7] have published similar protein contents and EPS<sub>p</sub>/EPS<sub>C</sub> ratios from seven Canadian Shield lakes (Table 7). The EPS<sub>p</sub>/EPS<sub>C</sub> ratios (0.1–3.2) determined in the present study are not as high as in wastewater flocs (0.5–21.2, [40]), thus

indicating microalgal contribution by carbohydrate production besides the metabolic activity of bacteria.

#### Microbial EPS Production

The present data set supports the hypothesis that the polymeric substances are mainly microbially produced because no correlation with macrofaunal abundances were found [28]. The microalgal biomass was correlated to the colloidal carbohydrates, with the strongest relation in the upper sediment layers (surface, 0–5 cm) during months of high metabolic activity (spring/summer) and at study sites with high chlorophyll *a* values, indicating healthy and productive microalgal communities (groyne fields, reservoir Deizisau). Higher chlorophyll *a* values reflected higher contents of colloidal carbohydrates, but this relation became increasingly decoupled with depth below the photic zone. These observations are in line with earlier studies

**Table 7** Overview of EPS contents in freshwater sediments from the literature compared with some studies from the intertidal areas

| Unit                      | Colloidal CH | Bound CH  | Bound protein | Study site          | Extraction | Reference     |
|---------------------------|--------------|-----------|---------------|---------------------|------------|---------------|
| (mg g <sup>-1</sup> DW)   | 0.10–2.40    | 1.60–6.00 | 0.90–12.9     | River/reservoirs    | Water/CER  | Present study |
| (mg g <sup>-1</sup> DW)   | 0.40–3.40    | 1.30–6.70 | 2.00–7.40     | River/groyne fields | Water/CER  | Present study |
| (μg C g <sup>-1</sup> DW) | –            | 2.50–7.50 | –             | River               | EDTA       | [2]           |
| (mg g <sup>-1</sup> FW)   | 0.01–0.38    | 0.07–0.31 | 0.01–0.11     | Lake                | Water/EDTA | [34]          |
| (mg g <sup>-1</sup> DW)   | 0.01–0.85    | 0.08–1.02 | 0.56–8.59     | Lakes               | Water/EDTA | [7]           |
| (mg g <sup>-1</sup> DW)   | 0.07–1.25    | 0.10–2.25 | –             | Intertidal          | Water/EDTA | [13]          |
| (mg g <sup>-1</sup> DW)   | 0.07–0.30    | –         | –             | Intertidal          | Water      | [22]          |
| (mg g <sup>-1</sup> DW)   | 0.01–2.30    | –         | –             | Intertidal          | Water      | [46]          |
| (mg g <sup>-1</sup> DW)   | 2.30–4.00    | –         | –             | Intertidal          | Water      | [68]          |
| (mg g <sup>-1</sup> DW)   | 0.01–0.35    | 0.15–1.30 | –             | Intertidal          | Water/EDTA | [64]          |



where colloidal carbohydrates were regarded as indicative of microphytobenthic production [46, 57]. Consequently, the model of Underwood and Smith [62] showing the relation between chlorophyll as a proxy of microalgal biomass and colloidal carbohydrates in intertidal areas was applicable to the present data set, with the best regression in the sediment surface layer. Presumably, colloidal carbohydrates are metabolic by-products of primary production and growth that are released passively as could be shown by work in the pelagic zone [4, 41, 44]. Other processes responsible for polymer secretion by microalgae are discussed in the literature (e.g. nutrient depletion, overflow mechanisms of excess of photosynthetic products [57, 65]), but this seems unlikely to induce EPS production at our eutrophic, light-limited study sites. The relation between microalgae and colloidal carbohydrates in deeper sediment layers beneath the photic zone became weaker (reservoirs) or did not hold (groyne fields). It has been shown that microalgae can produce colloidal carbohydrates under dark conditions [7, 48, 56] that has been linked to migration behavior [33, 55]. In contrast to the marine environment, little is known about migration of microalgae in freshwater sediments [25, 52, 67] and whether this is a regular feature. Although microalgae in freshwater sediments do not have to face emersion/immersion periods, there is a strong likelihood that they have to migrate in order to reposition themselves after being buried or in avoiding unfavorable conditions. This may explain the observed correlation between microalgae and colloidal carbohydrates in sediment layers without light.

Several studies suggest that the bound EPS fractions represent a refractory pool left behind following the biodegradation of labile, colloidal EPS components; the latter thus being indicative of recent production and more related to microalgal primary production [11, 15, 55, 60]. However, the present data set showed also a close relation between microalgal biomass and the more tightly bound carbohydrates in the upper sediment layers, as it was reported by Battin and Sengschmitt [2] and Cyr and Morton [7]. Whether bound carbohydrates are actually secreted by microalgae or whether this is rather an indirect effect (transformation of freshly produced colloidal to bound carbohydrates as discussed in [11]) remains open. The weak correlation between microalgae and EPS proteins, plus the inter-relation between chlorophyll, pheopigment, and proteins suggests that some protein might have been originated from algal cell lysis/debris.

High bacterial cell numbers were associated with high levels of EPS bound carbohydrates and proteins, but with lower colloidal carbohydrates contents, when considering all sediment depths (0–50 cm) investigated. This supports the idea of the elevated sensitivity of colloidal carbohydrates to biodegradation and bacterial involvement in their

modification by secretion of extracellular enzymes, especially in deeper sediment layers [29, 66]. With depth, the bound EPS components increasingly dominated the colloidal carbohydrates. Assuming that bound EPS represent the more refractory and less dynamic fraction [11], the data suggest accumulation of refractory material in deeper sediment layers as opposed to fresh production of labile/colloidal components in surface layers. Accordingly, in deeper layers, the bacterial cell numbers showed correlations to bound carbohydrates and proteins. Considering solely the upper layers, positive relations between bacteria and colloidal carbohydrates have been observed and were closest where high microalgal productivity led to secretion of colloidal EPS. This could indicate a “response” of bacterial cell numbers to the available nutrient source, but actual EPS secretion by the bacteria (overflow mechanism, attachment) cannot be ruled out. The latter is supported by the ratio of “bacteria to colloidal carbohydrates” that was fairly constant with depth over the first 15 cm and thus could indicate bacterial contribution to the fragile EPS pool in sediment layers below the photic zone. Overall, the bacteria showed a strong correlation to the proteins (except for upper sediment layers in most of the reservoirs) that could largely represent exoenzymes deployed to solubilize external organic matter [29, 66]. Thus, with protein secretion [16, 20], bacteria might have been involved in both EPS degradation and contribution to the EPS signal at the same time. However, there is also evidence that bacterial protein secretion might be an important feature for their strong attachment to sediment particles [40].

## Summary

The hydrodynamic regime and the season determined the biomass and composition of the microbial community in the upper sediment layers of the selected riverine sites and thus influenced EPS content and distribution. A freshly deposited, high microalgal biomass in the groyne fields seemed responsible for high levels of colloidal EPS. It is suggested that colloidal carbohydrates were produced by microalgae as a by-product of primary production in the upper sediment layers and were related to algal migration in deeper layers. The close relation between microalgae and colloidal carbohydrates showed that the models from the intertidal flats are applicable to riverine sediments. The positive relation between bacteria and colloidal carbohydrates in the upper layers (actual secretion or response to this potential nutrient source) is still debatable. However, the data set supports the hypothesis that bacteria contribute to the EPS signal by secretion of proteins that might represent degradation enzymes or attachment fibrils. The relative increase of bound EPS to colloidal EPS with depth

indicated fresh production of polymeric substances in the upper sediment layers and mainly accumulation of refractory material in the lower layers. Although a holistic approach to complex processes in natural sediments is highly preferable, it remains the case at present that different approaches are required to follow-up questions on the provenance and fate of varying EPS components in spatial, temporal, or seasonal terms or to distinguish between fresh microbial production and refractory compounds.

The EPS contents in the riverine sediments were in a similar range as values reported from intertidal areas where the ecological role of EPS has been well established. Thus, the EPS content determined here could have a nutritional role in the benthic pelagic food web [7, 17] or might affect nutrient or pollutant removal [40], but this requires confirmation. However, an involvement of microbially produced EPS in sediment stability, as tested for intertidal areas [63], has been shown for selected study sites [26–28]. Whether these results are transferable to the other study sites will be examined in future studies (Gerbersdorf, in preparation). Further work should investigate the possible ecosystem function of microbially produced EPS for the benthic habitat in freshwater sites and the drivers of microbial EPS secretion including locomotion, cell attachment, and protection against toxins or nutrient sequestration.

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