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

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	the resilience and recovery of the MPB community after physical disturbance from tidal currents and wave exposure. Canonical analysis of principal components (CAP) ordinations were used to illustrate the trends observed in bio-physical properties between the sites, while marginal and sequential distance-based linear models (DistLM) were used to identify key properties influencing sediment erodibility. While grain size was important for site differences in the CAP analysis, it contributed less to the variability in sediment erodibility than other 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 with diversity and biomass key predictors of sediment stability, contributing 9% and 10% respectively to the final model across all sites.

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Running head: Bio-physical mediation of sediment dynamics

Abstract

This study focuses on the interactions between the stability of different sediments and the biological and physical variables that influence the erodibility of the bed. Sampling at short-term temporal scales illustrated the persistence of the microphytobenthic (MPB) community even during periods of frequent, high physical disturbance. The role of MPB in biological stabilisation of the sediment along a changing sedimentary habitat was also assessed. Key biological and physical properties, such as the MPB biomass, composition and extracellular polymeric substances (EPS), were used to predict the sediment stability (erosion threshold) of muddy and sandy habitats within close proximity to one another over multiple days, as well as within emersion periods. This allowed the effects of dewatering, MPB growth and productivity to be examined as well as the resilience and recovery of the MPB community after physical disturbance caused by tidal currents and waves.

Canonical analysis of principal components (CAP) ordinations were used to visualise and assess the trends observed in bio-physical properties between the sites, and marginal and sequential distance-based linear models (DistLM) were used to identify the key properties influencing sediment 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 when MPB biomass was also considered in sequential tests. MPB diversity and biomass were both key predictors of sediment stability, contributing 9% and 10% respectively to the final model across all sites in comparison to 2% of the variance explained by sediment grain size.

Introduction

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

Biological stabilisation of cohesive (muddy) sediment is often attributed to microbially-produced extracellular polymeric substances (EPS) that increase the cohesion between sediment particles, often forming biofilms (Hubas et al., 2018). The production of EPS is typically attributed to bacteria and microphytobenthos (MPB) (Chen et al., 2017; Lubarsky et al., 2010). While MPB are more abundant on cohesive sediments, recent studies show that microbially-produced EPS can also hinder bedform development and inhibit erosion in non-cohesive (sandy) (Chen et al., 2017; Malarkey et al., 2015) 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 community, epipelic (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 homogenise 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 bio-physical feedbacks as the system becomes inherently more patchy leading to spatial self-organisation 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 also ‘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, biostabilisation 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 focussed on seasonal and inter-annual variability (Montani et al., 2003; 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 (Hewitt et al., 2006; Tolhurst et al., 2005a). However, short-term temporal dynamics that may influence EPS accumulation, biofilm development and biostabilisation have not been well characterised 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, biostabilisation, hydrodynamic stress and subsequent resistance to erosion over multiple emersion periods and within different habitats. This information is essential to assess the role of biostabilisation, both from ecological and dynamic perspectives (de Brouwer et al., 2000; Mariotti and Fagherazzi, 2012; Underwood and Paterson, 2003). 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 bentho-pelagic exchange of sediment (Chen et al., 2017); and nutrient cycling (McGlathery et al., 2004). MPB importance 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 biostabilisation potential (Aspden et al., 2004), however, an "inoculum" often remains in place (Chen et al., 2017) leading to rapid re-colonisation under suitable conditions.

We hypothesise that biofilm properties such as MPB biomass, colloidal carbohydrate concentrations and the MPB diversity will influence biostabilisation of various sediment types. Furthermore, we hypothesise 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 & 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 non-cohesive 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 three days at each site from 23rd – 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 supplementary material. 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 one 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 (Table S1).

At each site, 20 surface sediment samples were collected each day (n = 5 × 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 approx. 3 m^2. Sediment cores (2 mm depth, surface area = 250 mm^2) 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 epipelic and epipsammic microalgal cells, replicate surface scrapes (n = 5, 10×10×2 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 (SOM, %) was determined by loss-on-ignition at 450°C for 4 h. Chlorophyll a pigments were extracted with 90% acetone following the trichromatic method of Jeffrey & 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 in contents versus 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 analyser (Malvern Instruments Ltd, 2013) and summarised using GRADISTAT software (Blott and Pye, 2001) prior to statistical analysis with D_{50} and mud content (% <63µm) used for further analysis. The relative difference in erosion threshold required to suspend a user-defined erosion threshold 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 x100 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): (i) "motile" (fast moving and larger); (ii) "low profile" (short stature, prostrate, adnate erect and slow-moving species), and: (iii) "high profile" (tall stature, erect, filamentous branched or chain-forming and colonial centrics, largely non-motile or motile within tubes).

Low Temperature Scanning Electron Microscopy (LTSEM) – Fragments of contact core samples, frozen in liquid nitrogen (–196.8 ^{o}C, 1 atm), were mounted on mechanical stubs and examined using a JOEL 35CF SEM fitted with a 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, 2014) through the R studio graphical interface (v. 0.98.1083) and in PRIMER software (V.6, PRIMER-E, Ivybridge, UK). Differences in bio-physical variables were determined between sites, emersion times and days, after assumptions were tested (Pinheiro et al., 2012; Zuur et al., 2007). Necessary transformations were applied to conform to assumptions for parametric statistical testing (ANOVA), where possible or non-parametric Kruskal Wallace (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 for each day 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 bio-physical 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 (Somerfield, 2008). Differences in species between sites and days were tested using permutational multivariate analysis of variance (PERMANOVA) 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 bio-physical properties of the sediment across all sites, data was 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 bio-physical properties. Marginal and sequential tests were examined using Akaike's information criterion (AICc) and a backwards elimination process to identify the best combination of predictors, that maximised the explained variation with the most parsimonious model (Clarke and Gorley, 2006).

Results

Sediment bed properties and stability – The percentage of mud (< 63µm) 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 & 1%). For the sandy sites, clean particles were visually observed with very little associated organic matter (Fig 2 A-B) and the grain size distributions were similar (Supp. Fig S1). In contrast, the muddy site exhibited more varied and organic-rich sediments (Fig 2 C-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 chlorophyll 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 ($H_{(2)} = 27.12$, $P < 0.001$ 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 chlorophyll 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 ± 115 mg m^{-2} (day 1) to 229 ± 54 mg m^{-2} (day 3, Figure 3; $F_{2,59} = 6.57$, $P < 0.001$). At the muddy site, the erosion threshold was at its highest on day one (14.5 kPa) and the lowest on day two (2.7 kPa) also coinciding with the lowest colloidal carbohydrate concentration (428 ± 110 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 ± 3 µm on day one to 213 ± 10 µm on day three ($H_{(2)} = 25.88$, $P < 0.001$). 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 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 epipelic 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 (Figure 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% & 5%, respectively) were the greatest contributors to the overall dissimilarity (46%) between days at the muddy site.

Bio-physical influences on erodibility - Inspection of the CAP plots (Figures 5 & 6) and the resulting trace statistic ($P < 0.001$) confirmed a strong overall difference between the sites based on bio-physical properties. The first axis of the plot (CAP1) was partitioned between several biological variables relatively evenly, including chlorophyll 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 anti-correlation 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 from the superimposed scatter plot (Figure 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 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 chlorophyll a and organic content significantly explained the greatest variation (at 9% and 8% respectively). While chlorophyll 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 (chlorophyll 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 community maintain a key role in sediment dynamics, by surviving harsh environmental conditions, and quickly re-establishing biostabilisation. 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 biostabilisation 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 (Figure 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 create a stable matrix and therefore leads to a 'low biostabilisation' scenario. The lack of incorporation into the bed, explains the negative relationships between key biofilm properties and sediment erosion measurements observed and sandy site 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 biostabilisation potential (Aspden et al., 2004), an inoculum often remains in place (Chen et al., 2019) and this can still exert biostabilising effects on the sediment as we have illustrated. This persistence of the biofilm and its stabilising properties means that a biofilm can develop rapidly, if conditions become favourable (see Figure 7; Chen et al., 2019). Previous studies of relationships between EPS carbohydrates and sediment stability have estimated that 2-3 days 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 biostabilisation potential. The stabilising 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 (Figure 2a) whereas grains from ripple troughs were almost free of OM (Figure 2b) suggesting that OM was being 'caught' from the suspended sediment in the flow. In contrast, the surface sediment at the muddy site was characterised by a much thicker blanket of fine cohesive particles, rich in organic matter and MPB cells, confirmed by LSTEM images (Figure 2 C-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 (SSC). This stabilising 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 destabilise these beds. Organic material creates cohesion between sediment particles, stabilising 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 (chlorophyll 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 chlorophyll a and erosion threshold at sandy site 1. Substantially more EPS is produced and excreted by epipelic (motile) diatoms, like the taxa dominating the muddy site. While previously it has been thought that >50% of the MPB community must be epipelic species (Underwood et al., 1995; Underwood & Paterson, 2003), in the Dee Estuary it appears that the proportion may be much lower. These differences in the relationships often hinder attempts to generalise 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 biostabilisation, 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 biostabilisation (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 characterising the MPB community can help to explain these differences (Figure 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 (Figure 7B). Laboratory (Malarkey et al., 2015; Parsons et al., 2016) and field investigations (Baas et al., 2019; Lichtman et al., 2018) 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 towards 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 *Achnanthidium* 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 *Achnanthidium 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 bio-physical properties of the sediment can be useful for determining the differences in erosional resistance and potential biostabilisation across different habitats. Our results indicate 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 complimented 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 biostabilisation (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 timescales. 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 (Polst et al., 2018; Villeneuve et al., 2011) 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 adnately (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 biostabilisation (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 (Figure 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 epipelic 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 (Figure 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 homogenous and stable surface dominated by biofilms (Figure 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 focussed on the physical processes and the interaction with microbial organisms that are known to stabilise and disturb the bed. The close spatial association of visibly different sedimentological properties suggests bio-physical factors may contribute to the variation over short distances despite the similarity of dynamic context.

Understanding the bio-physical 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 bio-physical 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 (Figure 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; 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 biostabilisation 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 remain 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 emphasise 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.

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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 / median value, bottom number = Standard deviation / interquartile range.

	Colloidal carb conc (mg m^{-2})	Chl a 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)
23rd (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)
24th (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)
25th (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)
26th (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)
28th (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)
29th (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)
28th (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)
29th (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)
31st (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)

Table 2: Spearman rank correlation coefficients for all variables within and across sites. 1st number = sandy 1, 2nd = sandy 2, 3rd = muddy site, bottom number **(bold)** = all sites. Sig levels - '***' = P < 0.001, '**' = P < 0.01, '*' = P < 0.05. - = no significant correlation detected. *N* = 60 per site.

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

Table 3: The % variation in the erosion threshold of the sediments across all sites, explained by various bio-physical properties. Both marginal (single predictor) and step-wise sequential results for DistLM are presented. Significance levels indicated as '***' = P < 0.001, '**' = P < 0.01, '*' = P < 0.05, ¥ = marginally insignificant P<0.10 and NS = not significant.

	AICc	Pseudo-F	Expl. Variation (%)	Cumul. Expl. Variation (%)
Marginal tests				
Chl a (mg m^2)		17.32	9***	
Shannon (H) 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¥	
D_{50} (µm)		3.58	2¥	
Sequential tests				
Chl a (mg m^2)	-13.63	17.32	9***	9
Shannon (H) 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
D_{50} (µm)	-58.19	3.32	2¥	34

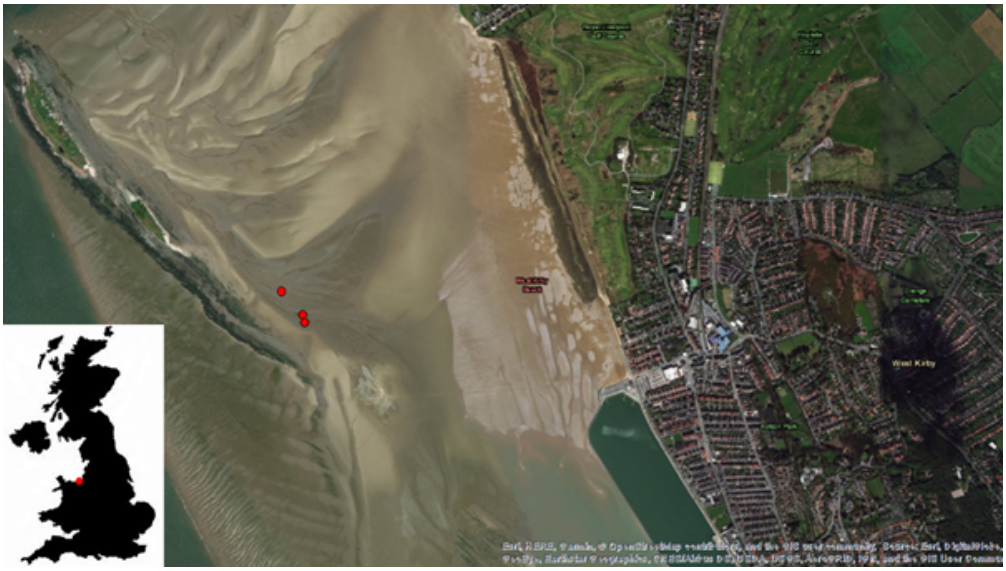

Figure 1: The location of the sampling sites on the intertidal flats near West Kirby and Hilbre Island. Inset - Position of the Dee Estuary, near West Kirby Liverpool, England.

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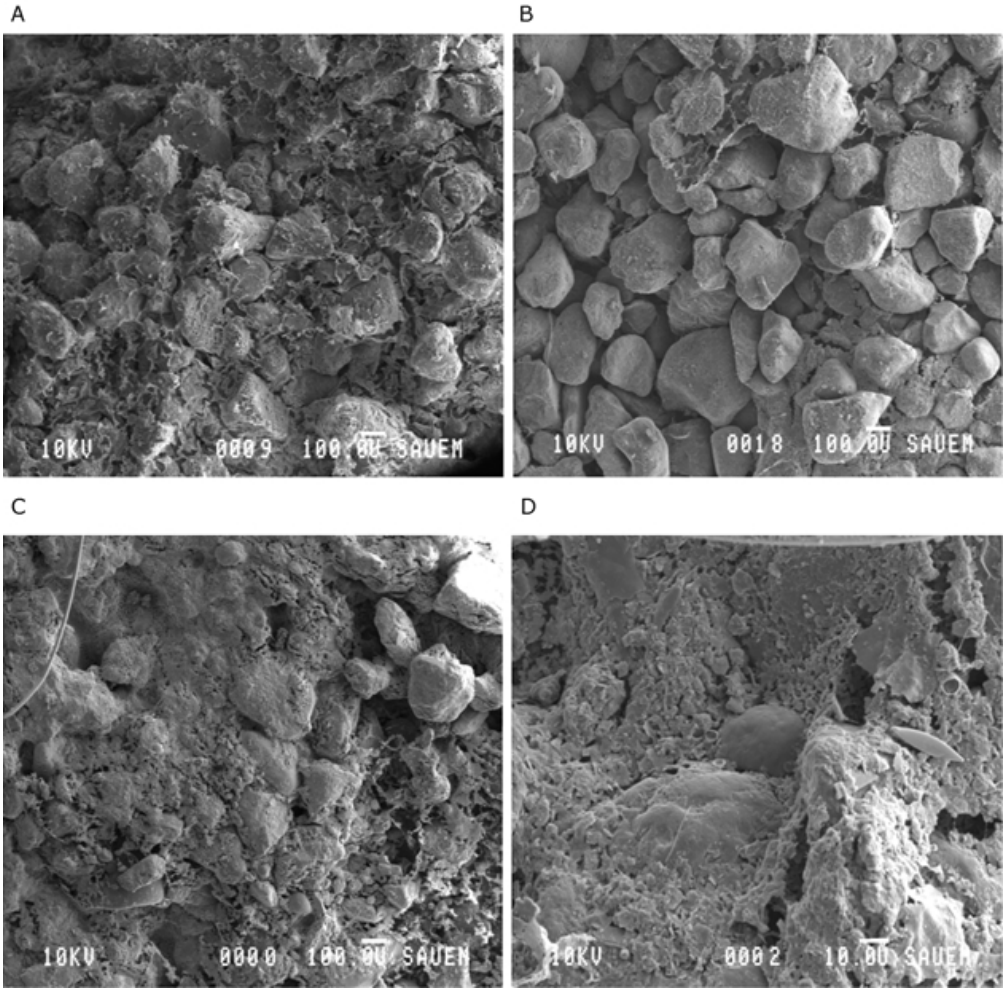


Figure 2: Low temperature scanning electron micrographs (LTSEM) of the intact sediment surface at A) Sandy site 2, crest of ripples B) Sandy site 2, troughs C) Muddy site, general surface and D) Muddy site, close-up image of organic material between sediment grains. Scale bars: 100μm for A-C and 10μm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

166x166mm (96 x 96 DPI)

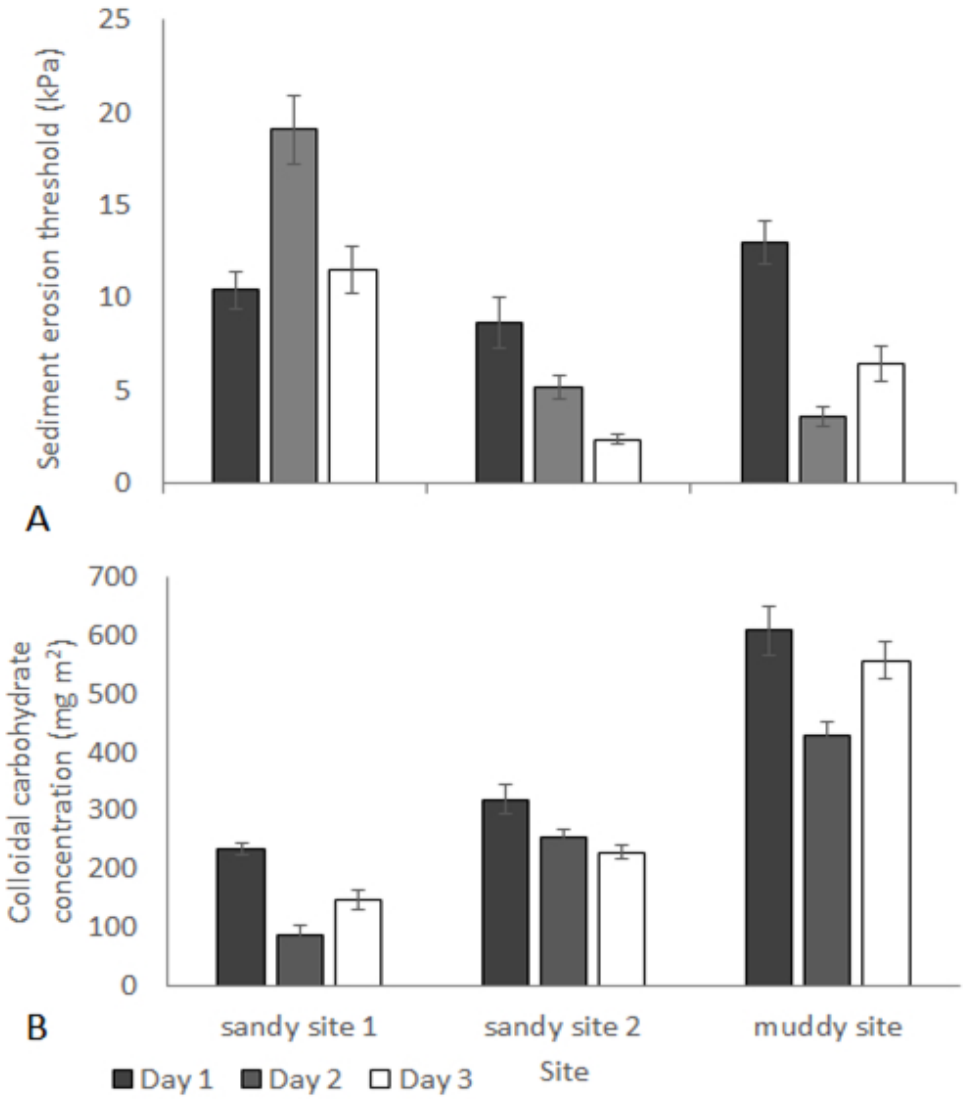


Figure 3: Biological and physical measurements from intertidal sediments of the Dee Estuary. A) Mean (± SE, n = 20) sediment erosion thresholds (kPa) for the three sites over 3 sampling days; Sandy site 1 on 23rd, 24th, 25th May. Sandy site 2 on 26th, 28th, 29th May 2013. Muddy site on 28th, 29th, 31st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

135x152mm (96 x 96 DPI)

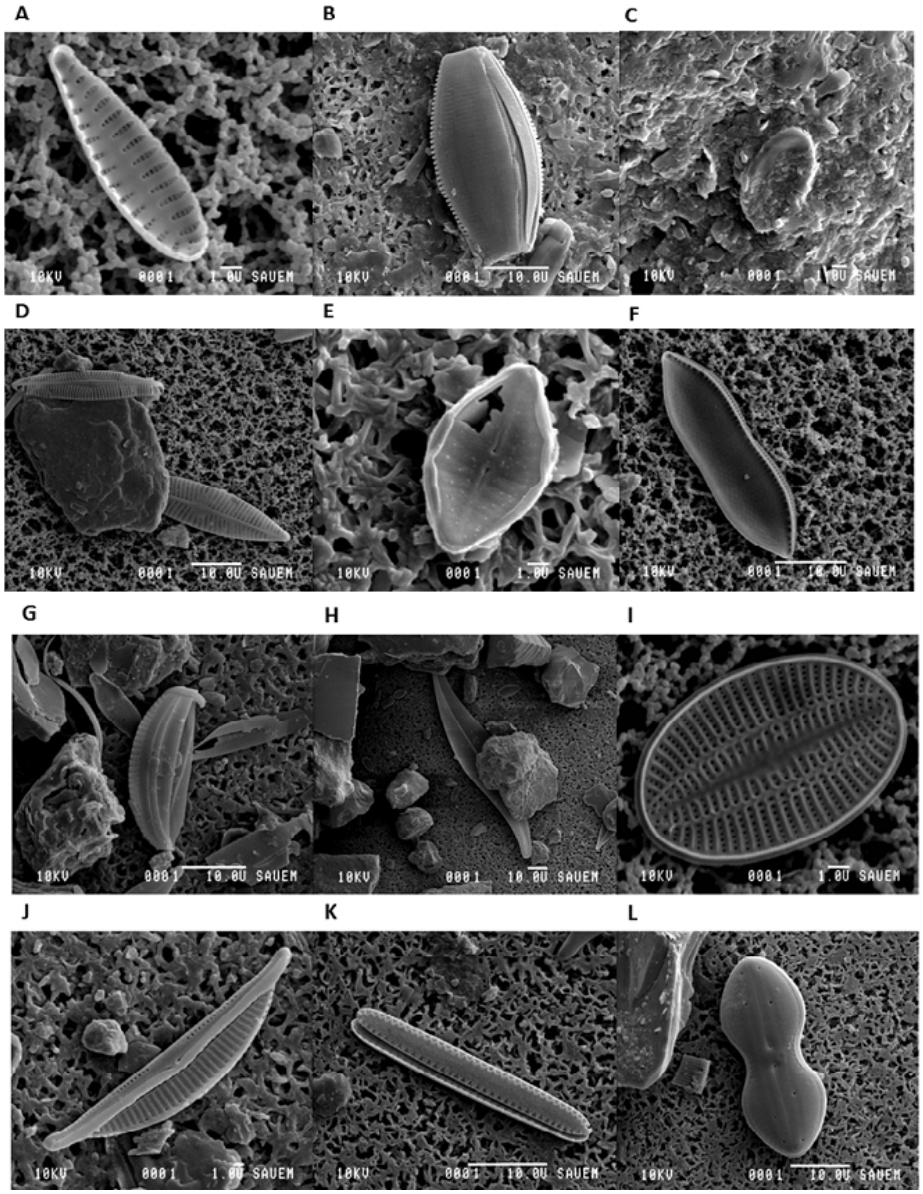


Figure 4: Low-temperature scanning electron micrographs of diatoms harvested from the surface sediment of the Dee Estuary. Scale bars = 1µm/10µm where stated. A) Opephora mutabilis (Grunow), B) Epipsammic sp 1, C) epipsammic cell embedded in a sediment particle in a matrix of EPS, D) Navicula gregaria (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) L) Diploneis spp.

186x228mm (96 x 96 DPI)

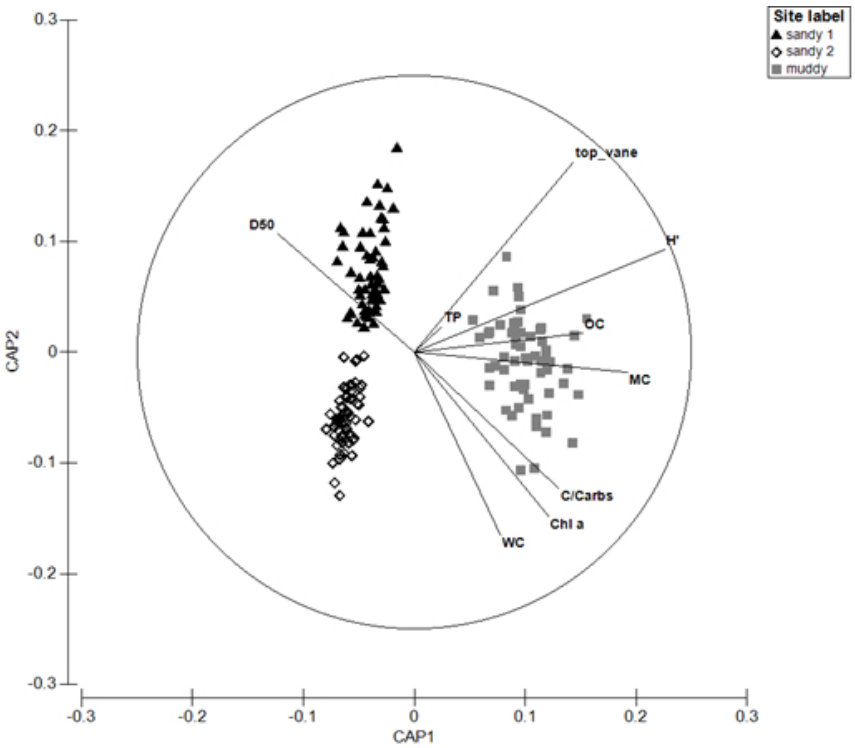


Figure 5: Canonical analysis of principal components (CAP) plot of euclidean distance similarities between samples. The correlation circle overlays measured variables that were influencing the similarity/dissimilatory between the samples. All data was square root transformed and normalised prior to analysis. n = 60. D50 - D50 of the particle size distribution, top_vane – undrained shear strength, TP – time point 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 (%).

159x119mm (96 x 96 DPI)

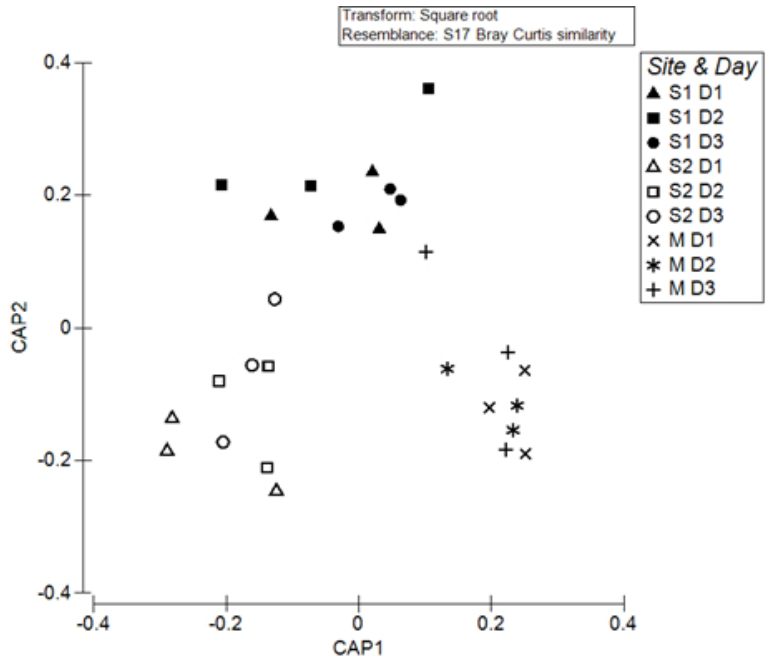
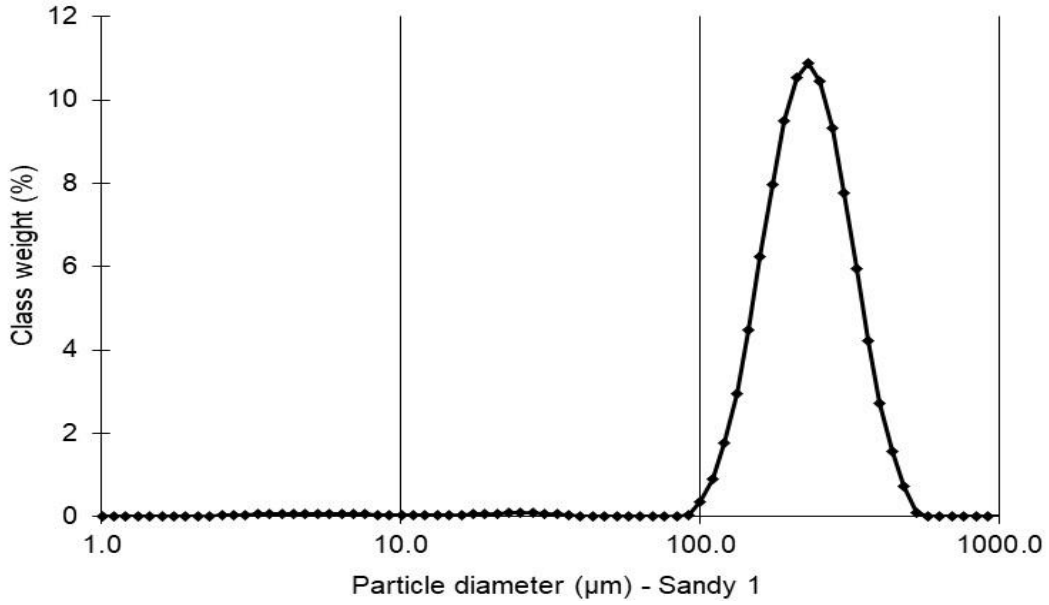
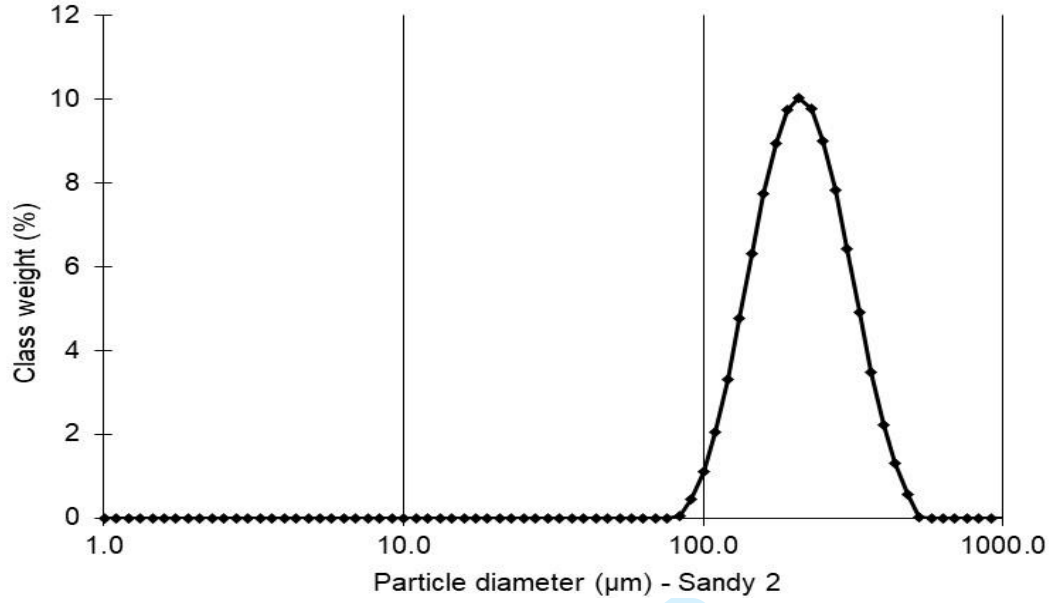


Figure 6: Canonical analysis of principal components (CAP) plot of Bray Curtis similarities in the microalgae community composition of the sediment surface between the two sandy sites (S1 and S2) and the muddy (M) site over three days at the Dee estuary.

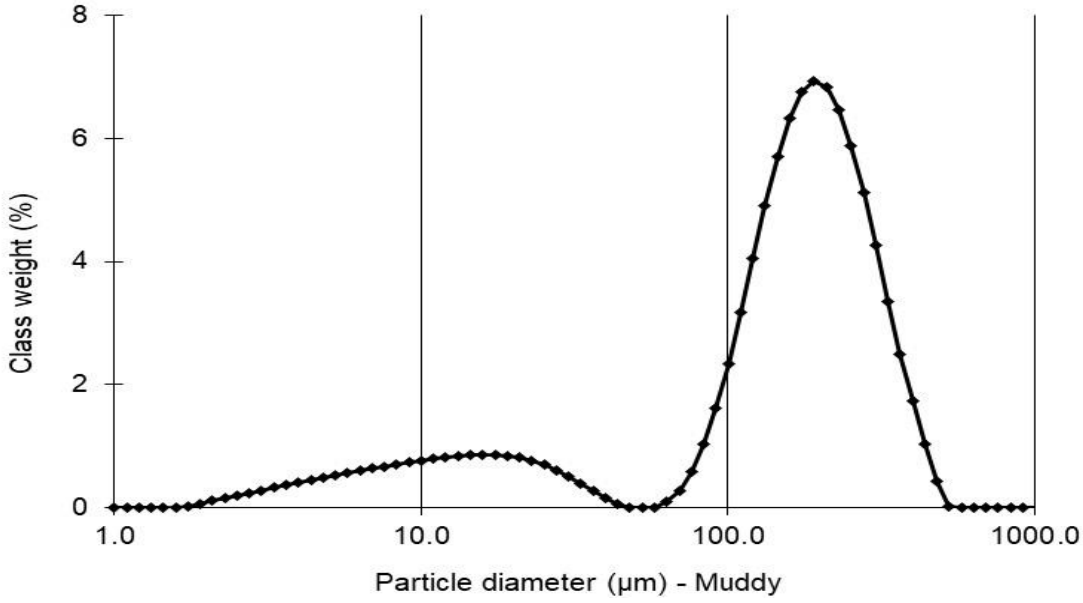
159x105mm (96 x 96 DPI)



A



B



C

Supplementary figure S1: Grain size distributions for A) sandy site 1. B) Sandy site 2. C) Muddy site. Values are averaged across all days at each site. Note that the y-axis of the muddy site is on a different scale.

Table S1: Peak hydrodynamic values during the inundation at the three sites based on the measurements of Lichtman et al. (2018). The current and wave-current bed shear stresses are the mean and maximum during a wave cycle (the closer they are to one another the weaker the effect of the wave).

Date	Site	Water depth (m)	Depth-averaged current (m s^{-1})	Significant wave height (m)	Wave period (s)	current bed shear stress (Nm^{-2})	Wave-current bed shear stress (Nm^{-2})
23/05	S1	2.23	0.49	0.38	7.8	0.41	1.09
24/05	S1	2.59	0.63	0.48	7.9	0.62	1.38
25/05	S1	2.88	0.56	0.11	8.3	0.51	0.59
26/05	S2	3.09	0.65	0.27	4.8	0.72	0.79
28/05	S2, M	3.34	0.71	0.28	6.1	0.84	0.93
29/05	S2, M	3.01	0.62	0.18	6.4	0.65	0.73
31/05	M	2.08	0.46	0.14	4.6	0.38	0.39

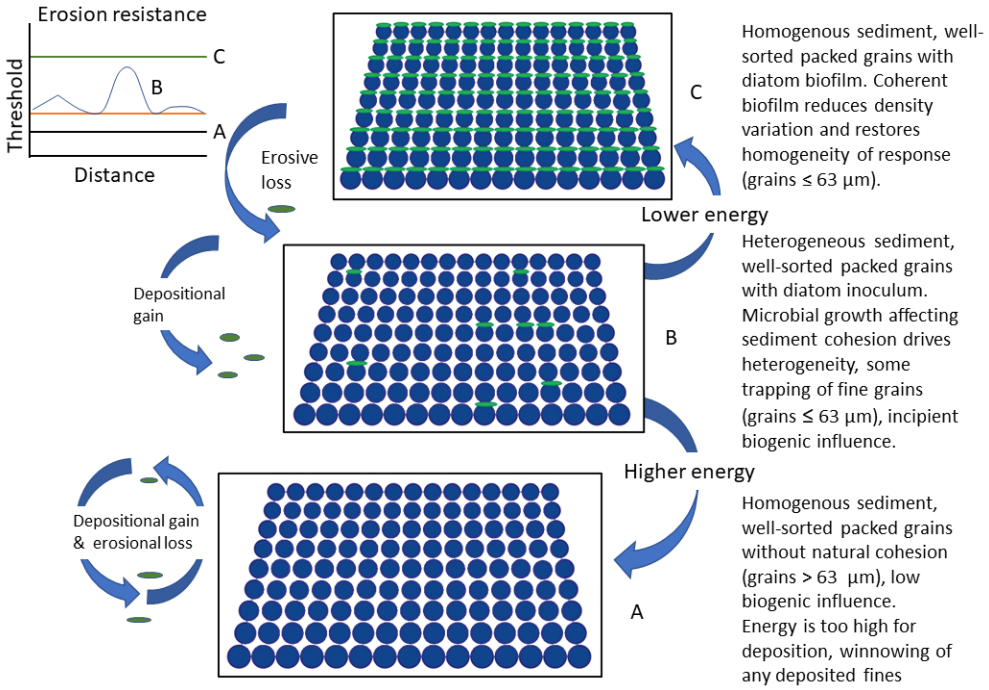


Figure 7. Conceptual diagram of microbially-induced variability in surface sediment erodibility. A) Non-cohesive sediment lacking biogenic influence that in theory would show little variability in surface behaviour forms a predictable and homogenous habitat. B) Colonisation of the non-cohesive bed by microbial cells producing extracellular polymeric substances (EPS) and the initial growth of small microbial colonies creates heterogeneity in the localised surface response to shear stress. Increasing the local resistance to erosion in some patches. C) A fully colonised 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 stabilising 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.

275x190mm (96 x 96 DPI)