



Interactions between sediment microbial ecology and physical dynamics drive heterogeneity in contextually similar depositional systems

J. A. Hope^{1,2*}, J. Malarkey^{3,4}, J. H. Baas³, J. Peakall⁵, D. R. Parsons⁴, A. J. Manning^{4,6,7,8}, S. J. Bass⁶,
I. D. Lichtman⁹, P. D. Thorne⁹, L. Ye^{4,8}, D. M. Paterson¹

¹Sediment Ecology Research Group, School of Biology, University of St Andrews, St Andrews, UK

²Institute of Marine Science, University of Auckland, Auckland, New Zealand

³School of Ocean Sciences, Bangor University, Bangor, UK

⁴Energy & Environment Institute, University of Hull, Hull, UK

⁵School of Earth and Environment, University of Leeds, Leeds, UK

⁶School of Biological and Marine Sciences, University of Plymouth, Plymouth, UK

⁷HR Wallingford, Wallingford, UK

⁸Department of Civil and Environmental Engineering, Center for Applied Coastal Research, University of Delaware, Newark, Delaware

⁹National Oceanography Centre, Liverpool, UK

Abstract

This study focuses on the interactions between sediment stability and biological and physical variables that influence the erodibility across different habitats. Sampling at short-term temporal scales illustrated the persistence of the microphytobenthos (MPB) biomass even during periods of frequent, high physical disturbance. The role of MPB in biological stabilization along the changing sedimentary habitat was also assessed. Key biological and physical properties, such as the MPB biomass, composition, and extracellular polymeric substances, were used to predict the sediment stability (erosion threshold) of muddy and sandy habitats within close proximity to one another over multiple days, and within emersion periods. The effects of dewatering, MPB growth, and productivity were examined as well as the resilience and recovery of the MPB community after disturbance from tidal currents and waves. Canonical analysis of principal components (CAP) ordinations were used to visualize and assess the trends observed in biophysical properties between the sites, and marginal and sequential distance-based linear models were used to identify the key properties influencing erodibility. While the particle size of the bed was important for differences between sites in the CAP analysis, it contributed less to the variability in sediment erodibility than key biological parameters. Among the biological predictors, MPB diversity explained very little variation in marginal tests but was a significant predictor in sequential tests when MPB biomass was also considered. MPB diversity and biomass were both key predictors of sediment stability, contributing 9% and 10%, respectively, to the final model compared to 2% explained by grain size.

Variability in response to physical forcing is an inherent property of natural systems (Black et al. 2002) and represents a significant challenge for modeling and predicting the behavior of natural sediment beds. It is also commonly suggested that an important source of this variability, biogenic stabilization (Tolhurst et al. 2009; Parsons et al. 2016), is largely confined

to fine cohesive sediments (mud flats) rather than more sandy substrata. However, this approach neglects the heterogeneous composition of natural beds that vary both spatially and temporally (Rainey et al. 2003; Chapman et al. 2010). Understanding the biogenic processes that generate variation and heterogeneity in natural systems will support our ability to model system behavior more accurately in the future.

Biological stabilization of cohesive (muddy) sediment is often attributed to microbially produced extracellular polymeric substances (EPS) that increase the cohesion between sediment particles, and forming biofilms (Hubas et al. 2018). The production of EPS is typically attributed to bacteria and microphytobenthos biomass (MPB) (Lubarsky et al. 2010; Chen et al. 2017). While MPB are more abundant on cohesive

*Correspondence: julie.hope@auckland.ac.nz

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

Additional Supporting Information may be found in the online version of this article.

sediments, recent studies show that microbially produced EPS can also hinder bedform development and inhibit erosion in noncohesive (sandy) (Malarkey et al. 2015; Chen et al. 2017) and mixed sediment beds (Parsons et al. 2016). The balance between physical disturbance (hydrodynamic stress) and bed erodibility is complex (Beninger et al. 2018). Regular physical forcing can restrict the accumulation of fine sediment and MPB on the bed (Mariotti and Fagherazzi 2012) preventing the MPB standing stock from developing fully (Blanchard et al. 2001). However, once developed, after a period of calm conditions, increasing erosive stress may be resisted. The biomass and nature of the MPB, epipellic (e.g., Paterson and Hagerthey 2001) or epipsammic (e.g., Harper and Harper 1967; Hickman and Round 2007), will contribute to the variability of the response to stress.

If a system is largely abiotic, then the introduction of biota can create greater heterogeneity or homogenize the system. For example, greater microphytobenthic diversity has been linked to higher grazer diversity (Balvanera et al. 2006) which through differences in bioturbation can increase habitat heterogeneity (Hale et al. 2015). Furthermore, patchy biofilm distribution and growth have been associated with positive biophysical feedbacks as the system becomes inherently more patchy leading to spatial self-organization and more fine sediment accretion and eventually influencing large geomorphological features (Weerman et al. 2010). However, the introduction of biota can also cause different sediments to become more similar to one another. For instance, the presence of large infauna can “smooth out” the effects of flow on sediment resuspension across different sediment types (Li et al. 2017). The former processes of increasing heterogeneity suggest that microbial growth and EPS accumulation can not only have a localized effect, but if growth becomes extensive, biostabilization is capable of influencing ecosystem functionality at various spatial and temporal scales (Orvain et al. 2012; Ubertini et al. 2015) and although variability may increase (Chapman et al. 2010) this can have system-wide implications. Many studies have focused on seasonal and interannual variability (Montani et al. 2003; van der Wal et al. 2010). However, the mechanisms that drive changes to the structure of the system (van de Koppel et al. 2001) and resilience and recovery from disturbance can occur on relatively short spatial (Spilmont et al. 2011) and temporal scales (Orvain et al. 2012). Furthermore, the variability observed from small spatial scales over short timeframes in intertidal environments can be of the same order of magnitude as both seasonal and annual variability (Seuront and Leterme 2006). The importance of considering temporal scales has been highlighted in previous soft sediment studies (Tolhurst et al. 2005a; Hewitt et al. 2007). However, short-term temporal dynamics that may influence EPS accumulation, biofilm development, and biostabilization have not been well characterized across different sediment types. The development of biofilms depends on the balance between growth and detachment,

with hydrodynamic stress being a primary driver of benthic biofilm detachment (Telgmann et al. 2004). We therefore require further information on the interactions between biofilm properties, biostabilization, hydrodynamic stress, and subsequent resistance to erosion over multiple emersion periods and within different habitats. This information is essential to assess the role of biostabilization, both from ecological and dynamic perspectives (de Brouwer et al. 2000; Underwood and Paterson 2003; Mariotti and Fagherazzi 2012). MPB influence on sediment stability and this key ecosystem function augments their important roles in; the transfer of energy to higher organisms (MacIntyre et al. 1996); the benthic-pelagic exchange of sediment (Chen et al. 2017); and nutrient cycling (McGlathery et al. 2004). The importance of MPB in these ecosystems highlights the need to understand the dynamics governing their presence across different habitats. Frequent resuspension of MPB cells and related EPS may prevent the formation of substantial biofilms, and therefore limit their biostabilization potential (Aspden et al. 2004); however, an “inoculum” often remains in place (Chen et al. 2017) leading to rapid recolonization under suitable conditions.

We hypothesize that biofilm properties such as MPB biomass, colloidal carbohydrate concentrations, and the MPB diversity will influence biostabilization of various sediment types. Furthermore, we hypothesize that the biogenic influence will persist over various temporal scales (emersion on consecutive days), as the microphytobenthic community and biotic characteristics tolerate regular, high intensity tidal inundation. As laboratory experiments cannot generally capture the natural variability in large, complex, and dynamic marine systems (Tolhurst et al. 2009), we examined these short-term dynamics in closely associated muddy and sandy habitats in the Dee Estuary, England. This estuary is subject to strong current velocities and frequent wave action, resulting in turbid waters with a high suspended load (Amoudry et al. 2014), but has various sedimentary habitats in close proximity to one another, making it an excellent model system. Suspended sediment often affects water quality, which limits light availability for sediment dwelling photosynthetic organisms during tidal inundation (Pratt et al. 2014), and the physical disturbance from flow itself may prevent the accumulation of EPS and biofilm development on the bed (Blanchard et al. 1997; Ubertini et al. 2015).

Methods

Study sites and sample collection

The Dee is a hypertidal estuary located on the border between England and Wales in the Eastern Irish Sea. The estuary is tidally dominated with a mean spring tidal range of 7–8 m (Moore et al. 2009). The geomorphology of the flats causes a tidal asymmetry that is flood dominated, resulting in significant accretion of fine sediments in the upper estuary (Halcrow 2013). Three sites were selected between Hilbre

Island and West Kirby (Fig. 1), based on the geomorphology of the bed surface at the sampling time. The first site (sandy 1) was dominated by noncohesive sediment (sand) with wave-influenced 2D current ripples. Site 2 (sandy 2) was similar but had active 2D and 3D ripples. The third site (muddy) was composed of muddy sand, with either a flat bed or relict current ripples (Lichtman et al. 2018).

Surface sediment samples were collected at four time points during tidal exposure over 3 d at each site from 23rd to 31st May 2013 (sandy 1: 23rd–25th, sandy 2: 26th, 28th–29th; and muddy: 28th–29th and 31st May). A full description of the physical conditions during the campaign at the adjacent sites can be found in Lichtman et al. (2018) and the Supporting Information. In brief, sampling dates at sandy 1 coincided with the tides transitioning from neaps into peak springs and there was also increased wave action due to high winds on 23rd–24th May. Despite the strong wave action at sandy 1, which caused the maximum wave-current bed shear stresses during wave cycles to be larger, the peak current bed shear stresses during inundation were greater at sandy 2 than sandy 1. Slightly weaker currents were observed at the muddy site on day 3, as the tides moved from peak springs toward neaps. Nonetheless, comparable maximum depth-averaged flood/ebb currents were measured across all sites (0.4 – 0.7 m s⁻¹) and the maximum water depth at each site ranged between 2 and 3.3 m (Supporting Information Table S1).

At each site, 20 surface sediment samples were collected each day ($n = 5 \times 4$ time points). The first sampling occurred 30–60 min after sediment exposure each day with sampling repeated quarterly during low tide until 60 min before inundation. Samples were collected within 5 m of rigs deployed by NOC Liverpool (Lichtman et al. 2018) and University of Plymouth across an area of approximately 3 m². Sediment cores (2 mm depth, surface area = 250 mm²) were frozen and stored in liquid nitrogen using the contact core method described in Ford and Honeywill (2002) and Brockmann et al. (2004). Cores were subsequently stored frozen (-80°C) in the dark until processed. To capture both epipelagic and epipsammic microalgal cells, replicate surface scrapes ($n = 5$, $10 \times 10 \times 2$ mm depth) were collected and stored in 2.5% w/w glutaraldehyde/filtered seawater solution from time point 2 (T2) only.

Sample processing

Water content (%) was calculated from wet and freeze-dried core weights before sediment organic matter (%) was determined by loss-on-ignition at 450°C for 4 h. Chlorophyll *a* (Chl *a*) pigments were extracted with 90% acetone following the trichromatic method of Jeffrey and Humphrey (1975). The colloidal and total carbohydrate fractions of the EPS were determined using the phenol-sulfuric acid assay (DuBois et al. 1956) following Underwood and Paterson (2003). Due to differences



Fig. 1. The location of the sampling sites on the intertidal flats near West Kirby and Hillbre Island. Inset—position of the Dee Estuary, near West Kirby Liverpool, England.

in contents vs. concentrations caused by the varied water content of sediment samples, both chlorophyll and carbohydrate measurements are expressed as concentrations per unit area (mg m^{-2} ; Tolhurst et al. 2005b). The effective particle size distribution (PSD; Grabowski et al. 2012) was determined using a Malvern Mastersizer 2000 laser diffraction analyzer (Malvern Instruments Ltd, 2013) and summarized using GRADISTAT software (Blott and Pye 2001) prior to statistical analysis with D_{50} and mud content ($\% < 63 \mu\text{m}$) used for further analysis. The relative difference in erosion threshold required to suspend a user-defined sediment concentration of 0.01 kg m^{-3} was measured using the portable in situ cohesive strength meter (CSM; Paterson 1989; Tolhurst et al. 1999). In addition to the surface erosion threshold, the undrained shear strength was measured using a 33 mm Pilcon shear vane (5 cm depth).

Microphytobenthic community composition

Microphytobenthic cells were extracted from sediment scrapes by adopting a modified isopycnic separation technique using silica sol Ludox TM-40[®] (Ribeiro et al. 2013). Diatom identification, by means of light microscopy (Zeiss Universal light microscope, phase and a Ph3-NEOFLUAR oil immersion objective $\times 100$ coupled to a 1.0 and 2.0 optivar), followed Hendey (1964), Hartley et al. (1996), and Round et al. (1990). Cells were identified to species level where possible and grouped into three ecological guilds (Passy 2007): (1) “motile” (fast moving and larger); (2) “low profile” (short stature, prostrate, adnate erect, and slow-moving species); and: (3) “high profile” (tall stature, erect, filamentous branched or chain-forming and colonial centrics, largely nonmotile or motile within tubes).

Low temperature scanning electron microscopy

Fragments of contact core samples, frozen in liquid nitrogen (-196.8°C , 1 atm), were mounted on mechanical stubs and examined using a JOEL 35CF SEM fitted with a low temperature scanning electron microscopy (LTSEM) (Oxford Instruments CT 1500B) following the procedure given in Paterson (1995).

Statistical analysis

Analyses were performed using “R” statistical software, version 3.1.1 (R Development Core Team 2013) through the R studio graphical interface (v. 0.98.1083) and in PRIMER software (V.6, PRIMER-E, Ivybridge, UK). Differences in biophysical variables were determined between sites, emersion times, and days, after assumptions were tested (Zuur et al. 2007; Pinheiro et al. 2012). Necessary transformations were applied to conform to assumptions for parametric statistical testing (ANOVA), where possible or nonparametric Kruskal–Wallis (H) tests were employed. Corresponding post hoc Tukey’s or Dunn-sidak tests were applied to detect differences between specific groups. The relationships between the different sediment properties and stability measurements were also assessed

using Spearman rank correlations. No significant differences were observed across the different timepoints during emersion for stability or biochemical properties, therefore timepoints were pooled resulting in 20 replicate samples from each day and site. Multivariate analysis of the data using canonical analysis of principal components (CAP) was employed on square-root transformed data to assess the response of multiple biophysical variables across the sites using constrained ordination taking account of the correlation structure of data (Andersen and Willis 2003).

The MPB community composition between the sites and days was also examined using CAP, based on Bray–Curtis dissimilarity matrices (Sommerfield 2008). Differences in species between sites and days were tested using permutational multivariate analysis of variance in addition to exploring Shannon’s diversity index (H') and Pielou’s evenness index (Magurran 2004).

To determine whether the variation in sediment erosion thresholds could be explained by differences in the measured biophysical properties of the sediment across all sites, data were pooled and distance-based linear models (DistLM) were employed (Anderson et al. 2008). Temporal factors (time since emersion and sampling day) were included as explanatory variables along with the various biophysical properties. Marginal and sequential tests were examined using Akaike’s information criterion (AICc) and a backward elimination process to identify the best combination of predictors, that maximized the explained variation with the most parsimonious model (Clarke and Gorley 2006).

Results

Sediment bed properties and stability

The percentage of mud ($< 63 \mu\text{m}$) was significantly higher at the third site (Muddy site, Table 1; 27%, $H_{(2)} = 93.93$, $p < 0.001$) compared to the sandy sites but there was no significant difference in mud content between the two sandy sites (0.8% and 1%). For the sandy sites, clean particles were visually observed with very little associated organic matter (Fig. 2A,B) and the grain size distributions were similar (Supporting Information Fig. S1). In contrast, the muddy site exhibited more varied and organic-rich sediments (Fig. 2C,D), but total organic content was relatively low across all sites ($< 2\%$) and no significant site differences were detected. The water content, colloidal carbohydrate, and Chl *a* concentrations were all significantly higher at the muddy site compared to both sandy sites (all $p < 0.001$, Table 1). Differences were between all three sites, with higher contents and concentrations at the muddy site, followed by sandy site 2 and then sandy site 1. The shear strength of the bed was also significantly different between sites, yet this was due to lower strength at sandy site 2, as strengths were similar between sandy site 1 and the muddy site ($p > 0.05$).

At sandy 1, both the colloidal carbohydrates (EPS) and erosion thresholds varied significantly over the sampling days

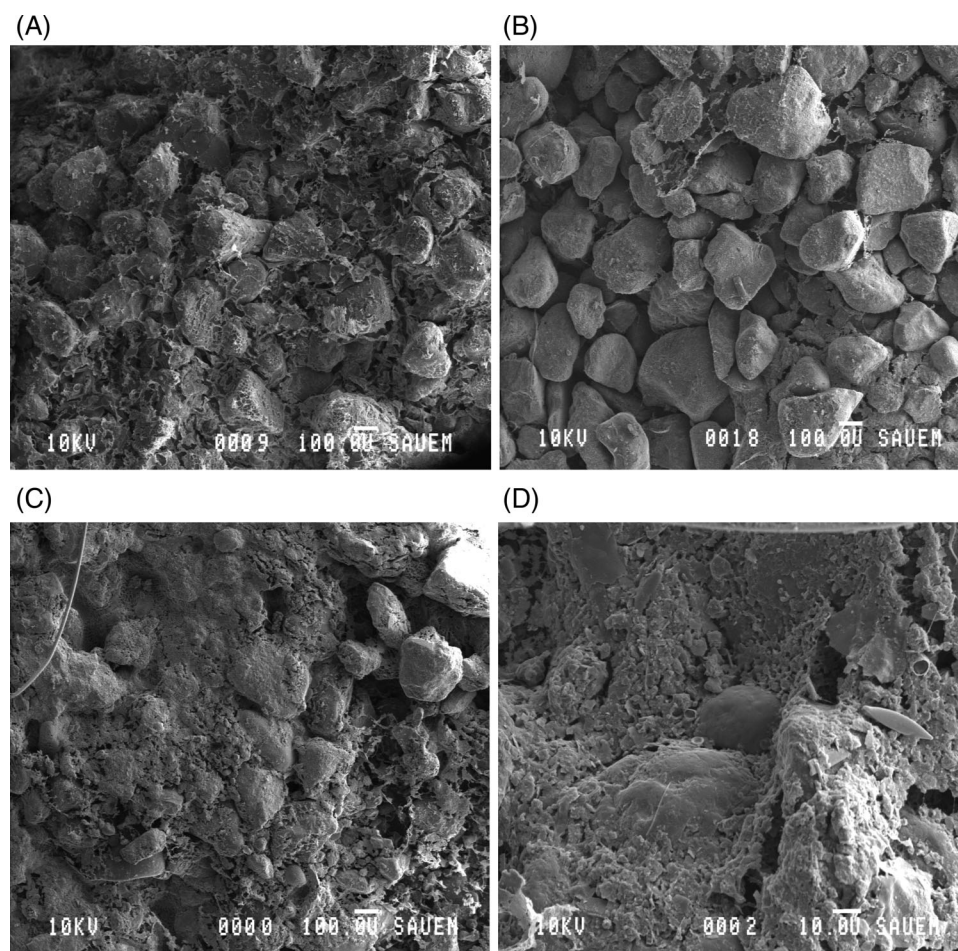


Fig. 2. Low temperature scanning electron micrographs of the intact sediment surface at (A) sandy site 2, crest of ripples, (B) sandy site 2, troughs, (C) muddy site, general surface, and (D) muddy site, close-up image of organic material between sediment grains. Scale bars: 100 μm for (A–C) and 10 μm for (D).

($H_{(2)} = 27.12$, $p < 0.001$ and $H_{(2)} = 13.76$, $p < 0.001$) but with opposing trends (Table 1). Colloidal carbohydrates were lowest on day two when the erosion threshold was highest, with a decrease in threshold coinciding with an increase in mud and organic content. Overall, the erosion measurements at sandy 1 correlated very poorly with all measured biological and physical variables, but there was a negative relationship between Chl *a* concentration (MPB biomass) and the erosion threshold at this site over the sampling days. At the second site (sandy 2), the erosion threshold decreased over the sampling days to its lowest on day 3 (1.8 kPa). This was alongside significant reductions in colloidal carbohydrate concentrations from $320 \pm 115 \text{ mg m}^{-2}$ (day 1) to $229 \pm 54 \text{ mg m}^{-2}$ (day 3, Fig. 3; $F_{2,59} = 6.57$, $p < 0.001$). At the muddy site, the erosion threshold was at its highest on day 1 (14.5 kPa) and the lowest on day 2 (2.7 kPa) also coinciding with the lowest colloidal carbohydrate concentration ($428 \pm 110 \text{ mg m}^{-2}$, $p < 0.001$) and the strongest wave action (Lichtman et al. 2018).

The D_{50} of the bed varied very little between days at sandy 1 (Table 1), although there was a small but statistically significant difference at sandy 2, D_{50} increasing from $202 \pm 3 \mu\text{m}$ on day 1 to $213 \pm 10 \mu\text{m}$ on day 3 ($H_{(2)} = 25.88$, $p < 0.001$). No significant differences in D_{50} were detected at the sites during different emersion points measured (data not presented).

Microalgae community analysis

Significant spatial and temporal differences were detected in the microalgae community across sites, as well as differences between individual days within the sites ($p < 0.01$). Diversity (H' index) and evenness (Pielou's index) were significantly different between sites ($F_{2,24} = 4.91$, $p < 0.05$) but differences were relatively small (H' at sandy 1 = 3.14, sandy 2 = 3.32 and muddy site = 3.62, and Pielou's index sandy 1 = 0.92, sandy 2 = 0.87 compared to muddy site = 0.94). A few cosmopolitan species such as *Nitzschia frustulum* var. *inconspicua* were present in almost all samples, across all sites, with *Achnanthes*

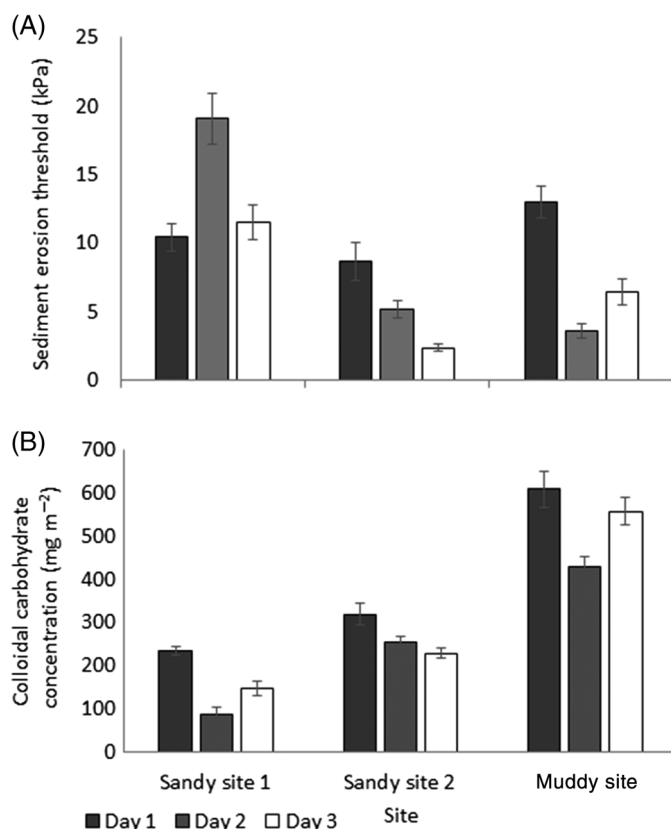
Table 1. Temporal variation in the mean/median values of biological and physical measurements at the sandy site and muddy site for all days combined and then each individual day of sampling. Top number = Mean \pm Standard deviation or Median (Interquartile range).

	Colloidal carb conc. (mg m ⁻²)	Chl <i>a</i> conc. (mg m ⁻²)	Water content (%)	Organic content (%)	D ₅₀ bed (μm)	Mud content bed (%)	Sed. erosion threshold (kPa)	Shear strength (kPa)
Sandy site 1	159 ± 87	13 ± 3	18 ± 1	1.5 ± 1.1	223 ± 6	0.8 ± 1.8	12.1 (8.8–17.5)	14.5 (8.9–16.7)
23 rd (D1)	235 ± 41	16 ± 2	18 ± 1	0.8 ± 0.3	222 ± 5	0.0 ± 0.0	11.0 (8.8–14.8)	15.0 (14.1–16.0)
24 th (D2)	88 ± 67	10 ± 2	18 ± 1	2.8 ± 2.8	221 ± 7	1.7 ± 2.2	18.6 (13.2–25.2)	14.0 (12.0–15.5)
25 th (D3)	147 ± 74	13 ± 2	18 ± 1	0.8 ± 0.2	225 ± 3	1.3 ± 1.9	11.0 (6.6–17.0)	14.5 (8.9–16.7)
Sandy site 2	269 ± 88	26 ± 4	19.9 ± 0.9	0.8 ± 0.2	204 ± 9	1.0 ± 2.0	4.4 (1.8–6.7)	6.8 (5.5–12.5)
26 th (D1)	320 ± 115	28 ± 3	20.4 ± 0.8	1.0 ± 0.9	202 ± 3	2.1 ± 2.6	6.6 (2.2–14.8)	6.2 (6.0–7.0)
28 th (D2)	255 ± 59	23 ± 4	20.1 ± 1.2	0.7 ± 0.2	197 ± 4	0.0 ± 0.0	4.9 (2.2–9.0)	6.5 (6.1–7.9)
29 th (D3)	229 ± 54	26 ± 3	19.6 ± 0.5	0.9 ± 0.1	213 ± 10	1.9 ± 2.4	1.8 (1.8–1.8)	10.2 (7.1–11.4)
Muddy site	532 ± 165	44 ± 11	21.0 ± 1.3	1.4 ± 0.5	156 ± 23	27.0 ± 7.0	5.4 (3.3–11.5)	13.2 (9.0–18.0)
28 th (D1)	609 ± 186	44 ± 14	21.6 ± 1.2	1.5 ± 0.4	140 ± 19	30.8 ± 5.0	14.5 (7.7–23.4)	14.0 (10.5–1.2)
29 th (D2)	428 ± 110	40 ± 8	21.1 ± 1.5	1.3 ± 0.4	159 ± 15	27.1 ± 3.9	2.7 (1.8–5.0)	13.2 (11.1–14.9)
31 st (D3)	557 ± 141	47 ± 9	21.0 ± 1.3	1.6 ± 0.6	169 ± 24	22.8 ± 8.2	5.5 (2.7–8.8)	13.0 (11.7–13.5)

punctulata present in greater numbers at the sandy sites. There was a greater abundance of low profile than motile species at the sandy sites and there were no high-profile species noted. Interestingly, the muddy site had a similar average abundance of low-profile epipelagic species to that of the sandy sites, had fewer small epipsammic cells, but had greater numbers of motile and high-profile species that dominated this site. The high proportions of *Navicula gregaria* (4%), *Amphora coffeaeformis* var. *perpusilla* (4%), and *Pleurosigma aestuarii* (4%) at the muddy site appeared to have the greatest effect on site differences in community composition (Fig. 4), whereas other smaller species such as *Opephora mutabilis* (4%) and *Cocconeis* sp 1 (4%) were more abundant at the sandy sites. Diversity and evenness did not change over time at the sites, but the abundance and turnover of key species did vary. At sandy 2, a decline in *Opephora mutabilis* contributed 9.3% to the overall dissimilarity (42%) between days while the decline in the large motile *Navicula digitoradiata* and *Pleurosigma aestuarii* (6% and 5%, respectively) were the greatest contributors to the overall dissimilarity (46%) between days at the muddy site.

Biophysical influences on erodibility

Inspection of the CAP plots (Figs. 5, 6) and the resulting trace statistic ($p < 0.001$) confirmed a strong overall difference between the sites based on biophysical properties. The first axis of the plot (CAP1) was partitioned between several biological variables relatively evenly, including Chl *a*, both carbohydrate fractions, the diversity and number of MPB species present, and water content. Together these variables and the D_{50} of the bed, which exhibited a strong anticorrelation to the biological properties, dominate this axis. On the second axis (CAP2), the D_{50} of the bed and the undrained shear strength were important factors. A clear spatial separation was observed

**Fig. 3.** Biological and physical measurements from intertidal sediments of the Dee Estuary. (A) Mean (\pm SE, $n = 20$) sediment erosion thresholds (kPa) for the three sites over three sampling days; Sandy site 1 on 23rd, 24th, and 25th May 2013. Sandy site 2 on 26th, 28th, and 29th May 2013. Muddy site on 28th, 29th, and 31st May 2013. (B) Mean (\pm SE, $n = 20$) colloidal carbohydrate concentrations (mg m⁻²), from the same sites and days.

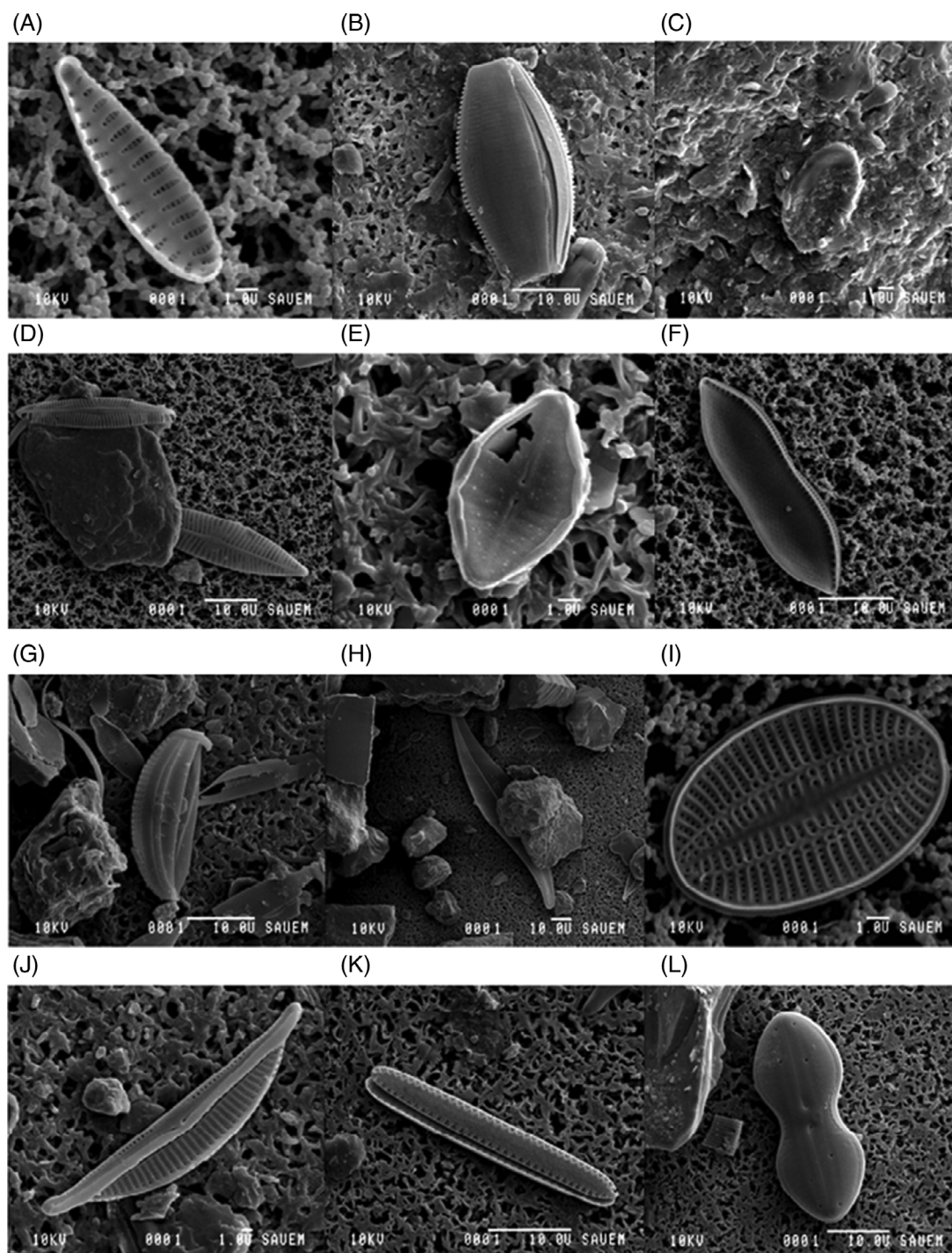


Fig. 4. Low-temperature scanning electron micrographs of diatoms harvested from the surface sediment of the Dee Estuary. Scale bars = 1 µm/10 µm where stated. (A) *Opephora mutabilis* (Grunow), (B) *Epipsammic* sp 1, (C) *epipsammic* cell embedded in a sediment particle in a matrix of EPS, (D) *Navicula gregaria* (Donkin), (E) *Planothidium haukiana* (Grunow), (F) *Nitzschia* sp 1, (G) *Amphora coffeaeformis* var. *coffeaeformis* (Agardh) Kützing, (H) *Pleurosigma aestuarii* (Brébisson ex Kützing) and several small *epipsammic* cells/sediment particles, (I) *Cocconeis peltoides* (Hustedt), (J) *Amphora tenerrima* (Aleem & Hustedt), (K) *Thalassionema* spp. (Grunow), and (L) *Diploneis* spp.

from the superimposed scatter plot (Fig. 5) as well as a temporal component based on draining throughout the emersion period. The close relationships between several variables in

the CAP plots were in agreement with correlation analysis (Table 2). As there were no clear dominant biological or physical factors, the majority of properties within both axes were

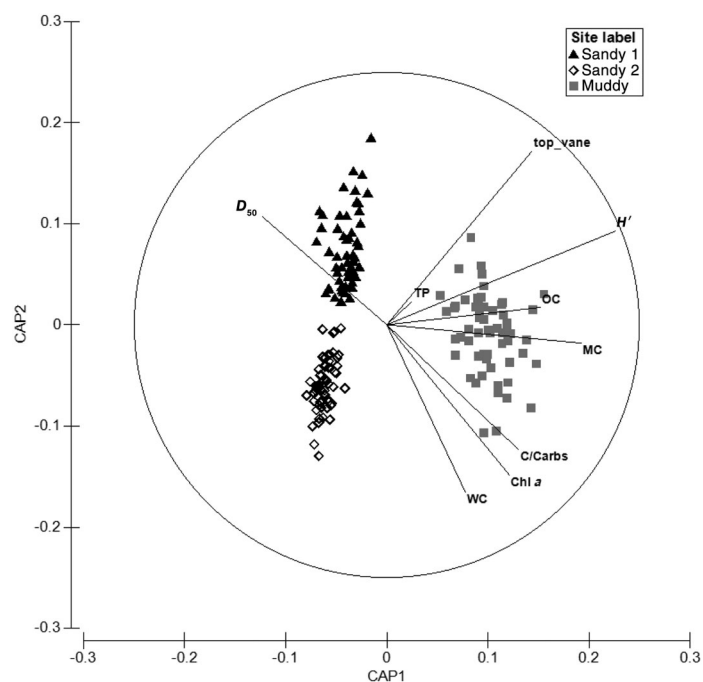


Fig. 5. Canonical analysis of principal components plot of Euclidean distance similarities between samples. The correlation circle overlays measured variables that were influencing the similarity/dissimilarity between the samples. All data were square-root transformed and normalized prior to analysis. $n = 60$. D_{50} , D_{50} of the PSD; top_vane, undrained shear strength; TP, timepoint since emersion; H' , Shannon diversity index; OC, organic content of sediment (%); MC, mud content of sediment (%); C/carbs, colloidal carbohydrate concentrations; Chl a , Chlorophyll a concentrations; WC, water content (%).

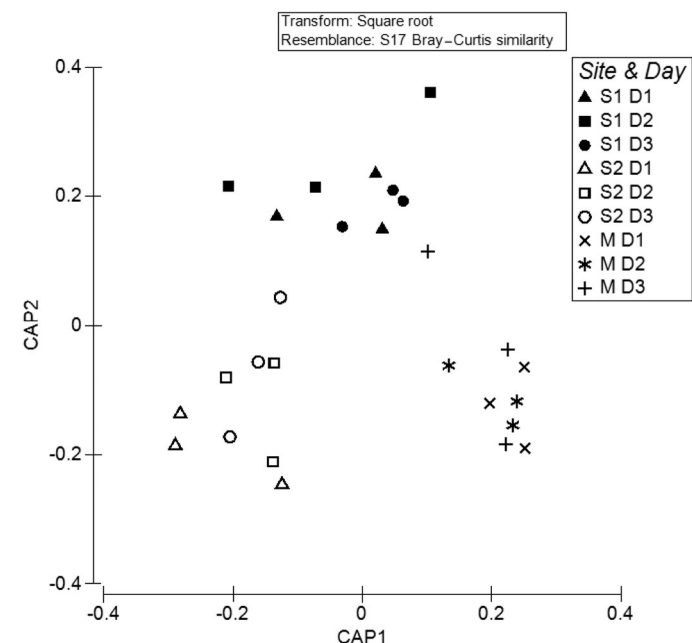


Fig. 6. Canonical analysis of principal components plot of Bray-Curtis similarities in the microalgae community composition of the sediment surface between the two sandy sites (S1 and S2) and the muddy (M) site over 3 d at the Dee Estuary.

retained for further exploration. Water content was highly correlated to several variables (Table 2) and there were no significant effects of dewatering detected from within each tidal exposure period. Therefore, water content was removed from further models. Various single and sequential predictor variables significantly explained the variation in the sediment erodibility across all sites in DistLM (Table 3). When properties were considered individually, both Chl a and organic content significantly explained the greatest variation (at 9% and 8%, respectively). While Chl a exhibited a negative effect across all sites, this was primarily driven by the negative relationship at sandy 1, and although MPB diversity and abundance were not good single predictors, they were valuable in sequential tests after consideration of the MPB biomass estimates (Chl a concentration). While the D_{50} was marginally insignificant in both marginal and sequential tests, it was important to retain in the latter yet surprisingly the mud content of the sediment was not selected as a good predictor of erosion threshold across the sites.

Discussion

Our results illustrate that the MPB maintain a key role in sediment dynamics, by surviving harsh environmental conditions, and quickly re-establishing biostabilization. MPB continued to exert an influence on a key ecosystem function; sediment stability across different sediments. Mariotti and Fagherazzi (2012) proposed that, given equal intensities of disturbance, the biomass of a biofilm determines whether or not it will be eroded and our results support this. Importantly, our results suggest that this biostabilization can exert influence on different sediment types. In energetic environments, the frequent turnover and reworking of the sediment may be expected to remove biofilms, hence these habitats are often depicted as abiotic (Fig. 7). In very energetic systems, like our sandy site 1, the formation of a fluffy biofilm or layer of cells and EPS may not have created a stable matrix and therefore leads to a “low biostabilization” scenario. The lack of incorporation into the bed explains the negative relationships between key biofilm properties and sediment erosion measurements observed at sandy 1. However, as grain size was reduced and mud content increased, even slightly, this positively influenced sediment stability, promoting stronger relationships between the biochemical properties of the biofilm and sediment stability at sandy 2 and the muddy site.

While frequent resuspension of MPB cells and related EPS may prevent the formation of substantial biofilms, and therefore limit their biostabilization potential (Aspden et al. 2004), an inoculum often remains in place (Chen et al. 2019) and this can still exert biostabilizing effects on the sediment as we have illustrated. This persistence of the biofilm and its stabilizing properties means that a biofilm can develop rapidly, if conditions become favorable (see Fig. 7; Chen et al. 2019). Previous studies of relationships between EPS carbohydrates and

Table 2. Spearman rank correlation coefficients for all variables within and across sites. First number = sandy 1, second = sandy 2, Third = muddy site, bottom number (bold) = all sites.

	Colloid carbs (mg m ⁻²)	Chl <i>a</i> (mg m ⁻²)	Water content (%)	Organic content (%)	<i>D</i> ₅₀ (μm)	Mud content (%)	Erosion threshold (kPa)	Undrained shear strength (kPa)
Colloid carbs (mg m ⁻²)	-							
Chl <i>a</i> (mg m ⁻²)	0.62***	-						
	0.64***							
	0.57***							
	0.83***							
Water content (%)	-	0.27*	-					
	-	0.33**						
	0.44***	0.59***						
	0.52***	0.62***						
Organic content (%)	-	-	-0.36**	-				
	-	-	-	-				
	0.34**	0.40**	0.43***					
	0.65***	0.68***	0.42***					
<i>D</i> ₅₀ (μm)	-	-	-	-	-			
	-	-	-	0.39**				
	-	-	-0.26*	-				
	-0.70***	-0.62***	-0.50***	-0.55***				
Mud content (%)	-	-	-	-	0.42***	-		
	-	-	-	-	0.44***			
	-	-	0.25*	-	-0.94***			
	0.72***	0.69***	0.47***	0.63***	-0.85***			
Erosion threshold (kPa)	-	-0.43***	-	-	-	-	-	
	0.35**	-	-	-0.31*	-	-		
	0.28*	-	-	-	-0.26*	-		
	0.35***	0.18*	0.20*	-	-0.29**	0.21*		
Undrained shear strength (kPa)	-	-	-0.3*	-	-	-	-	-
	-	-	-0.61***	0.30*	0.36**	-	-0.4**	
	-	-	-	-	-	-	-	
	0.65***	0.57***	0.20*	0.68***	-0.64***	0.72***	-	

Significant levels: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. - = no significant correlation detected. $N = 60$ per site.

sediment stability have estimated that 2–3 d are required (Lundkvist et al. 2007). However, as we have illustrated in situ that growth does not begin anew at the start of each tidal cycle and biofilms present across different sediment habitats, although invisible, maintain their biostabilization potential. The stabilizing effects of MPB may therefore take less time to develop and become more significant in the natural environment (Chen et al. 2017).

At the sandier sites, the fine sediment and organic matter which was captured in the suspended sediment traps (data not

presented) may have settled onto the sediment surface during slack water, but did not accumulate uniformly on the bed. At sandy 2, a fine organic coating was observed on larger sand grains on ripple crests (Fig. 2A) whereas grains from ripple troughs were almost free of OM (Fig. 2B) suggesting that OM was being “caught” from the suspended sediment in the flow. In contrast, the surface sediment at the muddy site was characterized by a much thicker blanket of fine cohesive particles, rich in organic matter and MPB cells, confirmed by LSTEM

Table 3. The percentage variation in the erosion threshold of the sediments across all sites, explained by various biophysical properties. Both marginal (single predictor) and step-wise sequential results for DistLM are presented.

	AICc	Pseudo-F	Expl. variation (%)	Cumul. expl. variation (%)
<i>Marginal tests</i>				
Chl <i>a</i> (mg m ⁻²)		17.32	9***	
Shannon (<i>H</i>) index of MPB diversity		0.34	2***	
MPB species abundance		0.77	4 ^{NS}	
Organic content (%)		14.81	8***	
Colloidal carbs (mg m ⁻²)		3.25	2 [‡]	
<i>D</i> ₅₀ (μm)		3.58	2 [‡]	
<i>Sequential tests</i>				
Chl <i>a</i> (mg m ⁻²)	-13.63	17.32	9***	9
Shannon (<i>H</i>) index of MPB diversity	-32.20	21.53	10***	19
MPB species abundance	-42.92	13.00	6***	25
Organic content (%)	-50.85	10.05	4**	29
Colloidal carbs (mg m ⁻²)	-56.94	8.14	3**	32
<i>D</i> ₅₀ (μm)	-58.19	3.32	2 [‡]	34

Significance levels indicated as *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ‡marginally insignificant $p < 0.10$ and NS = not significant.

images (Fig. 2C,D) and biochemical analysis (Table 1). The presence of this organic-rich material can result in positive feedbacks to the system, whereby the adhesive organic EPS and MPB cells trap and bind more fine material, maintaining a muddy bed and reducing suspended sediment concentrations. This stabilizing effect can often be limited to warmer seasons when MPB growth is higher (Borsje et al. 2008) and periods of lower physical disturbance (Widdows and Brinsley 2002) in temperate regions, but appears to prevail under higher shear stress in this instance. An increase in the *D*₅₀ of the bed and a decrease in the organic content at the muddy site 28th–31st was accompanied by a sharp decrease in bed stability (see Table 1), suggesting the removal of organic material and resulting increase in particle size can destabilize these beds. Organic material creates cohesion between sediment particles, stabilizing the sediment when it is bound to particles (Black et al. 2002; Manning et al. 2010; Zhang et al. 2018). However, MPB and OM transported to a particular area during the tide can also form a “fluff” layer on the bed surface that is easily resuspended if it is not incorporated into a biofilm (Orvain et al. 2003). This is likely the case at sandy site 1 as high MPB biomass (Chl *a* concentration) and EPS were observed on days when the erosion threshold was reduced. The cells and EPS detected in the sediment surface were therefore unlikely to have formed a protective film. Indeed, in sandy sediments (like sandy site 1), small diatoms tend to attach themselves to the grains and coat individual sand grains in EPS rather than forming a substantial biofilm per se.

The different mechanisms by which MPB and EPS develop in dynamic sandy sites may explain the negative relationship between Chl *a* and erosion threshold at sandy site 1. Substantially more EPS is produced and excreted by epipellic (motile) diatoms, like the taxa dominating the muddy site. While

previously it has been thought that > 50% of the MPB community must be epipellic species (Underwood et al. 1995; Underwood and Paterson 2003), in the Dee Estuary it appears that the proportion may be much lower. These differences in the relationships often hinder attempts to generalize MPB biomass effects on erodibility, and lead to significant differences between studies. Despite the importance of accurately forecasting erosion threshold parameters for sediment transport predictions (Sanford 2008), the influence of biological cohesion across different habitats is rarely considered in these models (Le Hir et al. 2007). This is primarily due to the complexity of intertidal systems and differences in biological and physical processes across sediment gradients. There is likely a threshold of development under which very coarse sandy sites may not be positively influenced by MPB biostabilization, or under extreme conditions like the significant wave action at sandy site 1 prior to sampling (Lichtman et al. 2018). Nonetheless, other sandy sites (such as our sandy site 2) can be positively influenced by biostabilization (Larson et al. 2009). Cells and EPS that are not incorporated as a biofilm can be easily suspended and recorded as erosion by the CSM system and these results suggest that characterizing the MPB community can help to explain these differences (Fig. 7A).

In this study, EPS (as colloidal carbohydrates) were positively related to sediment erosion thresholds, even at sandy site 2. At this site, the community was composed primarily of low profile pioneer species, and had a limited number of motile forms (discussed further in microalgal communities section). This relationship suggests that even when the EPS matrix does not form a substantial biofilm on the surface, it still offers some form of protection to the underlying sediment (Fig. 7B). Laboratory (Malarkey et al. 2015; Parsons et al. 2016) and field investigations (Lichtman et al. 2018; Baas et al. 2019)

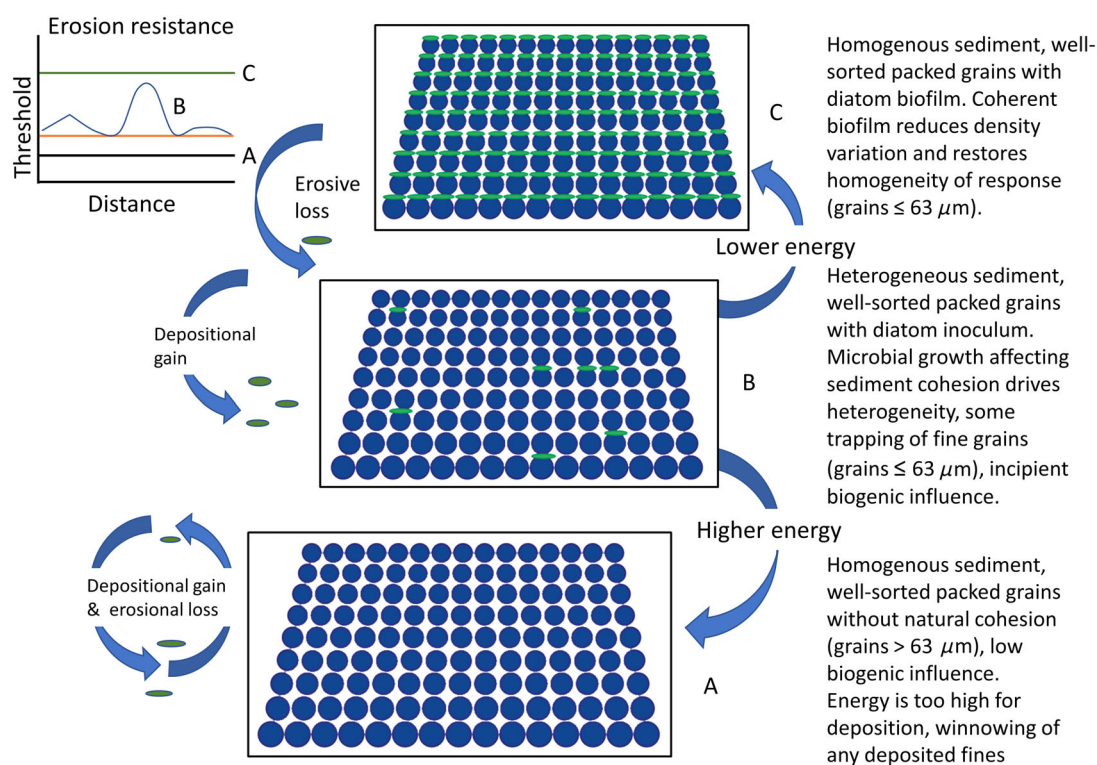


Fig. 7. Conceptual diagram of microbially induced variability in surface sediment erodibility. **(A)** Noncohesive sediment lacking biogenic influence that in theory would show little variability in surface behavior forms a predictable and homogenous habitat. **(B)** Colonization of the noncohesive bed by microbial cells producing EPS and the initial growth of small microbial colonies creates heterogeneity in the localized surface response to shear stress. Increasing the local resistance to erosion in some patches. **(C)** A fully colonized substratum where biofilm development has created a more uniform sediment surface, once again reduces the variability of the system but further increases the erosional resistance. Top left: Spatial variation in erosion resistance across the bed. Condition A = homogenous abiotic grains producing constant and predictable erosion thresholds. Condition B = a highly heterogeneous system with an erosion threshold influenced by the complexity of local conditions, and the patchy distribution of MPB and bacterial biofilms. Condition C = coherent biofilm increases sediment stability and reduces erodibility in a consistent manner across the bed. This creates a more homogenous response to erosional stress until bed failure. Erosive loss from areas of biofilm growth **(C)** can lead to the depositional gain of MPB at other sites **(B)**. This may lead to the development of a substantial biofilm **(C)** or the subsequent resuspension of the MPB again. At more energetic sites **(A)**, fine sediment and MPB are deposited during slack tide, but these are resuspended on the next tide, maintaining a relatively homogenous system where MPB may be present but there is no stabilizing effect of the biofilm due to frequent resuspension. These states may alter as local conditions change including seasonal, light, nutrient and temperature differences (which would stimulate the MPB and biofilm growth), and hydrodynamic conditions which increase erosional stress on the surface sediment.

have recently illustrated the influence of low EPS contents distributed deeper into the sediment bed. In these investigations, microbially produced EPS hampered sediment transport, bedform development, and bedform migration without the presence of a visible biofilm on the surface (see also Chen et al. 2017). These vectors of change are undoubtedly part of the short-term variation due to changes in the spring/neap cycle and daily weather fluctuations, but these changes can also be the first steps toward a transition toward an alternative state (van de Koppel et al. 2001).

The microalgal communities

Over the relatively short sampling period described here, the microalgae community at the sandy sites appeared to remain in early successional stages, whereas the community at the muddy site had already developed into a more vertically

structured community composed of stalked, filamentous, and motile microalgae (Winsborough and Golubic 1987). The latter forms can withstand stronger flow velocities and abrasion from moving sediments by migrating into the muddy sediment or creating filaments. However, adnate forms such as *Achnantheidium* are well equipped to resist flow (Passy 2007). Certain diatom species are indicators of the flow regime, with particular species exhibiting preference for high flow such as *Achnantheidium* spp. (Passy 2007). While flow differences can result in different communities, the effects can also be dampened by differences in turbidity (Soininen 2004). Nonetheless, information on community composition together with information on the biophysical properties of the sediment can be useful for determining the differences in erosional resistance and potential biostabilization across different habitats. Our results indicate that the number of species was altered over the

days at the different sites and species composition changed over short temporal scales. However, in each site the different guilds (low profile, high profile, motile, etc.) remained dominant due to their adaptations to the flow environment. While MPB community succession can be revealed through the microscopic identification of cells, this is time-consuming and could be complemented by next generation sequencing of the prokaryote and eukaryote communities for longer-term studies (Hicks et al. 2018). This would provide a more comprehensive microbial community analysis in relation to biostabilization (Paterson et al. 2018) as the diversity of prokaryotes has been linked to hydrodynamic regimes (Besemer et al. 2009). Such an approach would be incredibly useful for capturing the transformation of sites that are frequently disturbed and dynamic in nature, into more stable muddy habitats over longer time-scales. MPB composition and structure can reflect differences in flow regimes (Krajenbrink et al. 2019) and as the community changes, primary productivity, and the production of EPS exudates will vary, with knock on effects on various ecosystem structure and functions (Hope et al. 2019). As the hydrodynamic effects on MPB communities can modulate the effects of others stressors (Villeneuve et al. 2011; Polst et al. 2018), understanding the interaction between the community and hydrodynamics across different sediment habitats is essential. For instance, *Achnanthes* spp. and *Nitzschia inconspicua* were observed in all Dee Estuary sites. These cells are often one of the first species to inhabit recently disturbed sediment (Cardinale 2011), and are cosmopolitan (Sabater 2000). They can grow prostrate to the surface or adnatly (Berthon et al. 2011; Cardinale 2011), which helps them withstand high flow velocities (Passy 2007). We have however illustrated that these are not displaced by the development of the biofilm as they were still present in our muddier site. These could be key species to examine for the effects of other stressors in these environments. These pioneers promote the rapid recolonization of the sediment bed after physical disturbance, instigating a biological succession, which promotes greater biodiversity and heterogeneity in the biofilm and among higher organisms (Balvanera et al. 2006). This can pave the way for a more heterogeneous community of microorganisms and a complex habitat that can increase biostabilization (Paterson et al. 2018).

The survival of algal cells during tidal inundation or their deposition from the water column establishes a potential for microbial growth and eventual biofilm formation if conditions allow (Fig. 7B,C). This was evident from the differences between sites in the Dee Estuary. At sandy site 1, the MPB community was composed of pioneer species that turned over with the prevailing hydrodynamic conditions. At the opposite end of the spectrum, the MPB community at the muddy site was more stable and composed of larger epipellic species. However, at sandy site 2, the community was distinct and appeared to be intermediate between the two other sites. In these transitioning sites, the maximum variation in the sediment

surface erosion is expected (Fig. 7B) as the surface is patchy. As MPB communities develop and grow on the sediment surface, this drives the capture of more cohesive material and this positive feedback enhances the development of a more homogeneous and stable surface dominated by biofilms (Fig. 7C).

Disturbance from tidal flow can exert the same effects as large bioturbating fauna oxygenating the sediment surface layers (Huettel et al. 2003; Precht and Huettel 2003). These processes are important for soft sediment ecosystem functions such as sediment oxygenation, biogeochemical cycling and, depending on the organic enrichment of the sediment, degradation processes (Widdicombe and Austen 2001). The contribution of large infauna has recently be discussed elsewhere (Hillman et al. 2019) and are of course important to consider in many habitats. Low numbers of large fauna were observed at these sites, therefore we focused on the physical processes and the interaction with microbial organisms that are known to stabilize and disturb the bed. The close spatial association of visibly different sedimentological properties suggests that biophysical factors may contribute to the variation over short distances despite the similarity of dynamic context.

Understanding the biophysical factors influencing sediment stability across different habitats allows us to begin to discern how and why mixed beds occur and the mechanisms by which they alternate between muddy, mixed, and sandy habitats. Muddy sites can capture fine sediment, due to the cohesive nature of fine particles, MPB, and EPS in the surface layers (Table 1). This cohesiveness can prevent fine particles from winnowing during inundation and result in higher erosion thresholds. These biophysical properties can lead to the formation of a cohesive matrix that can effectively trap additional material from the water column and improve the clarity of the overlying water. Positive correlations between mud content, organic content, EPS carbohydrates, and MPB biomass/community indices were apparent at the muddy site (Fig. 5) indicative of biofilm development where higher numbers of motile diatoms were present. It has previously been suggested that relatively high proportions of motile diatoms, and hence high EPS concentrations, are required to trap new deposits of sediment (Underwood 1997) and counteract the physical forces that resuspend sediment, and this can lead to positive feedbacks. Van de Koppel et al. (2001) highlighted these feedbacks, and proposed that ecosystem engineering (Jones et al. 1994), principally by MPB, can mediate changes in bed sedimentology.

At the sandy habitats, the regular physical disturbance from waves and currents over the tidal cycle prevented the accumulation of larger MPB, which limits biofilm development (Blanchard et al. 2001). Over the course of this relatively high-resolution investigation, the D_{50} of the sandy site 1 increased despite the fine nature of the material frequently collected in suspension traps at the sites (data not presented). This was primarily due to the prevailing wind wave action at this site during this period (Lichtman et al. 2018; Supporting Information

Table S1). Fine cohesive sediment has to be removed frequently in sandier habitats, through resuspension or winnowing to impede the development of biofilm growth but MPB are still present and still exert influence over the sediment dynamics. When conditions are altered this can allow the MPB to proliferate, significantly increasing the erosion threshold and instigating a transition to finer sediment. These differences in the erosive nature of the bed and the fate of settling material is key to maintaining the differentiation between patches and increases overall habitat heterogeneity (Weerman et al. 2011) and functioning of soft sediment ecosystems (Thrush et al. 2008).

By investigating the short term, temporal dynamics influencing the MPB community, and the feedbacks between the biomass, community composition, exudates of MPB, and biostabilization potential, we can begin to understand the conditions required to instigate the changes that lead to transitions and postulate how the microbial organisms in these habitats can persist and continue to exert an influence on sediment stability.

Conclusion

The relative influence of MPB and EPS on sediment stability and transport remains poorly understood across different sediment habitats. The results of the study suggest various biological properties of the bed associated with the MPB significantly influence the short-term variability in the erodibility of different surface sediments. Importantly, we illustrate that while MPB diversity explained very little variation in marginal distance based linear tests, primary producer diversity was a significant predictor when MPB biomass was also considered in sequential tests. We emphasize the importance of considering the microbial diversity when assessing their influence on ecosystem functions such as sediment stability. Further evidence of biological cohesion across natural habitats of increasing complexity and at multiple spatial and temporal scales is required in order to understand the biological influence on sediment dynamics. Further data with natural gradients of sand and mud should be examined and the influence of larger benthic organisms included to document the influence of biological properties across different habitats, under differing physical conditions and with increasingly complex communities. This will facilitate the use of these variables in future sediment transport models.

References

- Amoudry, L. O., R. Ramirez-Mendoza, A. J. Souza, and J. M. Brown. 2014. Modelling-based assessment of suspended sediment dynamics in a hypertidal estuarine channel. *Ocean Dyn.* **64**: 707–722. doi:10.1007/s10236-014-0695-8
- Andersen, M. J., and T. J. Willis. 2003. Canonical analysis of principal coordinates: A useful method of constrained ordination for ecology. *Ecology* **84**: 511–525. doi:10.1890/0012-9658(2003)084[0511:CAOPCA]2.0.CO;2
- Anderson, M., R. N. Gorley, and K. R. Clarke. 2008. PERMANOVA+ for PRIMER. Guide to software and statistical methods, p. 213. PRIMER-E, Plymouth, UK.
- Aspden, R. J., S. Vardy, and D. M. Paterson. 2004. Salt marsh microbial ecology: Microbes benthic mats and sediment movements, p. 115–136. *In* S. Fagherazzi, M. Marani, and L. K. Blum [eds], *The ecogeomorphology of tidal marshes*. American Geophysical Union.
- Baas, J. H., et al. 2019. Integrating field and laboratory approaches for ripple development in mixed sand–clay – EPS. *Sedimentology* **66**: 2749–2768. doi:10.1111/sed.12611
- Balvanera, P., A. B. Pfisterer, N. Buchmann, J. S. He, T. Nakashizuka, D. Raffaelli, and B. Schmid. 2006. Quantifying the evidence for biodiversity effects on ecosystem functioning and services. *Ecol. Lett.* **9**: 1146–1156. doi:10.1111/j.1461-0248.2006.00963.x
- Beninger, P. G., D. Cuadrado, and J. Van De Koppel. 2018. Sedimentary and biological patterns on mudflats, p. 185–211. *In* P. G. Beninger [ed.], *Mudflat ecology*. Springer Nature Switzerland AG.
- Berthon, V., A. Bouchez, and F. Rimet. 2011. Using diatom life-forms and ecological guilds to assess organic pollution and trophic level in rivers: A case study of rivers in south-eastern France. *Hydrobiologia* **673**: 259–271. doi:10.1007/s10750-011-0786-1
- Besemer, K., G. Singer, I. Hödl, and T. J. Battin. 2009. Bacterial community composition of stream biofilms in spatially variable-flow environments. *Appl. Environ. Microbiol.* **75**: 7189–7195. doi:10.1128/AEM.01284-09
- Black, K. S., T. J. Tolhurst, D. M. Paterson, and S. E. Hagerthey. 2002. Working with natural cohesive sediment. *J. Hydraul. Eng.* **128**: 2–8. doi:10.1061/(ASCE)0733-9429(2002)128:1(2)
- Blanchard, F., J. Guarini, F. Orvain, and P. Sauriau. 2001. Dynamic behaviour of benthic microalgal biomass in intertidal mudflats. *J. Exp. Mar. Biol. Ecol.* **264**: 85–100. doi:10.1016/S0022-0981(01)00312-4
- Blanchard, G. F., P. G. Sauriau, V. Cariou-Le Gall, D. Gouleaul, M. J. Garet, and F. Olivier. 1997. Kinetics of tidal resuspension of microbiota: Testing the effects of sediment cohesiveness and bioturbation using flume experiments. *Mar. Ecol. Prog. Ser.* **151**: 17–25.
- Blott, S. J., and K. Pye. 2001. GRADISTAT: A grain size distribution and statistic package for the analysis of unconsolidated sediments. *Earth Surf. Process. Landf.* **26**: 1237–1248. doi:10.1002/esp.261
- Borsje, B. W., M. B. de Vries, S. J. M. H. Hulscher, and G. J. de Boer. 2008. Modeling large-scale cohesive sediment transport affected by small-scale biological activity. *Estuar. Coast. Shelf Sci.* **78**: 468–480. doi:10.1016/j.ecss.2008.01.009
- Brockmann, C., K. Stelzer, M. Consalvey, et al. 2004. HiMoM: A system of hierarchical monitoring methods for assessing changes

- in the biological and physical state of intertidal areas, p. 273–278. *In* M. A. Van Leeuwe, G. Morgan, and C. Brockmann [eds.], *Delivering sustainable coasts: Connecting science and policy*, Brockmann Consultancy Proceeding.
- Cardinale, B. J. 2011. Biodiversity improves water quality through niche partitioning. *Nature* **472**: 86–89. doi:10.1038/nature09904
- Chapman, M. G., T. J. Tolhurst, R. J. Murphy, and A. J. Underwood. 2010. Complex and inconsistent patterns of variation in benthos, micro-algae and sediment over multiple spatial scales. *Mar. Ecol. Prog. Ser.* **398**: 33–47. doi:10.3354/meps08328
- Chen, X. D., C. K. Zhang, D. M. Paterson, C. E. L. Thompson, and I. H. Townend. 2017. Hindered erosion: The biological mediation of noncohesive sediment behavior. *Water Resour. Res.* **53**: 4787–4801. doi:10.1002/2016WR020105
- Chen, X. D., C. K. Zhang, D. M. Paterson, I. H. Townend, C. Jin, Z. Zhou, Z. Gong, and Q. Feng. 2019. The effect of cyclic variation of shear stress on non-cohesive sediment stabilization by microbial biofilms: The role of 'biofilm precursors'. *Earth Surf. Process. Landf.* **44**: 1471–1481. doi:10.1002/esp.4573
- Clarke, K. R., and R. N. Gorley. 2006. *Primer v.6: User manual/tutorial*. PRIMER-E.
- de Brouwer, J. F. C., S. Bjelic, E. M. G. T. de Deckere, and L. J. Stal. 2000. Interplay between biology and sedimentology in a mudflat. *Cont. Shelf Res.* **20**: 1159–1177. doi:10.1016/S0278-4343(00)00017-0
- DuBois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **28**: 350–356. doi:10.1021/ac60111a017
- Ford, R. B., and C. Honeywill. 2002. Grazing on intertidal microphytobenthos by macrofauna: Is pheophorbide a useful marker? *Mar. Ecol. Prog. Ser.* **229**: 33–42.
- Grabowski, R. C., G. Wharton, G. R. Davies, and I. G. Droppo. 2012. Spatial and temporal variations in the erosion threshold of fine riverbed sediments. *J. Soils Sediments* **12**: 1174–1188. doi:10.1007/s11368-012-0534-9
- Halcrow. 2013. *North west estuaries processes reports. V3.1*, Sefton Council, UK.
- Hale, R., R. Boardman, M. N. Mavrogordato, I. Sinclair, T. J. Tolhurst, and M. Solan. 2015. High-resolution computed tomography reconstructions of invertebrate burrow systems. *Sci. Data* **2**: 150052. doi:10.1038/sdata.2015.52
- Harper, M., and J. Harper. 1967. Measurements of diatom adhesion and their relationship with movement. *Br. Phycol. Bull.* **3**: 195–207. doi:10.1080/00071616700650051
- Hartley, B., H. G. Barber, J. R. Carter, and P. A. Sims. 1996. *An atlas of British diatoms*. Biopress.
- Hendey, N. I. 1964. An introductory account of the smaller algae of British coastal waters. Part V. Bacillariophyceae (Diatoms). H. M. Stationery Office.
- Hewitt, J. E., S. F. Thrush, P. K. Dayton, and E. Bonsdorff. 2007. The effect of spatial and temporal heterogeneity on the design and analysis of empirical studies of scale-dependent systems. *Am. Nat.* **169**: 398–408. doi:10.1086/510925
- Hickman, M., and F. E. Round. 2007. Primary production and standing crops of epipsammic and epipelagic algae. *Br. Phycol. J.* **5**: 247–255. doi:10.1080/00071617000650311
- Hicks, N., X. Liu, R. Gregory, J. Kenny, A. Lucaci, L. Lenzi, D. M. Paterson, and K. R. Duncan. 2018. Temperature driven changes in benthic bacterial diversity influences biogeochemical cycling in coastal sediments. *Front. Microbiol.* **9**: 1730. doi:10.3389/fmicb.2018.01730
- Hillman, J. R., C. J. Lundquist, C. A. Pilditch, and S. F. Thrush. 2019. The role of large macrofauna in mediating sediment erodibility across sedimentary habitats. *Limnol. Oceanogr.* **65**: 683–693. doi:10.1002/lno.11337
- Hope, J. A., D. M. Paterson, and S. F. Thrush. 2019. The role of microphytobenthos in soft-sediment ecological networks and their contribution to the delivery of multiple ecosystem services. *J. Ecol.* **108**: 815–830. doi:10.1111/1365-2745.13322
- Hubas, C., C. Passarelli, and D. M. Paterson. 2018. Microphytobenthic biofilms: Composition and interactions, p. 63–90. *In* P. G. Beninger [ed.], *Mudflat ecology*. Springer Nature Switzerland AG.
- Huettel, M., H. Røy, E. Precht, and S. Ehrenhauss. 2003. Hydrodynamical impact on biogeochemical processes in aquatic sediments. *Hydrobiologia* **494**: 231–236.
- Jeffrey, S. W., and G. F. Humphrey. 1975. New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. *Biochem. Physiol. Pflanz.* **167**: 191–194. doi:10.1016/S0015-3796(17)30778-3
- Jones, C. G., J. H. Lawton, and M. Shachak. 1994. Organisms as ecosystem engineers. *Oikos* **69**: 373–386.
- Krajenbrink, H. J., M. Acreman, M. J. Dunbar, L. Greenway, D. M. Hannah, C. L. R. Laizé, D. B. Ryves, and P. J. Wood. 2019. Diatoms as indicators of the effects of river impoundment at multiple spatial scales. *PeerJ* **7**: e8092. doi:10.7717/peerj.8092
- Larson, F., H. Lubarsky, S. U. Gerbersdorf, and D. M. Paterson. 2009. Surface adhesion measurements in aquatic biofilms using magnetic particle induction: MagPI. *Limnol. Oceanogr.: Methods* **7**: 490–497. doi:10.4319/lom.2009.7.490
- Le Hir, P., Y. Monbet, and F. Orvain. 2007. Sediment erodibility in sediment transport modelling: Can we account for biota effects? *Cont. Shelf Res.* **27**: 1116–1142. doi:10.1016/j.csr.2005.11.016
- Li, B., F. Cozzoli, L. M. Soissons, T. J. Bouma, and L. Chen. 2017. Effects of bioturbation on the erodibility of cohesive versus non-cohesive sediments along a current-velocity gradient: A case study on cockles. *J. Exp. Mar. Biol. Ecol.* **496**: 84–90. doi:10.1016/j.jembe.2017.08.002
- Lichtman, I. D., and others. 2018. Bedform migration in a mixed sand and cohesive clay intertidal environment and

- implications for bed material transport predictions. *Geomorphology* **315**: 17–32. doi:10.1016/j.geomorph.2018.04.016
- Lubarsky, H. V., C. Hubas, M. Chocholek, F. Larson, W. Manz, D. M. Paterson, and S. U. Gerbersdorf. 2010. The stabilisation potential of individual and mixed assemblages of natural bacteria and microalgae. *PLoS One* **5**: e13794. doi:10.1371/journal.pone.0013794
- Lundkvist, M., U. Gangelhof, J. Lunding, and M. R. Flindt. 2007. Production and fate of extracellular polymeric substances produced by benthic diatoms and bacteria: A laboratory study. *Estuar. Coast. Shelf Sci.* **75**: 337–346. doi:10.1016/j.ecss.2007.04.034
- MacIntyre, H. L., R. J. Geider, and D. C. Miller. 1996. Microphytobenthos: The ecological role of the “secret garden” of unvegetated, shallow-water marine habitats. I. Distribution, abundance and primary production. *Estuaries Coast.* **19**: 186–201. doi:10.2307/1352224
- Magurran, A. E. 2004. *Measuring biological diversity*. Blackwell Publishing.
- Malvern Instruments Ltd 2013. Malvern Mastersizer web page. Available from <http://www.malvern.com/labeng/products/mastersizer/MS2000/mastersizer2000.htm> [accessed 2013 May 2].
- Malarkey, J., and others. 2015. The pervasive role of biological cohesion in bedform development. *Nat. Commun.* **6**: 6257. doi:10.1038/ncomms7257
- Manning, A. J., J. V. Baugh, J. R. Spearman, and R. J. S. Whitehouse. 2010. Flocculation settling characteristics of mud:sand mixtures. *Ocean Dyn.* **60**: 237–253. doi:10.1007/s10236-009-0251-0
- Mariotti, G., and S. Fagherazzi. 2012. Modeling the effect of tides and waves on benthic biofilms. *J. Geophys. Res. Biogeosci.* **117**: 1–14. doi:10.1029/2012JG002064
- McGlathery, K. J., K. Sundbäck, and I. C. Anderson. 2004. The importance of primary producers for benthic nitrogen and phosphorus cycling, p. 231–261. *In* S. L. Nielsen, G. T. Banta, and M. F. Pedersen [eds.], *Estuarine nutrient cycling: The influence of primary producers*. Springer.
- Montani, S., P. Magni, and N. Abe. 2003. Seasonal and inter-annual patterns of intertidal microphytobenthos in combination with laboratory and areal production estimates. *Mar. Ecol. Prog. Ser.* **249**: 79–91. doi:10.3354/meps249079
- Moore, R. D., J. Wolf, A. J. Souza, and S. S. Flint. 2009. Morphological evolution of the Dee Estuary, Eastern Irish Sea, UK: A tidal asymmetry approach. *Geomorphology* **103**: 588–596. doi:10.1016/j.geomorph.2008.08.003
- Orvain, F., R. Galois, C. Barnard, A. Sylvestre, G. Blanchard, and P.-G. Sauriau. 2003. Carbohydrate production in relation to microphytobenthic biofilm development: An integrated approach in a tidal mesocosm. *Microb. Ecol.* **45**: 237–251. doi:10.1007/s00248-002-2027-7
- Orvain, F., S. Lefebvre, J. Montepini, M. Sébire, A. Gangnery, and B. Sylvand. 2012. Spatial and temporal interaction between sediment and microphytobenthos in a temperate estuarine macro-intertidal bay. *Mar. Ecol. Prog. Ser.* **458**: 53–68. doi:10.3354/meps09698
- Parsons, D. R., and others. 2016. The role of biophysical cohesion on subaqueous bed form size. *Geophys. Res. Lett.* **43**: 1566–1573. doi:10.1002/2016GL067667
- Passy, S. I. 2007. Diatom ecological guilds display distinct and predictable behavior along nutrient and disturbance gradients in running waters. *Aquat. Bot.* **86**: 171–178. doi:10.1016/j.aquabot.2006.09.018
- Paterson, D. M. 1989. Short-term changes in the erodibility of intertidal cohesive sediments related to the migratory behavior of epipellic diatoms. *Limnol. Oceanogr.* **34**: 223–234. doi:10.4319/lo.1989.34.1.0223
- Paterson, D. M. 1995. Biogenic structure of early sediment fabric visualized by low-temperature scanning electron microscopy. *J. Geol. Soc.* **152**: 131–140.
- Paterson, D. M., and S. E. Hagerthey. 2001. Microphytobenthos in contrasting coastal ecosystems biology and dynamics. *In* K. Reise [ed.], *Ecological comparisons of sedimentary shores, ecological studies (Analysis and Synthesis)*, vol 151. Springer, Berlin, Heidelberg.
- Paterson, D. M., J. A. Hope, J. Kenworthy, C. L. Biles, and S. U. Gerbersdorf. 2018. Form, function and physics: The ecology of biogenic stabilisation. *J. Soils Sediments* **18**: 3044–3054. doi:10.1007/s11368-018-2005-4
- Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R Development Core Team. 2012. nlme: Linear and nonlinear mixed effects models. R package version 3.1-147, <https://CRAN.R-project.org/package=nlme>.
- Polst, B. H., C. Anlanger, U. Risse-Buhl, F. Larras, T. Hein, M. Weitere, and M. Schmitt-Jansen. 2018. Hydrodynamics alter the tolerance of autotrophic biofilm communities toward herbicides. *Front. Microbiol.* **9**: 1–12. doi:10.3389/fmicb.2018.02884
- Pratt, D. R., C. A. Pilditch, A. M. Lohrer, and S. F. Thrush. 2014. The effects of short-term increases in turbidity on sandflat microphytobenthic productivity and nutrient fluxes. *J. Sea Res.* **92**: 170–177. doi:10.1016/j.seares.2013.07.009
- Precht, E., and M. Huettel. 2003. Advective pore-water exchange driven by surface gravity waves and its ecological implications. *Limnol. Oceanogr.* **48**: 1674–1684. doi:10.4319/lo.2003.48.4.1674
- R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from <http://www.R-project.org/>.
- Rainey, M. P., A. N. Tyler, D. J. Gilvear, R. G. Bryant, and P. McDonald. 2003. Mapping intertidal estuarine sediment grain size distributions through airborne remote sensing. *Remote Sens. Environ.* **86**: 480–490. doi:10.1016/S0034-4257(03)00126-3
- Ribeiro, L., V. Brotas, Y. Rincé, and B. Jesus. 2013. Structure and diversity of intertidal benthic diatom assemblages in

- contrasting shores: A case study from the Tagus Estuary. *J. Phycol.* **49**: 258–270. doi:[10.1111/jpy.12031](https://doi.org/10.1111/jpy.12031)
- Round, F. E., R. M. Crawford, and D. G. Mann. 1990. The diatoms. Biology & morphology of the genera. Cambridge Univ. Press.
- Sabater, S. 2000. Diatom communities as indicators of environmental stress in the Guadiamar River, S-W Spain, following a major mine tailing spill. *J. Appl. Phycol.* **12**: 113–124. doi:[10.1023/A:1008197411815](https://doi.org/10.1023/A:1008197411815)
- Sanford, L. P. 2008. Modeling a dynamically varying mixed sediment bed with erosion, deposition, bioturbation, consolidation, and armoring. *Comput. Geosci.* **34**: 1263–1283. doi:[10.1016/j.cageo.2008.02.011](https://doi.org/10.1016/j.cageo.2008.02.011)
- Seuront, L., and S. Leterme. 2006. Microscale patchiness in microphytobenthos distributions: Evidence for a critical state, p. 167–185. *In* J. C. Kromkamp, J. F. C. de Brouwer, G. F. Blanchard, R. M. Forster, and V. Cr  ach [eds.], *Functioning of microphytobenthos in estuaries*. Royal Netherlands Academy of Arts and Sciences.
- Soininen, J. 2004. Assessing the current related heterogeneity and diversity patterns of benthic diatom communities in a turbid and a clear water river. *Aquat. Ecol.* **38**: 495–501. doi:[10.1007/s10452-004-4089-8](https://doi.org/10.1007/s10452-004-4089-8)
- Somerfield, P. J. 2008. Identification of the Bray-Curtis similarity index: Comment on Yoshioka (2008). *Mar. Ecol. Prog. Ser.* **372**: 303–306. doi:[10.3354/meps07841](https://doi.org/10.3354/meps07841)
- Spilmont, N., L. Seuront, T. Meziane, and D. T. Welsh. 2011. There's more to the picture than meets the eye: Sampling microphytobenthos in a heterogeneous environment. *Estuar. Coast. Shelf Sci.* **95**: 470–476. doi:[10.1016/j.ecss.2011.10.021](https://doi.org/10.1016/j.ecss.2011.10.021)
- Telgmann, U., H. Horn, and E. Morgenroth. 2004. Influence of growth history on sloughing and erosion from biofilms. *Water Res.* **38**: 3671–3684. doi:[10.1016/j.watres.2004.05.020](https://doi.org/10.1016/j.watres.2004.05.020)
- Thrush, S. F., J. Halliday, J. E. Hewitt, and A. M. Lohrer. 2008. The effects of habitat loss, fragmentation, and community homogenization on resilience in estuaries. *Ecol. Appl.* **18**: 12–21. doi:[10.1890/07-0436.1](https://doi.org/10.1890/07-0436.1)
- Tolhurst, T. J., K. S. Black, S. A. Shayler, S. Mather, I. Black, K. Baker, and D. M. Paterson. 1999. Measuring the in situ erosion shear stress of intertidal sediments with the cohesive strength meter (CSM). *Estuar. Coast. Shelf Sci.* **49**: 281–294. doi:[10.1006/ecss.1999.0512](https://doi.org/10.1006/ecss.1999.0512)
- Tolhurst, T. J., E. C. Defew, R. G. Perkins, A. Sharples, and D. M. Paterson. 2005a. The effects of tidally-driven temporal variation on measuring intertidal cohesive sediment erosion threshold. *Aquat. Ecol.* **40**: 521–531. doi:[10.1007/s10452-005-9001-7](https://doi.org/10.1007/s10452-005-9001-7)
- Tolhurst, T. J., A. J. Underwood, R. G. Perkins, and M. G. Chapman. 2005b. Content versus concentration: Effects of units on measuring the biogeochemical properties of soft sediments. *Estuar. Coast. Shelf Sci.* **63**: 665–673. doi:[10.1016/j.ecss.2005.01.010](https://doi.org/10.1016/j.ecss.2005.01.010)
- Tolhurst, T. J., K. S. Black, and D. M. Paterson. 2009. Muddy sediment erosion: Insights from field studies. *J. Hydraul. Eng.* **135**: 73–87. doi:[10.1061/\(ASCE\)0733-9429\(2009\)135:2\(73\)](https://doi.org/10.1061/(ASCE)0733-9429(2009)135:2(73))
- Ubertini, M., S. Lefebvre, C. Rakotomalala, and F. Orvain. 2015. Impact of sediment grain-size and biofilm age on epipellic microphytobenthos resuspension. *J. Exp. Mar. Biol. Ecol.* **467**: 52–64. doi:[10.1016/j.jembe.2015.02.007](https://doi.org/10.1016/j.jembe.2015.02.007)
- Underwood, G. J. C. 1997. Microalgal colonization in a saltmarsh restoration scheme. *Estuar. Coast. Shelf Sci.* **44**: 471–481. doi:[10.1006/ecss.1996.0138](https://doi.org/10.1006/ecss.1996.0138)
- Underwood, G. J. C., D. M. Paterson, and R. J. Parkes. 1995. The measurement of microbial carbohydrate exopolymers from intertidal sediments. *Limnol. Oceanogr.* **40**: 1243–1253.
- Underwood, G. J. C., and D. M. Paterson. 2003. The importance of extracellular carbohydrate production by marine epipellic diatoms. *Adv. Bot. Res.* **40**: 183–240.
- van de Koppel, J., P. M. J. Herman, P. Thoolen, and C. H. R. Heip. 2001. Do alternate stable states occur in natural ecosystems? Evidence from a tidal flat. *Ecology* **82**: 3449–3461. doi:[10.1890/0012-9658\(2001\)082\[3449:DASSO\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2001)082[3449:DASSO]2.0.CO;2)
- van der Wal, D., A. Wielemaker, and P. M. J. Herman. 2010. Spatial synchrony in intertidal benthic algal biomass in temperate coastal and estuarine ecosystems. *Ecosystems* **13**: 338–351. doi:[10.1007/s10021-010-9322-9](https://doi.org/10.1007/s10021-010-9322-9)
- Villeneuve, A., A. Bouchez, and B. Montuelle. 2011. In situ interactions between the effects of season, current velocity and pollution on a river biofilm. *Freshw. Biol.* **56**: 2245–2259. doi:[10.1111/j.1365-2427.2011.02649.x](https://doi.org/10.1111/j.1365-2427.2011.02649.x)
- Weerman, E. J., J. van de Koppel, M. B. Eppinga, F. Montserrat, Q. Liu, and P. M. J. Herman. 2010. Spatial self-organization on intertidal mudflats through biophysical stress divergence. *Am. Nat.* **176**: E15–E32. doi:[10.1086/652991](https://doi.org/10.1086/652991)
- Weerman, E. J., P. Herman, and J. van de Koppel. 2011. Macrobenthos abundance and distribution on a spatially patterned intertidal flat. *Mar. Ecol. Prog. Ser.* **440**: 95–103. doi:[10.3354/meps09332](https://doi.org/10.3354/meps09332)
- Widdicombe, S., and M. C. Austen. 2001. The interaction between physical disturbance and organic enrichment: An important element in structuring benthic communities. *Limnol. Oceanogr.* **46**: 1720–1733.
- Widdows, J., and M. Brinsley. 2002. Impact of biotic and abiotic processes on sediment dynamics and the consequences to the structure and functioning of the intertidal zone. *J. Sea Res.* **48**: 143–156. doi:[10.1016/S1385-1101\(02\)00148-X](https://doi.org/10.1016/S1385-1101(02)00148-X)
- Winsborough, B. M., and S. Golubic. 1987. The role of diatoms in stromatolite growth two examples from modern freshwater settings. *J. Phycol.* **23**: 195–201. doi:[10.1111/j.1529-8817.1987.tb04444.x](https://doi.org/10.1111/j.1529-8817.1987.tb04444.x)
- Zhang, N., C. E. L. Thompson, I. H. Townend, K. E. Rankin, D. M. Paterson, and A. J. Manning. 2018. Nondestructive 3D imaging and quantification of hydrated biofilm-sediment aggregates using x-ray microcomputed

tomography. *Environ. Sci. Technol.* **52**: 13306–13313. doi: [10.1021/acs.est.8b03997](https://doi.org/10.1021/acs.est.8b03997)

Zuur, A. F., E. N. Leno, and G. M. Smith. 2007. *Analysing ecological data*. Springer.

Acknowledgments

We are grateful to the NOC Ocean Technology and Engineering group, and field technicians from the University of Plymouth, Bangor University and St Andrews for instrument set up and deployment and sample collection. We thank Emily Carr and Stefano Schenone for their help with graphics. LTSEMs were produced by Mr Irvine Davidson, University of St Andrews, as his last SEM work before retirement. His phenomenal contributions over the years are greatly appreciated. We also wish to thank the editor, associate editor, and two anonymous reviewers for their valuable comments and suggestions on an earlier version of this manuscript. This

work was supported by the UK Natural Environment Research Council (NERC), grant NE/I027223/1 (COHBED). D.M.P. received funding from the Marine Alliance for Science and Technology for Scotland (MASTS), funded by the Scottish Funding Council (grant HR09011). J.M. and D.R.P. were partially funded by a European Research Council Consolidator Award (725955).

Conflict of Interest

None declared.

Submitted 09 October 2019

Revised 17 February 2020

Accepted 09 March 2020

Associate editor: Julia Mullarney