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Biofilms and Size Sorting of Fine Sediment During Erosion in Intertidal Sands

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Abstract The goal of this research was to investigate size-specific retention of clay and silt-sized grains by biofilms in sandy intertidal sediments. Sediment cores were collected from an intertidal flat in Cole Harbour, NS, and eroded at increasing shear stresses (0.08–0.60 Pa) with a Gust microcosm. Half of the cores were eroded without undergoing prior treatment, while sodium hypochlorite was added to the other cores to destroy biofilms. The disaggregated inorganic grain size distribution of sediment resuspended by the Gust microcosm was then obtained with a Multisizer™ 3 Coulter Counter®, and each treated core was compared with its corresponding untreated core. Overall, significantly less total sediment mass was resuspended from untreated cores than from treated cores. At intermediate shear stresses, the sediment resuspended from treated cores contained a greater proportion of fine and medium silts than the sediment resuspended from untreated cores. Very fine silts and clays were not retained preferentially by biofilms. The results show that biofilms stabilize the sediment, but they do not necessarily enhance the proportion of finest sediment sizes, as previously proposed.

Keywords Biofilms · Sediment sorting · Tidal flats · Grain size · Resuspension · Sediment mobility

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Introduction

Small changes in the fine sediment content in sands can have a strong effect on the erosion and sorting of sediments in the seabed. The transition from non-cohesive to cohesive sediment behavior occurs when clay content exceeds a 5–10 % threshold (Dyer 1986; van Ledden et al. 2004; Law et al. 2008). As the clay content grows to exceed this threshold, the erodibility of sediment decreases (Dyer 1986; van Ledden et al. 2004), as does sorting during erosion. The preferential retention and removal of sediment grains based on size is referred to as sorting, where sortable sediments display size-specific deposition and resuspension, while sediments that are not sortable deposit in the same proportion as found in suspension (Kranck et al. 1996; Curran et al. 2004; Milligan et al. 2007) or are resuspended in the same proportion as found in the seabed (McCave and Hall 2006; Law et al. 2008). Sediment sortability thus affects the size distribution of suspended particles as well as the evolution of seabed texture. As a result, sortability has an influence on light penetration in the water column (Baker and Lavelle 1984; Boss et al. 2001; Slade et al. 2011), is relevant to studies of sediment and contaminant transport because contaminants such as trace metals, PCBs, and PAHs preferentially adsorb onto fine particles (Milligan and Loring 1997; Wang and Mulligan 2006; George et al. 2007; Cadwalader et al. 2011), and has been invoked to explain the commonly observed abrupt transition from sand to mud on continental shelves (George et al. 2007; Law et al. 2008). Sortability associated with mud content has also been used to explain large-scale shifts in the facies associated with alluvial and coastal deposits that occurred early in the Paleozoic era of earth history. In short, the emergence of terrestrial plants at this time increased the production of mud via increased weathering, which then reduced sediment sorting and altered channel geometries in alluvial and coastal deposits (Davies et al. 2011).

Previous studies have shown that microorganisms found at the sediment surface, such as diatoms and cyanobacteria, can influence sediment erosion and sorting by secreting extracellular polymeric substances (EPS) that form biofilms and trap fine particles (Yallop et al. 1994, van de Koppel et al. 2001). Serving as buffer zones between microorganisms and their environment, biofilms offer protection from physical stresses such as desiccation, changes in salinity (Decho 2000), and damage from rolling grains (Delgado et al. 1991), as well as protection from grazing (Decho 1990). The interactions between biofilms and the sediment are extensive. The sticky nature of EPS secretions allows sessile species to adhere to the substrate and mobile species to navigate among sediment grains (Hoagland et al. 1993). These secretions increase the adhesion between sediment grains, which has been shown to limit erosion (Grant et al. 1986; Sutherland et al. 1998; Lundkvist et al. 2007). Adhesion creates a more stable substrate, which is thought to be advantageous to microorganisms by preventing physical damage and burial associated with moving sediment. EPS secretion may also represent an evolutionary advantage by reducing the amount of shading, both within the seabed and the water column. Shading is reduced in the seabed by the composition and structure of biofilms, which increase light scattering and spacing between sediment grains (Decho et al. 2003). In the water column, EPS secretion reduces shading by reducing the amount of sediment in suspension via two processes (Holland et al. 1974): biofilms reduce the amount of sediment resuspended during erosion and they increase flocculation, which allows sediment grains to sink more rapidly (Bender et al. 1994; Decho 2000; Stal 2010). Van de Koppel et al. (2001) suggested that the interactions between biofilms and the seabed are strongest when associated with fine sediment grains as the authors observed a positive correlation between net growth of biofilms and retention of fine particles (silt and clay, collectively known as "mud") in the seabed.

The preferential retention of fine sediment grains by biofilms could be a critical aspect of a positive feedback mechanism between surface biofilm growth and mud content in sediments (van de Koppel et al. 2001). Organic material adheres to the surface of particles (Keil et al. 1994), and because fine particles have a greater surface area per unit of volume than large particles, an increased retention of fine grains should result in enhanced nutrient availability in the sediment. Enhanced nutrient availability should, in turn, promote biofilm growth at the sediment surface, further increasing mud content. Such a positive feedback would lead to two alternate stable states: one with high mud content and high microbial growth (e.g., cohesive mud flats) and another with low mud content and low microbial growth (e.g., non-cohesive sand flats). At the core of the positive feedback mechanism described by van de Koppel et al. (2001) is the hypothesis that biofilms increase the proportion of fine sediment grains by increasing their deposition and/or their retention during erosion, although the study does not propose any size dependence for preferential accumulation of mud in the seabed, instead grouping all fine sediment sizes under the general term "silt." Implicitly, however, the effect should be strongest for the smallest particles sizes, which are the clays (<4 μm), and decrease for the progressively larger very fine silts (~4–8 μm), fine silts (~8–16 μm), medium silts (~16–32 μm), and coarse silts (>32 μm; Folk 1980). This size dependence would emerge under the assumption of van de Koppel et al. (2001) that nutrient availability scales with particle surface area in the bed and with the safer assumption that surface area scales with particle size.

This study focused on the effects of biofilms on the sorting of the fine fraction of intertidal sediments during erosion and sought to determine specifically what grain sizes, if any, are preferentially retained by biofilms. More precisely, the effect of biofilms on fine sediment sortability was investigated by comparing the size distribution of sediment artificially resuspended from cores with destroyed biofilms to that of sediment resuspended from intact cores. Sample cores were collected on a local intertidal flat and eroded with a Gust microcosm erosion chamber. A range of erodible grain sizes that included silts and very fine sands was necessary to compare erosional sorting over the full range of clay and silt sizes. As such, sandy sites as close to the sand-to-mud transition as possible were selected.

Material and Methods

Sample Collection

Sediment samples were collected biweekly from May to July 2010 from an intertidal flat by the Salt Marsh Trail in Cole Harbour, Nova Scotia (Fig. 1). Cole Harbour is a barred microtidal inlet on the Atlantic coast of Nova Scotia with semidiurnal tides, a maximum tidal range of 2 m, and a spring–neap variability of approximately 0.50 m. The area of the inlet is approximately 15 km^2 and is surrounded by single roads with limited land development, as well as provincial parks. Salinity at the sampling sites ranged from 24 to 30 and water temperature from 15 to 21 °C. During each collection, a set of three cores was obtained from site A and, when possible, additional cores were obtained from sites B and C (Fig. 1). Sites were selected in sandy areas near the sand-to-mud transition of the flat in order for samples to comprise a wide enough range in grain size to compare the erosion of sizes ranging from clays to coarse silts. Site A was located in a secondary channel, near a junction with the main channel, while both

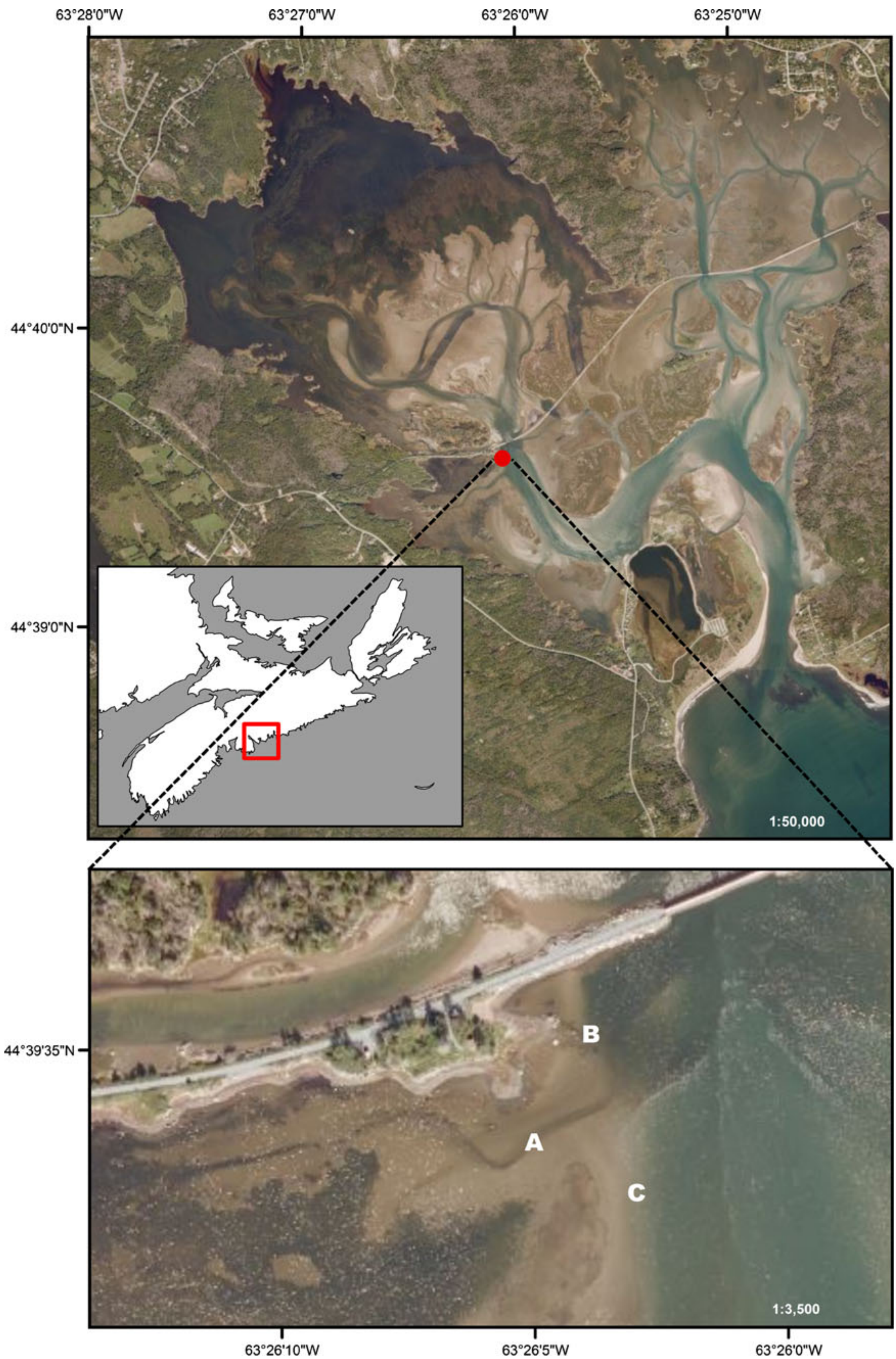

Fig. 1 Maps showing Cole Harbour and the study sites. The *red dot* shows where the samples were collected (*top map*) and *letters* represent each site (*bottom map*)

sites B and C were located on a bank of the main channel. The samples were obtained on flooding tides with a hand-held corer (water depth>50 cm). The cores contained sediments as well as some overlying water. Upon collection, they were sealed and transported back to the lab. To avoid disruption of the biofilm, care was taken to minimize disturbance to the sediment–water interface during coring and transport.

Treatment Overview

One core in each set was used to obtain samples of the intact sediment surface for grain size analysis. A second core remained untreated, while sodium hypochlorite (household bleach) was added to the third core in a concentration of 50 ml l^{-1} and left overnight. Sodium hypochlorite was selected in order to dissolve the biofilm without interfering with the physical cohesion of the seabed (Quaresma et al. 2004). Cores that were not immediately eroded were stored at 10 °C to preserve the surface biofilm. All untreated cores were eroded within 12 h of collection, while treated cores were eroded on the day following collection.

Erosion

Untreated cores were subjected to the erosion procedure before the treated ones in order to maintain the untreated surface biofilms as intact as possible while allowing the bleach to penetrate the treated sediment. The order between each site was, however, determined randomly.

A Gust microcosm (Tolhurst et al. 2000; Dickhudt et al. 2009) was used to erode untreated and treated cores. The Gust microcosm fits directly onto core tubes and contains an electronically controlled rotating head as well as water input and output. The instrument is calibrated so that the rotation and pumping rates are automatically adjusted to apply a given shear stress uniformly across the sediment surface (Law et al. 2008). Each eroded core was progressively subjected to shear stresses of 0.01, 0.08, 0.16, 0.24, 0.32, 0.40, 0.48, and 0.60 Pa, with the first step used to flush the water inflow and outflow lines. Shear stresses were applied on the sediment for 20 min, except for 0.48 and 0.60 Pa which were applied for 5 min. For the shear stresses selected for this experiment, intertidal sediments typically experience depth-limited erosion (Amos 1995). This means that at any given shear stress, only a fixed mass of sediment can be eroded from the seabed, and once that mass has been eroded, no further erosion occurs. Depth-limited erosion has been reported in mud flats (Mehta et al. 1982; Amos 1995; Sanford and Maa 2001) as well as in non-cohesive sediments where bed armoring took place (Gomez 1983; Wiberg et al. 1994). Bed armoring occurs when shear stresses are too weak to induce motion of all particle sizes in the seabed. In such cases, finer particles at the surface are resuspended while coarser grains remain immobile, eventually forming a coarser-grained lag layer that prevents the resuspension of finer grains found deeper in the sediment. The erosion times for this study were selected based on prior experiments, which found them sufficient to resuspend the available erodible mass (Law et al. 2008). Water collected at the site flowed through the Gust microcosm and into the core, while water containing sediment resuspended by the erosion flowed out of the core and into collecting bottles. The water collected was then filtered using pre-weighed Millipore™ 8.0-μm SCWP (cellulose acetate) filters, allowing for the mass of the sediment resuspended at each stress to be calculated. These filters have previously been shown to have a much smaller nominal pore size once filtration has started, allowing for the retention of sediment grains <1 μm (Sheldon 1972).

Surface and Eroded Grain Size Distributions

Seabed surface samples (<5 mm deep) were obtained from the first core in each set and digested in an excess of 30 % H_2O_2 to remove all organic matter. The remaining inorganic fraction was suspended in a 1 % NaCl solution and disaggregated with a sapphire-tipped ultrasonic probe. The samples were then filtered through a 100-μm screen, and disaggregated inorganic grain size (DIGS) distributions of the filtrates were obtained using a Multisizer™ 3 Coulter Counter® equipped with aperture tubes of 30 and 200 μm.

The 100-μm cutoff was selected because particles with larger diameters would not be fully resuspended by the Gust microcosm. In the Gust microcosm, the outflow is located 6.5 cm above the sediment surface, which implies that sediment grains need to be continuously in suspension and uniformly distributed in the water column to be adequately represented in our samples. Transport as suspended load, as opposed to bed load or mixed load, occurs when the ratio of settling velocity to shear velocity is <0.3 (Dade and Friend 1998). Shear velocity (u_*, in meters per second) is given by the equation

$$u_* = (\tau/\rho)^{1/2} \tag{1}$$

where τ is the boundary shear stress (in pascals or kilograms per meter per square second) and ρ is fluid density (in kilograms per cubic meter). Settling velocity (w_s, in meters per second) is a function of particle diameter, density, sphericity and roundness, as well as the density of the suspending medium (Dietrich 1982). Table 1 shows the ratios of settling velocity to shear velocity calculated for various grain sizes subjected to the shear stresses used in this experiment, assuming spheres of quartz density (2,650 kg m^{-3}) with a Powers roundness scale of 2 (Dietrich 1982) and fluid density of

Table 1 Values for w_s/u_* associated with various grain sizes when subjected to shear stresses relevant to this experiment, assuming quartz spheres with a Powers roundness scale of 2

Diameter (μm)	w_s/u_*						
	0.08 Pa	0.16 Pa	0.24 Pa	0.32 Pa	0.40 Pa	0.48 Pa	0.60 Pa
16	0.02	0.01	0.01	0.01	0.01	0.01	0.01
32	0.08	0.05	0.04	0.04	0.03	0.03	0.03
64	0.29	0.21	0.17	0.15	0.13	0.12	0.11
100	**0.64**	**0.46**	**0.37**	**0.32**	0.29	0.26	0.24
128	**0.98**	**0.70**	**0.57**	**0.49**	**0.44**	**0.40**	**0.36**

The assumption that the particles are adequately represented in the samples fails at $w_s/u_*>0.3$, shown in bold (Dade and Friend 1998)

1,023 kg m^{-3} (experimental water at 10 °C and 30 ppt). These calculations support the application of a 100-μm cutoff since particles with greater diameters will not be transported as a fully suspended load. More generally, this implies that Gust microcosms should be used with caution when trying to monitor the erosion of sands.

DIGS distributions were obtained for the samples resuspended during each erosion stress. The filters on which the sediment was retained were first ashed using a gas plasma system. The sediment samples were then digested with H_2O_2 and the remaining inorganic fraction was disaggregated before being processed with the Multisizer™ 3. Size distributions were based on the volume occupied by each size class in the sample (normalized to the total sediment volume) and ranged from particle diameters of 1 μm to a maximum of 100 μm. The size data were binned in 1/5 phi increments ($\phi=-\log_2 d$, where d is diameter in millimeters). For additional information, refer to Milligan and Kranck (1991).

Mobility

The mobility of each size class (at a given shear stress) was calculated by dividing its volume fraction in the resuspended sediment by its volume fraction in the original seabed, as described in Law et al. (2008).

$$M_{i,\tau} = \frac{V_{i,\tau}\ \text{resuspended}}{V_i\ \text{in original seabed}} \tag{2}$$

where M indicates mobility, i a given size class, τ the stress applied by the Gust microcosm, and V the volume occupied in the sediment sample (normalized to the total sediment volume). A mobility distribution for each core and each erosion stress was then obtained by plotting mobility as a function of diameter (in micrometers). Flat distributions with values near unity indicate low sortability, while distributions with distinct peaks indicate high sortability.

Sortability Index

For the purpose of this study, a sortability index (S_I) was developed to quantify the mobility distributions obtained for each erosion stress. The index needed to reflect the magnitude of peaks present in mobility distributions (indicative of sorting) as well as allow for a distinction between peaks located at finer and coarser grain sizes. As a first step, the total sum of squares was obtained for each mobility distribution.

$$|S_I| = \sum_{i=1}^{n\,class} \left(M_{i,\tau} - \overline{M}_\tau\right)^2, \tag{3}$$

where $\overline{M}_\tau$ represents the average mobility at a given stress. This value was then made positive when grains larger than the mid-size showed mobilities greater than finer grains and negative in the opposite case. This was achieved by comparing the total variation of the data points above and below the midpoint within a single mobility distribution.

$$v_b = \sum_{i=1}^{m-1} \left(M_{i,\tau} - \overline{M}_\tau\right) \tag{4}$$

$$v_a = \sum_{i=m+1}^{n\,class} \left(M_{i,\tau} - \overline{M}_\tau\right)$$

where v_b is the variation below the midpoint, v_a is the variation above the midpoint, and m is the midpoint (i.e., mid-size class). If $v_b<v_a$, then larger grains were preferentially resuspended and the previously calculated S_I was assigned a positive value. Alternately, if $v_b>v_a$, then preferential resuspension of finer grains occurred and S_I was assigned a negative value. The sortability index thus increased in value when larger peaks were observed and became more negative/positive when small/large grains were more mobile. To facilitate understanding, the index can be thought of as a slope, but given that the data were not linear, some variability between distributions could not effectively be captured by a simple slope calculation.

Results

Site Characteristics

Initially, multiple sites were selected to analyze the effects of biofilms on sediments with different cohesive properties. Physical cohesion is largely determined by the amount of clay (grains <4 μm) present within the sediment (Dyer 1986; van Ledden et al. 2004; Law et al. 2008). Sediments have been found to behave cohesively when their clay fractions exceeded a threshold between 5 and 10 % and non-cohesively otherwise (van Ledden et al. 2004, Law et al. 2008). The clay fraction at all study sites was consistently below that threshold (Table 2), implying non-cohesive behavior. All sediments collected were classified as sands or within the upper range of silty sands, according to the classification of Folk (1980). Detailed size analysis of the sands was not conducted, but all sediment passed through a 125-μm sieve, indicating that the sediments at the sites are appropriately classified as very fine sands. Because of the textural similarity among sites, results were pooled for analysis. Statistical analyses were conducted using R.

DIGS distributions for the original seabed samples were plotted as a function of diameter (Fig. 2). These distributions were all found to have similar slopes at small diameters, indicating a common sediment source (Kranck et al. 1996; Curran et al. 2004). Some distributions from site A, as well as the one from site B, showed a peak at approximately 40 μm. The other distributions from site A, as well as those from site C, however, flattened around 10 μm, with the latter showing a moderate peak at this diameter. All of the surface distributions tailed off at sizes smaller than the 100-μm cutoff, indicating that particles with an equivalent spherical diameter below the cutoff of the screen may have been retained on the screen due to irregular shapes.

Table 2 Grain size characteristics of the original seabed for all three study sites

Sites	Date	%<4 μm	% 4–63 μm	% 63–125 μm
A	18-May	2.88	11.15	85.96
	31-May	2.64	11.25	86.11
	17-June	2.45	15.03	82.52
	29-June	2.66	13.61	83.73
	15-July	2.58	13.06	84.36
	29-July	1.71	6.92	91.36
B	29-June	1.64	8.58	89.77
C	15-July	1.04	4.06	94.90
	29-July	2.21	9.49	88.30

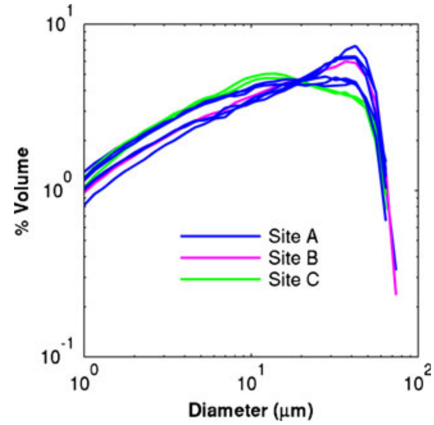

Fig. 2 Surface DIGS distributions (after 100-μm screening) for cores collected at all three study sites. An average of two or three surface samples is plotted for each collection day and site

Amount of Sediment Resuspended

The cumulative mass of sediment resuspended at or below a given shear stress was calculated by adding the masses of sediment resuspended at stresses less than or equal to that stress (Fig. 3). Overall, the total mass of sediment resuspended from the treated cores was significantly greater than the mass of sediment resuspended from the untreated cores (paired *t* test: $P \leq 0.05$), with the treatment producing approximately five times greater erosion. Each stress increment also consistently resuspended a greater mass of sediment from the treated cores than from the cores left intact (paired *t* test: all $P \leq 0.05$), except for shear stress of 0.08 Pa ($P=0.20$) where the mass of sediment was similar to that collected when flushing the lines (stress of 0.01 Pa). This indicates that a shear stress between

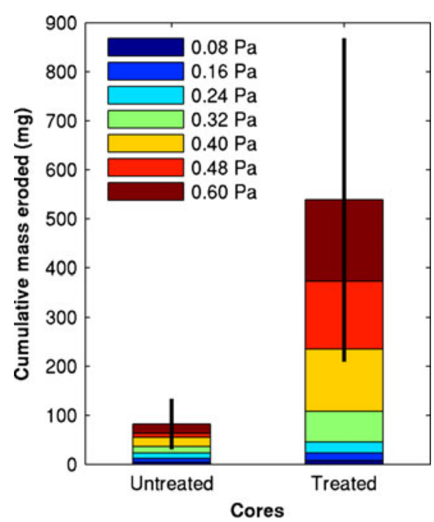

Fig. 3 Cumulative mass of sediment eroded (in milligrams) by the Gust microcosm from 0.08 to 0.60 Pa for the treated and untreated cores. Average values are shown. *Error bars* indicate the standard deviations for the total mass of sediment resuspended

0.08 and 0.16 Pa was required to resuspend some particles from the seabed.

Plots of cumulative mass eroded (in milligrams) as a function of particle diameter (in micrometers) were generated for each core by multiplying the cumulative mass eroded at a given stress with the volume fraction as a function of diameter, as obtained from the DIGS distributions for that stress. Similar trends were observed in all cores, so only one set was selected for illustration (Fig. 4a). For every grain size, the mass eroded increased with stress. At low to moderate stresses (0.08–0.24 Pa), the masses of fine particles eroded were comparable between the treated and untreated cores. The total mass eroded from the treated cores at these low to moderate shear stresses, however, were larger than from untreated cores. This indicates that larger particles accounted for the difference in masses eroded from the treated and untreated cores. At higher shear stresses, a greater mass of sediment was eroded from the treated cores for all size classes, not just for the larger particles.

DIGS Distributions and Mobilities

DIGS distributions were generated for each sample by plotting volume fraction (in percent) as a function of diameter

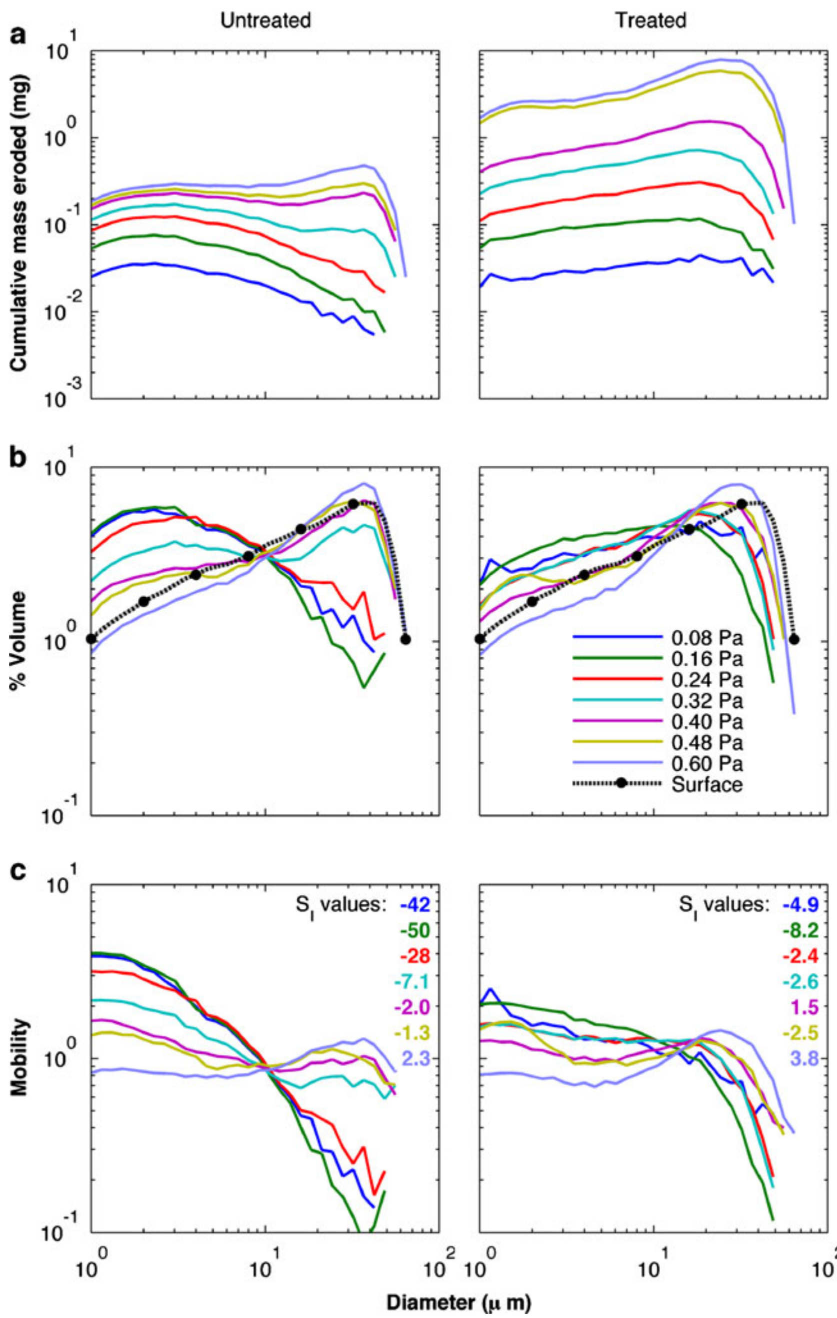

Fig. 4 **a** Cumulative mass eroded (in milligrams) as a function of diameter (in micrometers). **b** DIGS distributions. **c** Mobility plots for an untreated core and its corresponding treated core, both collected at site A on July 15, 2010. *Solid lines* represent various shear stresses (in pascals)

(in micrometers), while mobility distributions were obtained by plotting mobility (fraction in sample/fraction in original seabed) as a function of diameter (in micrometers). Figure 4 shows all data obtained for one set of cores, while Fig. 5 shows the mobility plots associated with low, moderate, and high shear stresses (0.16, 0.32, and 0.48 Pa) for all cores. At low shear stresses, finer particles were resuspended by the Gust microcosm (Figs. 4 and 5). As stress increased, however, the maximum diameter of the particles resuspended increased, as well as the proportion of large particles. Overall, greater shear stresses increased the mobility of larger grains. At low and high shear stresses (0.08–0.16 and 0.48–0.60 Pa, respectively), the untreated and treated cores showed similar particle mobility curves (e.g., blue and red lines in Fig. 5). At intermediate shear stresses (0.24, 0.32, and 0.40 Pa), the mobility of large particles was greater in the treated cores than in the untreated ones (e.g., green line in Fig. 5). A possible seasonal trend was evident at site A, where the mobility of larger grains in the untreated cores decreased with the progression of summer.

Sortability Indexes

S_I values were calculated from mobility curves as outlined in "Sortability Index." The difference in sortability index between the treated and untreated cores, at a given stress, was calculated by subtracting the S_I value of each untreated core from that of its corresponding treated core (Fig. 6). At intermediate erosion stresses (0.24, 0.32, and 0.40 Pa), the sortability indexes of the treated cores were significantly greater than those of the untreated cores (Wilcoxon signed-rank test: all $P \leq 0.05$). This trend was not observed at lower and higher stresses.

Seasonal Trends

In order to further investigate the apparent seasonal trend observed at site A, where larger grains appeared to become less mobile as summer progressed (Fig. 5), correlation analyses between the properties of the untreated cores collected at site A (sortability index and cumulative mass eroded) and their collection date were carried out. At low to moderate shear stresses (0.08–0.32 Pa), the sortability indexes of the untreated cores became more negative with increasing year day, indicating that larger grains were less mobile later in the season (Fig. 7). The correlations, however, are not statistically significant.

Discussion

The greater mass of sediment resuspended in treated cores is consistent with the body of literature that found biofilms to limit erosion (Holland et al. 1974; Grant et al. 1986; Sutherland et al. 1998; Decho 2000; Lundkvist et al. 2007). Although no direct measurements of the biofilms were carried out, these results suggest that the bleach treatment was effective at destroying the biofilms present in the cores.

The positive differences in the sortability index between the treated and untreated cores at intermediate shear stresses (0.24, 0.32, and 0.40 Pa; Fig. 6) indicate that coarser sediment grains were resuspended from cores without biofilms. Alternatively, given the formulation for mobility (Eq. 2), higher mobilities for larger grains in the treated cores may have arisen from depletion of the smaller grain sizes in the bed sediments of treated cores. Extensive removal of clay and very fine silt at low stresses would make them unavailable for resuspension at intermediate stresses, thereby leading to the incorrect interpretation that these sizes were being retained in the seabed at intermediate stresses. Plots of cumulative mass eroded for specific grain sizes (Fig. 8), however, demonstrate that the mass of resuspended clay and very fine silt increased monotonically with stress in treated and untreated cores, thus showing that depletion of the smaller grain sizes did not occur. Instead, at intermediate stresses in treated cores, the resuspension of fine and medium silts (8 and 16 μm) was enhanced relative to that of clay and very fine silt (Fig. 8), supporting the argument that biofilms preferentially retain these intermediate silt sizes. Since physical cohesion in the seabed is determined by clay content (Dyer 1986; van Ledden et al. 2004; Law et al. 2008) and biofilms were found to preferentially retain intermediate silts, the results from this study suggest a potential decoupling between physical and biological cohesion. Although these findings further our understanding of natural sediment transport, the exact mechanism by which biofilms preferentially retain intermediate silt sizes remains unknown. Results are consistent with biofilms affecting sediment consolidation and, hence, the depth profile of critical shear stress for erosion, but they are also consistent with biofilms enhancing bed armoring by anchoring large grains more firmly.

The tendency of biofilms to limit the resuspension of fine and medium silt refines the earlier observations of van de Koppel et al. (2001) that showed simply that silt content and net growth of diatom biofilms were correlated. The observations here, however, question the proposed cause of this observed correlation. Van de Koppel et al. (2001) link higher net growth to enhanced nutrient supply, which is in turn linked to particle surface area. Under this scenario, selective advantage would exist for the retention of particles with the highest surface area-to-volume ratios, which are the clays and very fine silts. Billerbeck et al. (2007) challenge the concept that small particle size leads to enhanced nutrient availability with observations of higher nutrient levels in

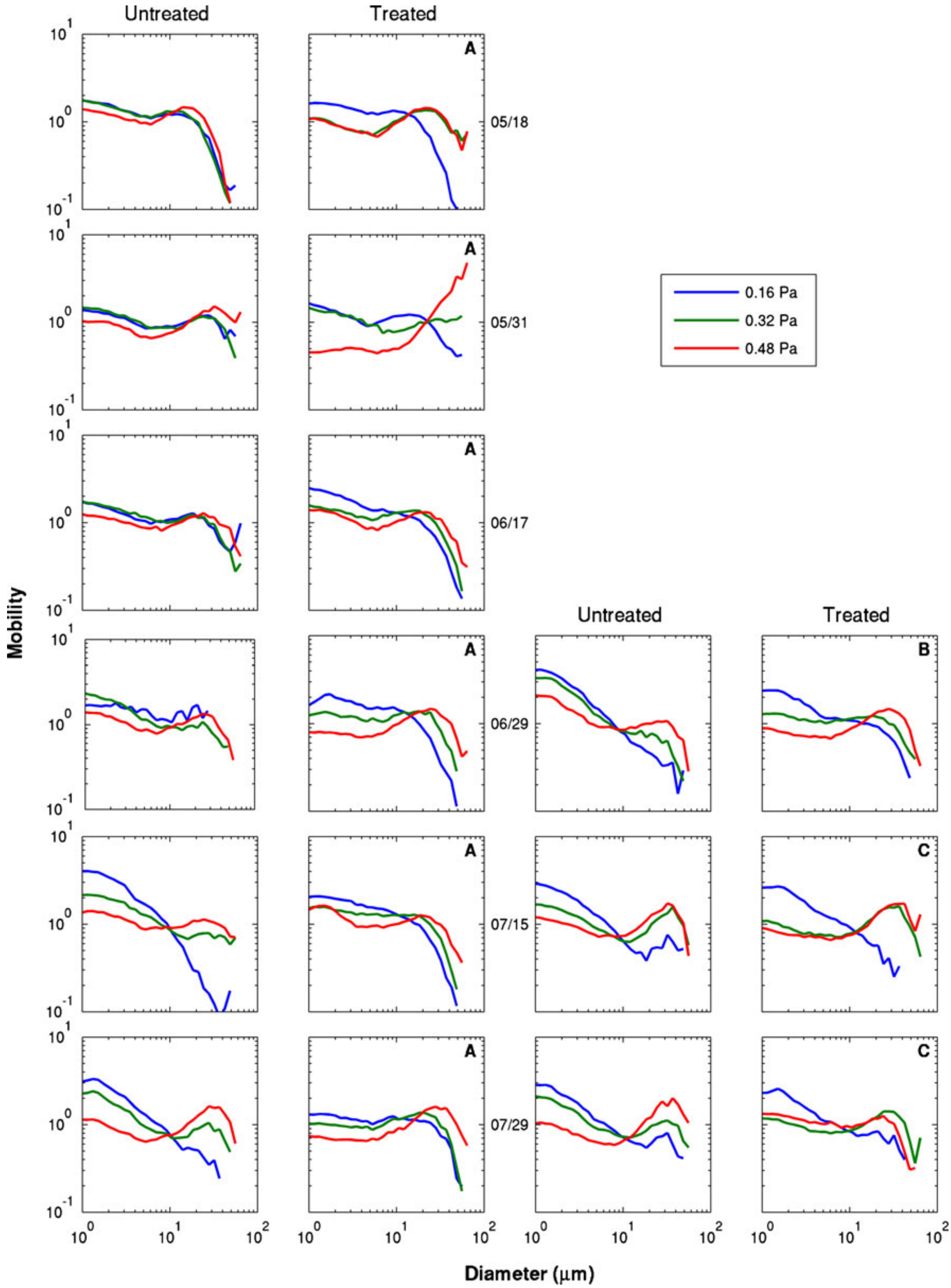

Fig. 5 Mobility plots obtained for low, intermediate, and high erosion stresses (0.16, 0.32, and 0.48 Pa, respectively) for all cores. Collection sites are indicated in the *top right corner* of the treated core plots. Plots were organized by collection date (shown in the *middle of rows*)

sandy sediment with low mud content due to tidal flushing and high permeability. These findings, as well as those resulting from this study, suggest that the positive feedback previously described between microbial growth, mud content, and nutrient availability (van de Koppel et al. 2001) may not be applicable to all environments.

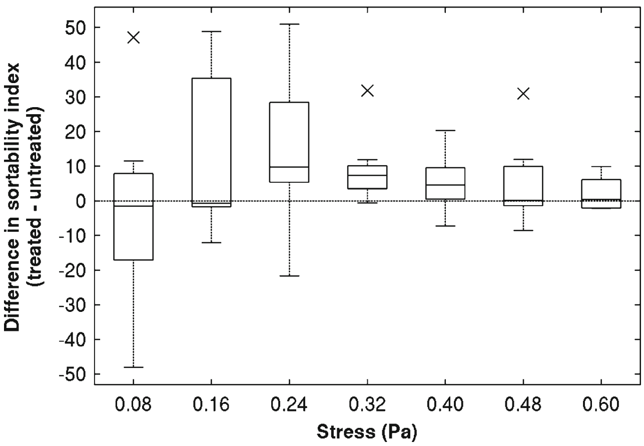

Fig. 6 Box plot representation for the differential sortability between the treated and untreated cores based on the stress applied at the sediment surface (N=9 for each stress). The *whiskers* extend from the minimum to the maximum value within ±1.5 times the interquartile range, the *box* covers the second and third quartiles, while the *midline* shows the median. Outliers are shown as *X*'s

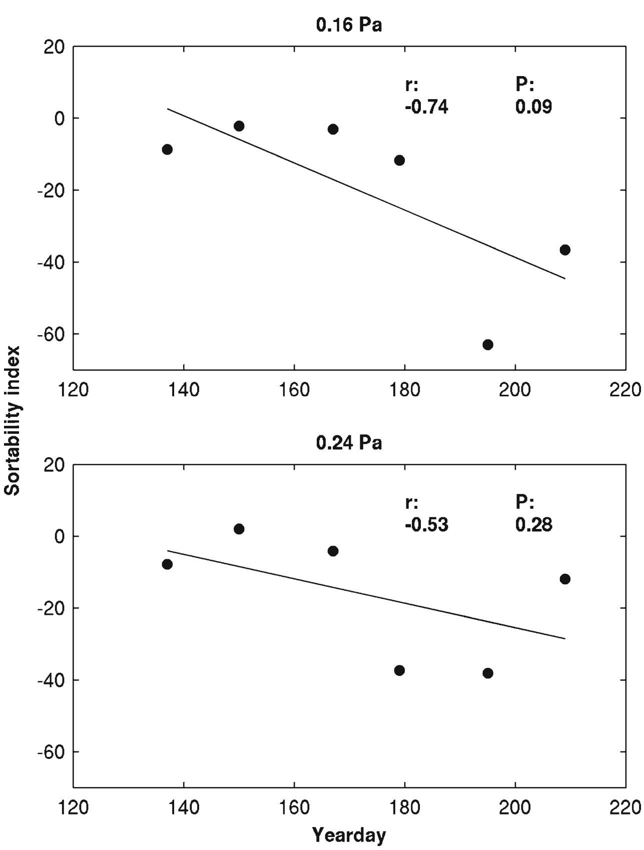

Fig. 7 Correlation between untreated sediment sortability index at site A and collection date for shear stresses of 0.16 Pa (*top*) and 0.24 Pa (*bottom*). The correlation coefficient is indicated as *r* and the *P* value as *P*. Positive/negative values indicate that larger/smaller grains are more mobile

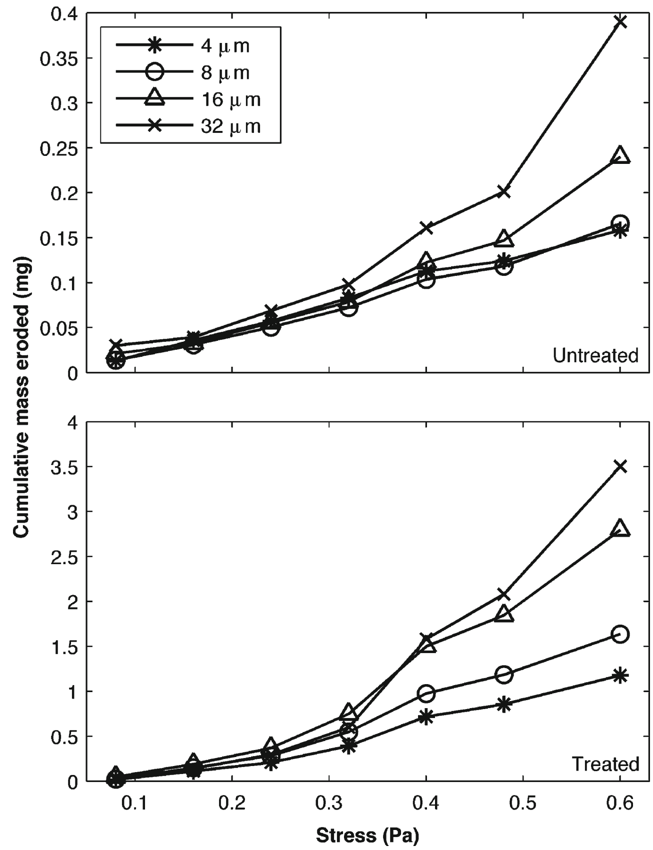

Fig. 8 Cumulative mass eroded (in milligrams) as a function of stress (in pascals) for sediment grains with diameters of 4, 8, 16, and 32 μm in a single set of cores. Note the change in order of magnitude between the treated and untreated cores

Diatom biofilms are most often associated with mud flats, while cyanobacteria are dominant in fine sand environments such as the ones studied here (Stal 2010). Sandier sediments are found in higher energy environments, where large rolling grains can cause physical damage to fragile microorganisms (Stal 2003). In such environments, the advantages of gluing down large grains may supersede those associated with increasing sediment surface area. Additionally, in energetic environments, it may be beneficial for organisms to anchor themselves to particles that are less likely to be resuspended, resulting in connective strands of EPS being secreted preferentially between large grains. Such linkage between sand grains has, in fact, been observed by Grant and Gust (1987). Reid et al. (2000) also described young cyanobacteria biofilms as consisting mostly of vertical filaments between sand grains, with the more traditional perception of biofilms as covering the entire sediment surface occurring at a later growth stage. Various microorganisms, or biofilms at various stages, could, therefore, have different effects on sediment sortability. One hypothesis is that diatoms are found higher on the flats, where the environment is less energetic and mud content is greater. The positive feedback described by van de Koppel et al. (2001) would maintain this environment in a stable state. Cyanobacteria would be found lower on the flats, where the environment is more energetic and the sediment coarser. In this environment, the positive feedback mechanisms would not apply and cyanobacteria biofilms would mainly prevent the motion of larger sediment grains.

Although no statistically significant seasonal trends were found, there is some evidence that the mobility of fine and medium silts at low to moderate shear stresses decreased as summer progressed (Fig. 7). This reduction in the mobility of larger grains occurred over a period of time where cyanobacteria are thought to become more abundant (Jesus et al. 2009), which is consistent with the hypothesis that cyanobacteria retain larger grains. Further characterization of microbial assemblages in relation to sortability measurements is required to evaluate this hypothesis.

The lack of significant differences between the sortability indexes observed at low shear stresses (0.08 and 0.16 Pa) can be explained by the small mass of sediment resuspended by the Gust microcosm. The low concentrations obtained led to a high level of variation between samples. At high stresses (i.e., >0.40 Pa), however, the lack of differences in the sortability indexes was likely due to the indiscriminate resuspension of all clay and silt grains from the seabed, leading to similar sortability indexes for both the untreated and treated cores. The fact that the DIGS distributions obtained at these stresses cover similar size ranges to those obtained from surface samples (Fig. 4b) is in accordance with this hypothesis. The presence of a biofilm appeared to have no effect on sorting during resuspension at high stresses (0.48 and 0.60 Pa), suggesting that an erosion threshold exists above which biofilms no longer affect size-specific sediment resuspension of clays and silts. At high stresses, biofilms may serve mainly to limit the overall amount of sediment resuspended.

Estimates of critical shear stresses for surface samples fall within the range of shear stresses selected for artificial erosion by the Gust microcosm. Based on Wiberg and Smith (1987), the critical shear stress for a quartz sphere of 125 μm resting on a flat uniform bed with no cohesion is 0.25 Pa. This implies that the experimental erosions to which sediments and biofilms were subjected were likely realistic simulations of natural conditions to which microorganisms could have adapted over time. It is conceivable that low to moderate shear stresses (0.08–0.40 Pa) would be routinely encountered by microorganisms, driving the evolution of adaptive mechanisms, such as EPS secretion, that limit the mobility of large sediment grains. Higher shear stresses (0.48 and 0.60 Pa), however, are likely encountered less frequently, during storm events for instance. The rarity of these events over the generation time of most microorganisms may make size-specific grain retention at these shear stresses relatively costly due to its limited benefits and, thus, prevent the evolution of size-specific grain retention mechanisms acting at such high stresses.

It is worth mentioning that this study focused on the effects of biofilms on erosion without addressing flocculation and deposition. In order to fully understand the interactions between microorganisms and the sediment, the effects of biofilms on these latter processes should also be considered.

Conclusion

This study found that biofilms reduce sediment resuspension and preferentially retain fine and medium silts at intermediate shear stresses. The latter result does not support the hypothesis that biofilms preferentially retain the finest sediment sizes with the largest surface area-to-volume ratios. Various microorganisms may have different effects on grain retention, with cyanobacteria retaining larger grains and diatoms finer grains, but this hypothesis requires further research. Preferential retention of fine and medium silts did not occur once the shear stress was sufficiently high (>0.40 Pa), suggesting that a threshold exists above which biofilms no longer result in size-specific grain retention. A more complete characterization of biofilms should help in determining whether the effects observed in this study are predictable.

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