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Grain sizes retained by diatom biofilms during erosion on tidal flats linked to bed sediment texture



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ABSTRACT

Size-specific sediment retention by diatom biofilms was measured by eroding intertidal muds at increasing shear stresses (0.01–0.60 Pa) using a Gust microcosm. The grain sizes eroded from biofilmcovered sediment were compared to those from control cores from which the biofilms were destroyed using bleach. Biofilms were quantified using carbohydrate measurements. Cores from an intertidal mud flat in the Minas Basin of the Bay of Fundy (Canada) showed biofilms preferentially retained clays and very fine silts relative to fine and medium silts. In contrast, prior field observations on an intertidal sand flat indicated that fine and medium silts were preferentially retained by biofilms relative to clays and very fine silts. These contrasting results suggest a link between size-specific sediment retention and sediment texture, where sand biofilms retain coarser, non-cohesive sediment grains, while mud biofilms retain finer, cohesive sediment grains. This relationship implies that biofilms could contribute to a positive feedback that would preserve existing sediment texture.

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1. Introduction

Diatoms and other benthic microbes secrete extracellular polymeric substances (EPS) that form a sticky web among sediment grains (Grant et al., 1986). These molecular networks of EPS are known as sediment biofilms. Previous studies (Holland et al., 1974; van De Koppel et al., 2001) have linked biofilms and sediment texture, noting that biofilm-covered sediment was associated with increased clay ($< 4 \,\mu m$) and silt content ($\sim 4-63 \,\mu m$). This association is due, in part, to the redistribution of EPS in the water column, which enhances flocculation, a process that increases the settling velocities of clays and silts, and thus their depositional fluxes to the seafloor (Bender et al., 1994; Decho, 2000; Stal, 2010). More importantly, because EPS increase the cohesion between sediment grains, they increase sediment erosion thresholds. Cohesion affects the erosion thresholds of finer sediment sizes more than coarser sizes because smaller particles have larger surface-area-to-mass ratios. As a result, biofilms reduce winnowing of fine sediment (Sutherland et al., 1998; van De Koppel et al., 2001).

A better understanding of the interactions between biofilms

E-mail addresses: jgarwood@ucsd.edu (J.C. Garwood), Paul.Hill@dal.ca (P.S. Hill), Hugh.MacIntyre@dal.ca (H.L. MacIntyre), Brent.Law@dfo-mpo.gc.ca (B.A. Law). and fine sediment in coastal areas is crucial to assess and predict water quality. Focusing on fine sediment is a priority because contaminants, such as trace metals, adsorb preferentially to fine particles (Milligan and Loring, 1997) and bind to biofilms (Sutherland, 1990). Recently, both biofilms and suspended particles have been shown to increase contaminant retention by the seabed, notably with DDT (Guo et al., 2012). Other studies have shown an increased survival of pathogenic bacteria in the sediment when biofilms were present (Decho, 2000; Piggot et al., 2012). Fine sediment retention by biofilms may affect sensitive benthic communities. For instance, a reduction in silt content can decrease organic matter availability (e.g., Thrush and Dayton, 2002). The link between organic matter and grain size can forge complex links between grain size and the abundance of important species in intertidal ecosystems. In the Bay of Fundy, the abundance of Corophium volutator, which is considered a keystone species in the area, has been linked to the grain size of mudflats (Trites et al., 2005). A vulnerable population of semi-palmated sandpipers (Calidris pusilla) rely on Corophium as their main food source during migration (Shepherd et al., 1995). Biofilms in the area are dominated by diatoms (Daborn, 1991; Amos et al., 1992) and an association between local biofilms, Corophium, semipalmated sandpipers and sediment stability has been reported (Daborn et al., 1993). More specifically, it was observed that biofilm grazing by Corophium can destabilize the sediment. When sandpipers started feeding on the amphipods, a trophic cascade allowed biofilms to recover and stabilized the sediment (Daborn et al.,

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1993). Although the trophic cascade hypothesis has been questioned, the interdependence of biofilms, *Corophium*, sandpipers and sediment texture is clear (Hamilton et al., 2006).

Studies of the role of biofilms on size-specific sediment retention generally have considered broad size classifications, and they have not distinguished the behaviors of cohesive versus non-cohesive fractions (Holland et al., 1974; van De Koppel et al., 2001). McCave et al. (1995) argued that aggregates smaller than $\sim 10\,\mu m$ are not broken up by shear in the viscous bottom boundary layer and, as such, their constituent grains are not subject to hydrodynamic sorting, while grains larger than $10 \,\mu m$ can be sorted hydrodynamically. Increasing abundance of constituent sediment grains smaller than 10 um may be associated with reduced erodibility (van Ledden et al., 2004) and reduced erosional sorting (Law et al., 2008) of sediment. An accurate understanding of erosion and sorting of a sediment bed, therefore, relies on an accurate understanding of the processes that control the abundance of fine sediment in the seabed. The goal of this work is to resolve the effect of biofilms on detailed, size-specific retention of sediment in the seabed, focusing particularly on the fine sediment fractions.

Previous research on size-selective erosion from sandy sediment with biofilms showed that clay-sized ($< 4 \mu m$) particles are not preferentially retained in the seabed during erosion. In one experiment, biofilms grown on sand were shown to preferentially retain 5-µm very-fine-silt-sized microspheres relative to the 1-µm clay-sized microspheres that were simultaneously released into a recirculating flume (Arnon et al., 2010). The authors argued that biofilm pore sizes allowed both particle sizes to deposit within the biofilm, but they reduced the resuspension of the larger particles more effectively than that of the finer particles. In another study, Garwood et al. (2013) used a Gust microcosm to apply a sequence of shear stresses to sediment cores from a sandy intertidal flat. They demonstrated that biofilms preferentially retained fine and medium silts (8-16 µm) relative to clays and very fine silts $(< 8 \,\mu m)$. These two studies are inconsistent with the hypothesis that diatom biofilms always retain the finest grain sizes (Holland et al., 1974; van De Koppel et al., 2001). Garwood et al. (2013) speculated that the biofilms at their site did not retain the finest grain sizes because the biofilms were formed by cyanobacteria, whereas previous research had examined sorting associated with diatom biofilms. Alternatively, differences in sorting may have

been reinforced by the substrates themselves, with mud biofilms preferentially retaining the finest, most cohesive grain sizes and sand biofilms preferentially retaining coarser, non-cohesive grain sizes.

Because previous studies reporting an association between biofilm and mud (clay and silt) content of the sediment focused on diatom biofilms (Holland et al., 1974; van De Koppel et al., 2001), and because none of the studies addressing size-specific sediment retention explicitly involved muddy substrates and diatom biofilms (Arnon et al., 2010; Garwood et al., 2013), a field study was conducted to quantify the effect of diatom biofilms on size-specific sediment retention in muds. Cores were collected and eroded biweekly over an 8-month period from an intertidal mudflat in the Minas Basin of the Bay of Fundy, Canada. This site was selected because previous research showed that diatom biofilms dominated the muddy sediment at the site (e.g., Daborn, 1991).

2. Methods

2.1. Field site

Natural sediment was eroded to test whether the effects of diatom biofilms on size-specific sediment retention in mud differed from that observed in sand. Sediment cores were collected from a macrotidal flat near Kingsport, Nova Scotia, in the Minas Basin of the Bay of Fundy (45.15°N, 64.37°W, Fig. 1). The landward edge of the site was located one meter (in horizontal distance) beyond the lower edge of a salt marsh, where the high marsh was dominated by *Spartina patens*, and the low marsh by *Spartina alterniflora*. The surface sediment at the site was composed of mud (see Section 3.2). The intertidal flats in this region of the basin experience slightly asymmetric semi-diurnal tides, with a stronger flood than ebb, and an average tidal range of 11.5 m (Faas et al., 1993).

2.2. Sample collection

Sediment cores were collected biweekly from April through November, 2012. To minimize diurnal and tidal influence on biofilm properties caused by migration of microorganisms in the



Fig. 1. Maps of the Minas Basin and intertidal flats. The field site is indicated by the red box and red dot, while the intertidal zones are shown in gray. The left panel is based on data from the Atlantic Climate Adaptation Solutions Association published in van Proosdij and Pietersma-Perrott (2012). For additional characterization of the site, including a LIDAR survey, see Law et al. (in preparation).

sediment (Smith and Underwood, 1998) or changes in sediment erodibility caused by flat exposure (Paterson et al., 1990), samples were always collected 5 h after high tide. Collection days were selected such that sampling occurred at the same point in the spring-neap cycle, and at the same time of day.

The sampling site was divided into a 4 alongshore by 6 acrossshore grid of 24 quadrats $(1 \text{ m} \times 1 \text{ m})$, each of which had one edge in contact with one of two sampling piers that consisted of wooden planks resting on wooden piles. The piles were driven into the mud prior to the sampling period and were left in place for the duration of the study, while the planks were laid down for sampling and removed immediately after. The sampling piers allowed for collection of cores with minimal disturbance of the surrounding mud. Quadrats were randomly selected for sampling, without replacement. On each sampling day, six sediment cores (10-cm diameter) were collected from the randomly-selected quadrat. Core tubes were inserted 10 cm into the sediment, dug out, and sealed prior to transportation to the lab. Nearby sediment was used to refill the holes in order to minimize disturbance to flow over the flats.

In the lab, two of the six large cores were subsampled for biofilm and surface sediment measurements using 13-mm syringe cores. The surficial 5 mm of each syringe was stored at $-80 \,^{\circ}\text{C}$ until analysis. The other four 10-cm cores were gently filled with filtered seawater, taking care not to disturb the sediment surface. Two of the cores were eroded immediately using a double-head Gust microcosm (similar to the single Gust microcosm described in Tolhurst et al. (2000)), as described by Garwood et al. (2013). Household bleach (c. 6% NaOCl) was added to the two remaining cores (50 ml bleach per liter of seawater) to destroy the biofilms while minimally impacting the physical cohesion among sediment grains (Quaresma et al., 2004). The two treated cores were sealed, stored at 4 °C overnight to allow full reaction of the bleach, and were then eroded with the Gust microcosm. This schedule was followed because it allowed for the erosion of biofilms that were not degraded in untreated cores and for the bleach to penetrate the sediment surface in bleached cores. Preliminary tests showed that the sediment mass eroded from bleached cores was always greater than the mass eroded from untreated cores, implying that any compaction effect in the treated cores, which presumably would decrease mass eroded, was less than the effect associated with the destruction of the biofilm, which increased mass eroded. Preliminary tests also showed similar masses eroded for untreated cores eroded upon return to the laboratory and for untreated cores eroded the following morning, again implying a minimal effect of compaction.

2.3. Erosion

The head of a Gust microcosm comprises a magnetically-driven, rotating shear plate that is mounted on top of a core tube. By electronically controlling the rotation of the shear plate and the pump rate, a uniform shear stress can be applied at the sediment surface (Tolhurst et al., 2000). Shear stresses ranging from 0.01 to 0.60 Pa were applied incrementally to each core for 20 min, as this time was sufficient for all erodible sediment to be resuspended (i.e., depth-limited erosion; Amos, 1995). Throughout the erosion process, filtered seawater was pumped into the system, and the compensating outflow carried the resuspended sediment. For each stress step, the outflowing seawater was filtered through Millipore 8.0 μm SCWP (cellulose acetate) pre-weighted filters to obtain the mass and grain size distribution of eroded sediment. These filters were selected because at the concentrations observed (of order $10-100 \text{ mg l}^{-1}$), they have effective pore sizes that are much lower than the nominal size, and they combine excellent trapping efficiency while minimizing clogging (Sheldon, 1972; Law et al., 2008). No residual sediment was observed in the filtrate, confirming the filters' trapping efficiency. A sample of the inflowing filtered seawater was also collected for each erosion experiment in order to measure background sediment concentration. The background sediment concentration was then subtracted from the eroded mass concentration.

2.4. Grain size analysis

The disaggregated inorganic grain size (DIGS) distributions of resuspended sediment, as well as that of surface sediment, were obtained using a Multisizer 3 Coulter Counter (Beckman Coulter, Brea, CA, USA) following Milligan and Kranck (1991) and Garwood et al. (2013). Sediment samples were first digested using excess 30% hydrogen peroxide and then resuspended into a NaCl electrolyte (0.15 mol 1^{-1}). Immediately prior to obtaining size spectra with the Coulter Counter, the samples were disaggregated using an ultrasonic probe. Aperture sizes of 30 and 200 µm were selected to measure disaggregated grains falling in a size range of 1–120 µm. This size range is adequate for the muddy sediments at the site (see Section 3.2).

Grain size mobility for a given core was calculated (Law et al., 2008) for each stress step using both resuspended and surface DIGS:

$$M_{i,\tau} = \frac{V_{i,\tau} \text{ resuspended}}{V_i \text{ in seabed}}$$
(1)

where *M* (dimensionless) is the mobility, *i* is a given size class, τ is the stress applied by the Gust microcosm, and *V* is the volume fraction of a size class in the total sediment sample. Mobility is a measure of preferential resuspension or retention in the seabed. Mobility values below 1 indicate that the sediment grain size was preferentially retained in the seabed during erosion, while mobility values above 1 indicate that the sediment grain size was preferentially resuspended from the seabed.

Mobility distributions, where mobility is plotted as a function of grain diameter (μ m), were described using a sortability index (S_l):

$$|S_I| = \sum_{i=1}^{n \text{ class}} \left(M_{i,\tau} - \bar{M_r} \right)$$
(2)

where $\overline{M_r}$ is the average mobility at a given stress. The index is a total sum of squares of the mobility distribution, and it is assigned a positive value if higher mobility values are found toward larger grain sizes, and a negative value if higher mobility values are found toward finer grain sizes (Garwood et al., 2013). Large positive values, therefore, indicate that coarser grains were preferentially resuspended, while large negative values indicate preferential resuspension of finer grains. S_I values close to zero indicate that sediment grain sizes were resuspended in proportion to their abundance in the seabed.

Average mobility distributions, as a function of diameter (μ m), were obtained from both the natural and bleached core duplicates. The sortability index of each distribution was calculated, and the index of natural cores was subtracted from the index of their corresponding bleached cores in order to perform a Wilcoxon signed-rank test (Randles, 1988). The Wilcoxon signed-rank test is a non-parametric paired-difference statistical test that was used to test whether the mean sortability index of bleached cores. All statistical analyses were conducted in Matlab.

2.5. Biological properties of the sediment

The bulk carbohydrate content of the sediment was measured

with the phenol–sulfuric method (Dubois et al., 1956), as modified by Sun et al. (1984). Samples were freeze-dried, ground and homogenized, and triplicate 10–15 mg subsamples each of the forty-two syringe cores (3 syringe cores for each 14 collection days) were used for analysis. Following reaction, samples were centrifuged at 21,000g for 10 min and optical density (350– 800 nm) in the supernatant was measured within a Cary 400 spectrophotometer. D-Glucose (Sigma Aldrich, St. Louis, MO, USA) was used as a standard and concentrations in the samples were calculated from optical density at the product peak (487 nm). To be consistent with the nomenclature in Tolhurst et al. (2005), bulk carbohydrate was expressed as carbohydrate content for units of mass per mass (mg Glucose Eq g⁻¹) and as carbohydrate concentration for units of mass per volume (mg Glucose Eq cm⁻³).

Pigment analyses were conducted using high performance liquid chromatography, following the methods of Wright et al. (1991), in order to ascertain the dominant biofilm taxa. The pigments were extracted in 100% acetone. chlorophylls *a* and *c*, and fucoxanthin. These pigments were interpreted as evidence of a diatom-dominated biofilm (Jeffrey and Vesk, 1997), as previously reported for the area (Daborn, 1991).

3.1. Sediment eroded

Sample mobility distributions for natural and bleached cores show that grains with diameters less than $\sim 10 \,\mu m$ were preferentially retained during erosion of natural sediment when compared to control (bleach-treated) cores (solid and dashed line respectively; Fig. 2). A Wilcoxon signed-rank test revealed that mobilities in natural cores were significantly different from mobilities in control cores only when eroded at surface stresses of 0.08 and 0.16 Pa (p=0.004 and p=0.008, respectively; Fig. 2). Data from October and November were excluded from this and subsequent analyses of sediment resuspension because virtually no erosion took place on natural cores, leading to sediment concentrations that did not meet the minimum coincidence threshold for the Multisizer 3 Coulter Counter (5–10%). In fact, during these months, the total mass eroded from one natural core was comparable to the mass of sediment eroded at the lowest shear stress in other months (mass eroded from 0.001 to 0.01 kg m⁻²). October and November were not, however, excluded from the surface grain

3. Results

The only pigments detected in the field samples were



Fig. 2. Comparison of mobility in natural and bleached cores. *Top*: Sample mobility as a function of diameter (μ m) for cores collected on May 26 (blue, n=4) and July 24 (red, n=4), 2012. The solid and dashed lines represent natural and bleached core, respectively. *Bottom*: Difference between the sortability index of natural sediment cores and bleached cores eroded from April to September 2012 (n=34). Negative values indicate that fine sediment was preferentially retained by biofilms. The gray lines show the sortability difference for each field day, the black line is an average of all field days, while the blue (circles) and red (diamonds) lines show the data for May 26 and July 24, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



Fig. 3. Average mobility in cores with and without a natural biofilm. *Top*: Average mobility of sediment grains as a function of diameter (μ m) for cores with a natural biofilm and for bleached cores (solid and dashed lines, respectively) eroded at (A) 0.08 and (B) 0.16 Pa (n=34). The shaded area represents the grain sizes preferentially retained by biofilms. *Bottom*: For comparison, similar average mobility plots were included for cores collected on a sandy intertidal flat (from Garwood et al., 2013) and eroded at (C) 0.24, (D) 0.32, and (E) 0.40 Pa (the shear stresses that produced a significant difference in the Garwood et al. (2013) study). Markers were added to the solid line to show the grain size bin mid-points, but were omitted for all other mobility plots.

size analysis.

The grain sizes for which mobilities in bleached cores exceeded those in the corresponding natural cores (shear stresses of 0.08 and 0.16 Pa) were isolated to identify more precisely the grain sizes preferentially eroded in bleached cores (shaded area in Fig. 3). The sediment preferentially resuspended from bleached cores covered a range of 1.00–6.06 μ m, when eroded at 0.08 Pa; and a range of 1.00–9.19 μ m at 0.16 Pa. The means of these ranges were calculated to be 2.4 \pm 0.4 and 3.3 \pm 0.7 μ m, respectively. When a Wilcoxon signed-rank test was performed, the means were found to be statistically distinct (*p*=0.02). This is in contrast with the overlapping ranges of the two means, but the Wilcoxon signed rank-test is a paired statistical test and thus accounted for any variation in surface grain size over time.

3.2. Field surface grain size

Surface DIGS spectra were obtained every collection day to identify any seasonal change in seabed texture. A time series of these measurements, as well as monthly-averaged DIGS spectra show surface sediments to be finer in May and June than in July, August and September 2012 (Fig. 4A–C). The coarsest sediment grain size distributions were observed in October, while surface sediments became finer once again in November, returning to textures similar to those observed in the spring. Based on Folk's classification (Folk, 1980), the flat can be described as silt for the duration of the sampling season, except for October, when seabed texture reached the lower limit of sandy silt (Table 1).

Given that biofilms on the tidal flat preferentially retained



Fig. 4. Sediment surface analysis. (A) Fine fraction ($\% < 10 \,\mu$ m) of disaggregated inorganic surface sediment over time, as mean \pm standard deviations of triplicate measurements (n=42). (B) Monthly average of surface DIGS (n=42). (C) Anomaly (value – mean) from volume (%) for all DIGS over time. The zero contour is shown in bold. D. Time series of the carbohydrate content (black, mg g⁻¹) and concentration (red, mg cm⁻³) as means \pm standard deviations over the entire study period (n=126, from 42 syringe core samples, subsampled 3 times).

Table 1

Grain size characterization of surface sediment. Consecutive months with similar average surface DIGS were grouped, as identified in Fig. 4. Mean values \pm standard deviation are shown, but note that sample size varies from one group to the other.

Months	$\% \leq 4\mu m$	% 4–10 μm	% 10–63 μm	$\% \ \geq 63 \ \mu m$	Folk's classification
May–June $(n=12)$ July–Sept. $(n=18)$ October $(n=6)$ November $(n=6)$	$\begin{array}{c} 21.6 \pm 3.2 \\ 16.2 \pm 2.1 \\ 13.9 \pm 3.0 \\ 23.3 \pm 6.0 \end{array}$	$\begin{array}{c} 19.1 \pm 1.8 \\ 14.5 \pm 1.2 \\ 12.9 \pm 1.8 \\ 19.7 \pm 4.3 \end{array}$	$\begin{array}{c} 56.7 \pm 4.4 \\ 63.4 \pm 1.9 \\ 62.7 \pm 3.0 \\ 53.3 \pm 6.8 \end{array}$	$\begin{array}{c} 2.5 \pm 0.9 \\ 5.9 \pm 2.2 \\ 10.4 \pm 2.0 \\ 3.6 \pm 3.7 \end{array}$	Silt Silt Silt/sandy silt Silt

sediment grains $< 10 \mu m$ (Fig. 3), the evolution of the fine fraction of the surface sediment (% $< 10 \mu m$, by volume) over time was investigated (Fig. 4A). Values for May, June and November were overall distinct from those from July to October, except for a transition day on November 6 where the large error bar is explained by one DIGS distribution similar to those in October. Using the *runstest* function in Matlab, it was found that the trend was not statistically random (p < 0.01), suggesting that the grain size anomaly trend was significant (Fig. 4C). The means of the two groups (May, June, November vs. July–October) were statistically distinct (*t*-test, p < 0.01).

Time series of the carbohydrate content (mg g⁻¹) and the surface grain size anomaly (value – mean, from volume %) showed covariance between surface sediment size spectra and carbohydrate content (Fig. 4C, D). The correlation between the fine volume fraction of the surface sediment (% < 10 μ m, by volume) and carbohydrate content (mg g⁻¹) was significant (Spearman's rank correlation, *r*=0.82, *p* < 0.001). However, there was no significant correlation (*p* > 0.05) between the fine volume fraction of the surface sediment and carbohydrate concentration (mg cm⁻³).

4. Discussion

Results from the field study showed that natural mud biofilms preferentially retained clays ($< 4 \mu m$) and cohesive very fine and fine silts (4–10 µm) (sensu McCave et al., 1995; Folk, 1980) at low shear stresses (0.08 and 0.16 Pa). Observations from this study are in contrast with results from Garwood et al. (2013) who found biofilms to preferentially retain fine and medium silts when sands were eroded at intermediate shear stresses (0.24, 0.32 and 0.40 Pa). van Ledden et al. (2004) argued that clay content (% $< 4 \,\mu$ m) of the sediment, as opposed to mud content (% $< 63 \,\mu$ m), is the best predictor of cohesive vs. non-cohesive sediment behavior. More precisely, sediment mixtures were found to behave cohesively when clay content exceeded a threshold of \sim 7.5%, and non-cohesively otherwise. Taken together, these results help refine the positive feedback proposed by van De Koppel et al. (2001). These authors described a positive feedback between sediment silt content and diatom growth, where diatom mats increased the silt content of the sediment via increased silt flocculation and retention, which then supported higher microbial growth due to enhanced nutrient availability. This feedback led to either silt- or sand-dominated environments, persisting in this state until sufficient silt was removed or supplied by physical factors. The results of the study presented here, however, suggest that biofilms display different behavior in muds and sands, but still support a positive feedback mechanism via clay (as opposed to silt) retention. The natural mud biofilms in this study retained finer particles than sand biofilms (Table 2), with sorting taking place at lower shear stresses in the former. These characteristics are effective for preserving sediment texture, as the grain sizes retained in mud make the substrate behave more cohesively, but those retained in fine sands do not (van Ledden et al., 2004; McCave and Hall, 2006). It is important to note that it remains uncertain exactly how the sediment behavior observed in cores transported to and eroded in

Table 2

Sediment and biofilm properties. For comparison, the sediment and biofilm properties of this study are shown with those from Garwood et al. (2013).

Folk's classification	Sand	Silt
Study % < 4 μm Biofilm Dominant organisms Shear stress with significant bio- film effect Average grain size retained by biofilm (μm)	$\begin{array}{c} \mbox{Garwood et al. (2013)}\\ 2.20 \pm 0.61\\ Natural\\ N/A\\ 0.24 \mbox{ Pa}\\ 0.32 \mbox{ Pa}\\ 0.40 \mbox{ Pa}\\ 213 \pm 3.7 \ (0.24 \mbox{ Pa})\\ 25.2 \pm 4.1 \ (0.32 \mbox{ Pa})\\ 19.5 \pm 3.8 \ (0.40 \mbox{ Pa}) \end{array}$	This study 18.43 \pm 3.63 Natural Diatom 0.08 Pa 0.16 Pa 2.3 \pm 0.4 (0.08 Pa) 3.3 \pm 0.7 (0.16 Pa)

the lab relates to in situ behavior. Nevertheless, the results were compared to a study that used an identical procedure, which reduces effects associated with factors other than the natural sediment.

Deflaun and Mayer (1983) observed that microorganisms in intertidal sediment were not found on grains smaller than 10 μ m, suggesting that the size-specific sediment retention observed in this study may not be due exclusively to direct bonding of particles, but instead to biofilm pore size, as suggested by Arnon et al. (2010). Rather than picturing biofilms as uniform mats, it may be more appropriate to view them as webs. Sediment texture may determine how closely the EPS strands of the biofilm are interwoven when microorganisms migrate around particles. Coarser sediment would then lead to larger pore sizes, and finer sediment to smaller pore sizes. Although both $1-\mu m$ and $5-\mu m$ particles were able to settle within the biological matrix in Arnon et al.'s (2010) experiment, the coarser ones were preferentially retained, potentially because their larger sizes made it more difficult to escape through the biofilm pores. Unlike Arnon et al.'s (2010) experiment, however, the grains eroded in this study likely were mainly resuspended as flocs, and not as individual grains. A similar reasoning still applies if fine sediment biofilms retained smaller flocs with smaller constituent grains than coarse sediment biofilms. This study addressed the size of constituent grains, but not the size of resuspended flocs. The floral community structure might also affect size-specific retention by biofilms because of consistent differences between the minimum dimensions of the dominant taxa, cyanobacteria and diatoms. Garwood et al. (2013) speculated that they observed preferential retention of fine and medium silts by biofilms on a sand flat because their uncharacterized biofilms were formed by cyanobacteria rather than by diatoms, but this remains to be tested.

Associations between surface sediment grain size and carbohydrates were stronger than those between surface sediment grain size and chlorophyll *a*, which is consistent with other studies of biofilm–sediment interactions (Grant et al., 1986; Daborn, 1991). Over the 8-month sampling period, surface sediments were finer when carbohydrate content was greater (Fig. 4), a correlation that may indicate a causal relationship between sediment texture and biofilm carbohydrate content in the sediment. The possibility that biofilm carbohydrate content was simply correlated with another compound in biofilms and that the latter was responsible for gluing down sediment grains cannot be rejected, but to simplify the discussion, the hypothesized mechanisms will be discussed with regards to carbohydrate content. Given that the mud biofilms were shown to preferentially retain fine sediments ($< 10 \,\mu m$), the accumulation of biofilm may have caused the accumulation of fine, cohesive sediment on the seabed. Under this scenario, carbohydrate content was high in the late spring because of elevated production and minimal grazing, by analogy with the spring bloom observed in temperate phytoplankton populations (Lima and Doney, 2004). The biofilm retained clays and very fine silts, leading to a fining of the surficial sediment. During the summer, carbohydrate content of the sediment decreased, perhaps due to active grazing of the biofilms. Reduced biofilm led to less retention of clays and very fine silts, causing a coarsening of the surficial sediment. In the fall carbohydrate content rebounded, again perhaps due to reduced grazing of the biofilms. The increased biofilm enhanced retention of clays and very fine silts and, again, caused fining of the surficial sediment. Finer sediments provided a better growth environment for diatoms, which increased production of biofilm carbohydrates, allowing the sediments to retain more fine sediment, which further improved growing conditions (van De Koppel et al., 2001). These hypothesized causal links between surface sediment texture and biofilm production are speculative and require more research to examine their validity.

Tolhurst et al. (2005) advocated the use of carbohydrate concentration (mass per volume) instead of carbohydrate content (mass per mass) when studying biofilm and sediment properties because sediment mass is used to measure carbohydrate content and, thus, the two measurements are covariant. Part of the correlation obtained between fine sediment and biofilms (as measured with carbohydrate content) can, therefore, likely be explained by this covariance. Nevertheless, results from this study provide direct evidence for fine sediment retention by biofilms, which would strengthen this correlation. At this time, the two contributions to the correlation cannot be separated. Biological properties, such as chlorophyll a, can vary over depth scales less than 1 mm (Kelly et al., 2001), which implies that the carbohydrate sampling depth in this study was quite coarse. Finer scale measurements, using a Cryolander for instance (Wiltshire et al., 1997), would be required to test the hypothesized scenario presented here and quantify the contributions of specific biofilm properties and floral community on fine sediment retention.

Although patterns in content and concentration can oppose each other (Perkins et al., 2003), the carbohydrate concentration and content measurements in this study were generally in agreement, except for a few data points. A decrease in carbohydrate concentration in late fall when the sediment fine fraction increased, and a peak in carbohydrate concentration in midsummer, when sediment fine fraction was relatively constant, combined to make the correlation between carbohydrate concentration and fine fraction not significant (Fig. 4). The stronger correlation between sediment fine fraction and carbohydrate content than between fine fraction and carbohydrate concentration also may have arisen because content and fine fraction have the same units (Flemming and Delafontaine, 2000). Carbohydrate content is a mass fraction. Because the Coulter Counter measures the volume of individual grains, the fine fraction was obtained with respect to the solid inorganic portion of the sediment only, and not with respect to the bulk sediment, preventing the conversion of the measurement to a concentration. Under the assumption that sediment grain density is not a function of grain size, fine fraction is proportional to sediment mass fraction and can be expressed as a content.

Considering that EPS strands are attached to and connect sediment grains (Grant et al., 1986; Taher and Abdel-Motelib, 2014), the concept of biofilms as a web at the sediment surface is consistent with the significant correlation between fine fraction and carbohydrate content. Surficial webs would grow denser primarily by constricting pore sizes, which would lead to better retention of fines. Assuming that the underlying sediment has ample storage space for fine sediments, the mass fraction of fines and the mass fraction of the biofilm web would correlate, regardless of the volume of the underlying sediment.

Low erosion rates were observed in October and November, but the cause of reduced erodibility in the fall is not clear. One possibility is that increased biofilms and associated carbohydrate levels provided additional stability, which is consistent with results from previous studies that suggested a biological mediation of seasonal stability patterns in temperate intertidal muds (e.g., Frostick and McCave, 1979). The carbohydrate levels in the fall were, however, comparable to those in the spring. It is, therefore, possible that other, unmeasured characteristics of the biofilm, such as hydration state, stickiness, or chemical composition, affected seabed erodibility more strongly. Reduced deposition and increased erosion due to fall storms also may have played a role as an older and more compacted surface would be more difficult to erode. The HPLC analysis did not show a consistent increase in chlorophyll degradation products (chlorophyllide, phaeophytin and phaeoforbide) over the sampling period, which suggests that the biofilm was of recent formation rather than a relict horizon. These results suggest that erosion timescales were shorter than those associated with biofilm growth, but further research is necessary to better evaluate the cause of reduced erodibility in the fall.

5. Conclusions

Intertidal mudflat biofilms preferentially retained clays (< 4 μ m) and cohesive silts (4–10 μ m) when subjected to low erosion shear stresses (0.08 and 0.16 Pa). These results are in contrast with intertidal sand biofilms that were previously reported to preferentially retain fine and medium silts when subjected to moderate erosion shear stresses (0.24–0.40 Pa, Garwood et al., 2013). An association between carbohydrate and fine content (% < 10 μ m) in the surface sediment was also identified throughout the 8-month sampling period. This association is consistent with mud biofilms retaining finer grains and influencing sediment texture but the causal links remain speculative. Because the grain sizes retained in muds contribute more to cohesion than those retained in fine sands, a positive feedback between size-specific sediment retention by biofilms and seabed texture likely contributes to preserving sediment texture.

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