PRIMARY RESEARCH PAPER

Changes in cohesive sediment properties associated with the growth of a diatom biofilm

T. J. Tolhurst · M. Consalvey · D. M. Paterson

Abstract Microalgal biofilms are associated with considerable variability in the properties of natural sediments, yet little effort has been made to isolate micro-scale spatial and temporal changes in sediment properties caused by the growth of a biofilm. Understanding the changes associated with biofilm growth and quantifying the time scales over which these changes occur is important for developing suitable experimental designs and for understanding how biofilms mediate sediment properties and processes. The development of a microphytobenthic biofilm and associated changes in the sediment was investigated over 45 days in the laboratory. The biogeochemical properties of the sediment: bulk density, water content, chlorophyll a concentration and colloidal carbohydrate concentration were measured on a sub-millimetre scale in the top 2 mm. The erosion threshold was measured with a Cohesive Strength Meter (CSM). Biofilm development was rapid, with changes in the properties occurring after 1 day and a visible film forming after just 3 days. The largest changes in sediment properties tended to occur in the surface 200 µm through time, with some variables also showing a differing response with depth. There were significant changes in water content, chlorophyll a concentration, colloidal carbohydrate concentration and erosion threshold in the surface 2 mm, with a general trend to increase with time. Bulk density was highly variable and did not show a consistent pattern of change with time. Erosion threshold was positively correlated with water content, chlorophyll a and colloidal carbohydrate in the surface 200 µm and these were also positively correlated with each other. Low Temperature Scanning Electron Microscopy (LTSEM) images revealed changes in the surface sediment structure and the formation of a thick multi-layer biofilm. The rapidity of biofilm growth and development and the associated changes to the sediment should be considered when designing experiments that investigate biofilms and properties of sediments and/or that involve biocide treatments or disturbance to the sediment.

T. J. Tolhurst (✉)
Centre for Research on Ecological Impacts of Coastal Cities, Marine Ecology Laboratories A11, University of Sydney, Sydney, NSW 2006, Australia
e-mail: ttolhurs@eicc.bio.usyd.edu.au

M. Consalvey
National Institute of Water and Atmospheric Research, Private Bag 14-901, Wellington, New Zealand

D. M. Paterson
Sediment Ecology Research Group, Gatty Marine Laboratory, St. Andrews University, St. Andrews, Fife KY16 8LB, Scotland

Introduction

The growth of diatom biofilms has been shown to be important in mediating the properties of and processes in muddy sediments (Paterson, 1997; Black et al., 2002). The net effect of biofilm growth is usually considered to be stabilising, however, diatoms can destabilise sediments (de Jonge & van der Bergs, 1987; Sutherland, 1998) and natural biofilms exhibit a wide range of erosion thresholds (Defew et al., 2002). The stabilising effect of biota is usually attributed to the secretion of extracellular polymeric substances (EPS) (Dade et al., 1990; Decho, 1990; Tolhurst et al., 2002), however, recent work indicates that the relationship may be more complex, with the organisms *themselves* being important in structuring or stabilising the EPS and sediment (de Brouwer et al., 2002; Tolhurst et al., 2003; de Brouwer et al., 2005).

Unfortunately many studies of muddy sediments are confounded by temporal and spatial variability (Chapman & Tolhurst, 2004). There is limited information on the temporal scales at which biofilms mediate sediment properties and processes and how quickly this mediation could begin after a disturbance event. Such information is essential for experimental design and for including biosedimentological processes in models of sediment dynamics. This article presents data from a laboratory study examining the micro-scale changes in the properties of sediment associated with the growth and development of a biofilm over a period of 7 weeks. Whilst the data are primarily relevant to muddy estuarine sediments, they are also indicative of potential changes due to biofilm growth and development in sandy sediments and other sedimentary habitats such as lakes (Cyr & Morton, 2006).

Materials and methods

Cohesive sediment was collected from the Eden Estuary, Scotland (UK) (56°22′ N, 02°51′ W). Surface sediments were collected by removing the upper few cm of sediment with a shovel. The sediment was sieved in seawater through a 1 mm sieve to remove larger macrofauna and macroalgae to form one composite sample and then frozen at −70°C to kill organisms (Ford et al., 1999). The sediment was thawed, homogenised and divided amongst 35 petri dishes, then divided amongst four aquarium tanks with a flow through of natural seawater, so a biofilm could develop. The samples were constantly immersed and illuminated with a 300 W halogen light source (12 Light:12 Dark) which provided ~ 650 μmol m^{-2} s^{-1}.

On days 0, 1, 3, 5, 7, 13 and 45 the sediments in five randomly selected replicate dishes were tested for stability using the Cohesive Strength Meter (CSM). After measurement with the CSM three of the dishes were randomly selected and quench frozen in liquid nitrogen and stored at −20°C for later biogeochemical analysis.

Epipelic diatom activity is usually limited to the top 2 mm of intertidal sediments (Paterson et al., 1986; Hay et al., 1993; Consalvey et al., 2004). One reason for the poor progress in understanding biota—sediment interactions is a failure to measure on relevant scales (Wheatcroft & Butman, 1997; Flemming & Delfontaine, 2000; Kelly et al., 2001). If the mechanisms by which diatom biofilms influence sediment properties are to be understood, it is vital to investigate these properties at a scale relevant to diatom influence i.e. the sub-millimetre scale. A section of the frozen sediment samples undisturbed by the CSM measurement was cut using a diamond tipped lapidary saw into a block of approximately 1 cm^2. The exact size of the block was measured, so that biogeochemical data could be expressed as a concentration where appropriate. These blocks were micro-sectioned on a freezing microtome in 200 μm sections down to 1000 μm, then 500 μm sections to 2000 μm. The wet weight was measured before biochemical analysis and the dry weight after analysis. For the graphs, data are expressed as the midpoint of the section, so the top 200 μm section is designated 100 μm the next 300 μm and so on. The sections were analysed for bulk density, water content, chlorophyll *a* and colloidal carbohydrate to determine the distribution of these variables with depth.

Previous work has shown that biogeochemical data from soft sediments expressed as contents can be confounded by spatial and/or temporal variability in grain size, sample density and dewatering (Flemming & Delafontaine, 2000; Perkins et al., 2003; Tolhurst et al., 2005). Thus colloidal carbohydrates and chlorophyll *a* were expressed as concentrations. Water was expressed as % content.

Chlorophyll *a* concentration (as a proxy for biomass) was determined using High Performance Liquid Chromatography (HPLC) analysis (Wiltshire et al., 1998). The Dubois assay (Dubois et al., 1956 as modified by Taylor & Paterson, 1998) was used to measure the amount of water-extractable colloidal carbohydrates in each sectioned sample. The sectioned sediment samples were also used to ascertain water content and wet bulk densities were calculated using the following formula:

$$\text{Bulk density} = \frac{\text{Wt}_{\text{wet}}}{V} \qquad (1)$$

where Wt_{wet} = wet mass in grams, V = volume in cm^3.

A CSM was used to measure the critical erosion threshold of the sediments immediately prior to sampling for the biogeochemical analysis (Tolhurst et al., 1999). The CSM measures over spatial (cm) and temporal (minutes) scales impossible with traditional flume systems, this is vital for measuring changes without spatially or temporally confounding the data (Tolhurst et al., 2000, 2006). In this case, it enables the measurement of laboratory cultures on a scale impossible with any other device.

The CSM consists of a 30-mm diameter chamber that is pushed into the sediment and filled with local seawater (Tolhurst et al., 1999). The jet of water comes from a downward directed nozzle in the chamber, the velocity of the jet is increased systematically through each experiment. Bed erosion is inferred from the drop in the transmission of infrared light across the chamber caused by the suspension of sediment. A drop in transmission below 90% is taken as a critical drop (Tolhurst et al., 1999), and is approximately equal to erosion of 0.01 kg m^{-2} of cohesive sediment. The slope of the averaged erosion profiles was used to give a relative measure of the erosion rate, the suspension index (S$_i$).

The microstructure of the sediment/biofilm as well as the occurrence of diatoms and the distribution and microstructure of the EPS in relation to the diatoms was investigated using Low Temperature Scanning Electron Microscopy (LTSEM; Paterson, 1995). Sediment samples were fractured and mounted on to specially constructed cryostubs under liquid nitrogen. The cryostubs were then placed on to an adapted stage of a JOEL 35CF SEM fitted with a LTSEM (Oxford Instruments CT 1500B). Samples were freeze dried under vacuum and sputter coated with gold before being examined at −170°C. Further details of the examination of frozen sediment/biofilms under LTSEM are given by Paterson (1995).

Data were analysed using the Gmav5 programme for analysis of variance and SNK tests for sequential pairwise comparison of ranked means. For the CSM erosion threshold there was one factor (time) with 7 levels, orthogonal and fixed, $n = 5$. The S$_i$ value was an average of the profiles, thus it was not possible to do ANOVA. For the biogeochemical properties two analyses were done, the first was on the average of all the sections i.e. the top 2 mm of the sediment. This analysis had one factor (time) with 7 levels, was orthogonal and fixed, $n = 3$. For the analysis of changes in the depth profile of the biogeochemical variables with time, time and core depth are both fixed factors, thus it was not valid to do an ANOVA on these data. Instead, the slope of the depth profile was calculated for each variable in the three replicate cores at each time and ANOVA was done on these data to determine if there were any changes in the depth profile with time. This analysis had one factor (time) with 7 levels, was orthogonal and fixed, $n = 3$. Differences were determined using SNK tests. Pearson's correlation analysis was done for the whole 2 mm section and the 100 μm and 1750 μm sections to investigate relationships at the surface and deeper in the sediment.

Results

For the 2 mm section, erosion threshold, water content and colloidal carbohydrate concentration were all significantly positively correlated with time (Table 3a, Figs. 1, 2). Erosion threshold was significantly positively correlated with water content and colloidal carbohydrate concentration, which were positively correlated with each other. For the top 200 μm section, erosion threshold, water content, chlorophyll *a* and colloidal carbohydrate were all significantly positively correlated with time (Table 3b). Erosion threshold was significantly positively correlated with water content, chlorophyll *a* and colloidal carbohydrate. Water content and chlorophyll *a* were significantly positively correlated with colloidal carbohydrate. For the 1750 μm section, erosion threshold and water content were
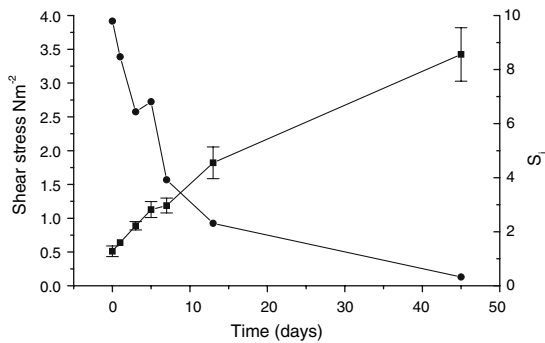
Fig. 1 CSM erosion thresholds (squares) show that stability increases with time as the laboratory diatom biofilm develops. Error bars are standard error, $n = 5$. The relative erosion rate, S_i values (circles), decreases with time

significantly positively correlated with time (Table 3c). Erosion threshold was significantly positively correlated with water content and bulk density was significantly positively correlated with chlorophyll a and colloidal carbohydrate.

Low Temperature Scanning Electron Microscopy images of the sediments revealed changes in sediment structure as the biofilm developed with time. Samples from day 0 and day 1 exhibited the typical open "card-house" structure (Paterson, 1995) with only the odd solitary diatom (Fig. 7A, B, C). However by day 3 large numbers of small pennate diatoms had colonised the sediment surface forming a biofilm (Fig. 8A, B). The numbers of diatoms and thickness of the biofilm increase throughout the experiment and by day 45 the biofilm had become multilayered, epipelic diatoms are found on the surface of the biofilm, with chain-forming species below (Fig. 8C), harpacticoid copepods were also present in the biofilm at this time (Fig. 8D).

There was a significant increase in the erosion threshold of the sediment over time (Fig. 1 and Table 1). Initially the increase in erosion threshold was linear, but the rate of increase slowed after 5 days. The SNK test showed that day 0 = day 1 < day 3 = day 5 = day 7 < day 13 < day 45. The increase in erosion threshold was accompanied by a
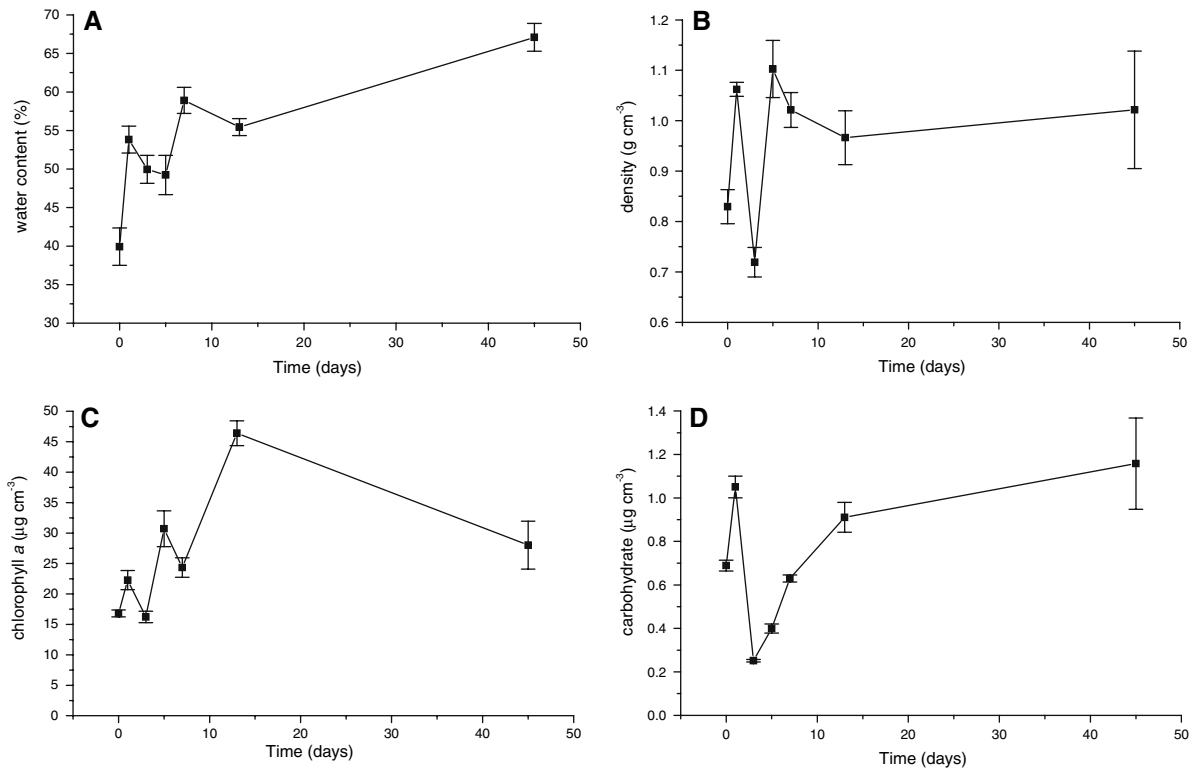

Fig. 2 Changes in the biogeochemical properties of the sediment with time, averaged over the surface 2 mm, (**A**) water content, (**B**) bulk density, (**C**) chlorophyll a concentration, (**D**) colloidal carbohydrate. Error bars are standard error, $n = 3$

Table 1 Results of GMAV analysis of variance on changes in biogeochemical variables with time, $n = 5$ for CSM and 3 for other variables

Source	df	CSM		df	Density		Water		Chlorophyll *a* concentration		Carbohydrate concentration	
		MS	F		MS	F	MS	F	MS	F	MS	F
Time	6	2.06	35.82***	6	0.07	4.38*	216.4	11.64***	319.1	12.95***	0.33	8.89***
RES	28	0.06		14	0.02		18.59		24.64		0.04	
TOT	34			20								

Significance shown at the 0.05 level (*), 0.01 level (**), 0.001 level (***)

decrease in the S_i values with time, showing that the erosion rate decreases as the biofilm develops (Fig. 1).

The water content of the surface 2 mm increased significantly with time (Fig. 2A, Table 1), the SNK test showed that day 0 < day 5 = day 3 = day 1 = day 13 = day 7 < day 45. Analysis of the water content depth profiles showed that there was a significant change with a general increase over time (Table 2). The SNK test could not, however, determine where the differences were, sometimes water content decreased with depth and sometimes it increased and there was no consistency to this pattern (Fig. 3). Bulk densities in the surface 2 mm were highly variable (Fig. 2B, Table 1), there was a significant effect of time, but the SNK test could not determine where the differences were. The bulk density depth profiles showed no significant change with time (Fig. 4, Table 2). There was a significant change in chlorophyll *a* concentration in the top 2 mm with time (Fig. 2C, Table 1). Chlorophyll *a* concentration generally increased with time, the SNK test showed that day 3 = day 0 = day 1 = day 7 = day 45 = day 5 < day 13. The chlorophyll *a* depth profiles were highly variable and whilst there was a general trend for chlorophyll to increase, showed no significant change with time (Fig. 5, Table 2). There was a significant change in colloidal carbohydrate concen-

tration in the top 2 mm with time (Table 1), it initially increased, followed by a decrease then a steady increase (Fig. 2D). However, the SNK test could not determine where the differences were. The carbohydrate concentration depth profiles showed a significant change with time (Fig. 6, Table 2), although with inconsistent differences between depths from day to day. The SNK test showed that day 45 = day 1 < day 7 = day 13 = day 0 = day 5 = day 3.

Discussion

The growth of a biofilm significantly altered the biogeochemical properties of the sediment. There was an increase in microphytobenthos with time, the LTSEM images and chlorophyll *a* data demonstrated that diatoms rapidly colonise sediment and form a biofilm. The formation of the biofilm was accompanied by changes in the other measured sediment properties over time and there was strong correlation between chlorophyll *a*, colloidal carbohydrate, water content erosion threshold and time in the top 200 μm. This agrees with previous work showing such correlations (see for example: Underwood and Smith, 1998; Paterson et al., 2000; de Brouwer et al., 2000; Bellinger et al., 2005), supporting the conclusion that changes in these properties can be caused by growth

Table 2 Results of GMAV analysis of variance on changes in the slopes of the biogeochemical depth profile with time, $n = 3$

Source	df	Carbohydrate concentration		Chlorophyll *a*		Density		Water	
		MS	F	MS	F	MS	F	MS	F
time	6	203257	12.43***	235488439	2.52	81468	2.40	91389709	3.75*
RES	14	16347		93390866		33917		24384873	
TOT	20								

Significance shown at the 0.05 level (*), 0.01 level (**), 0.001 level (***)
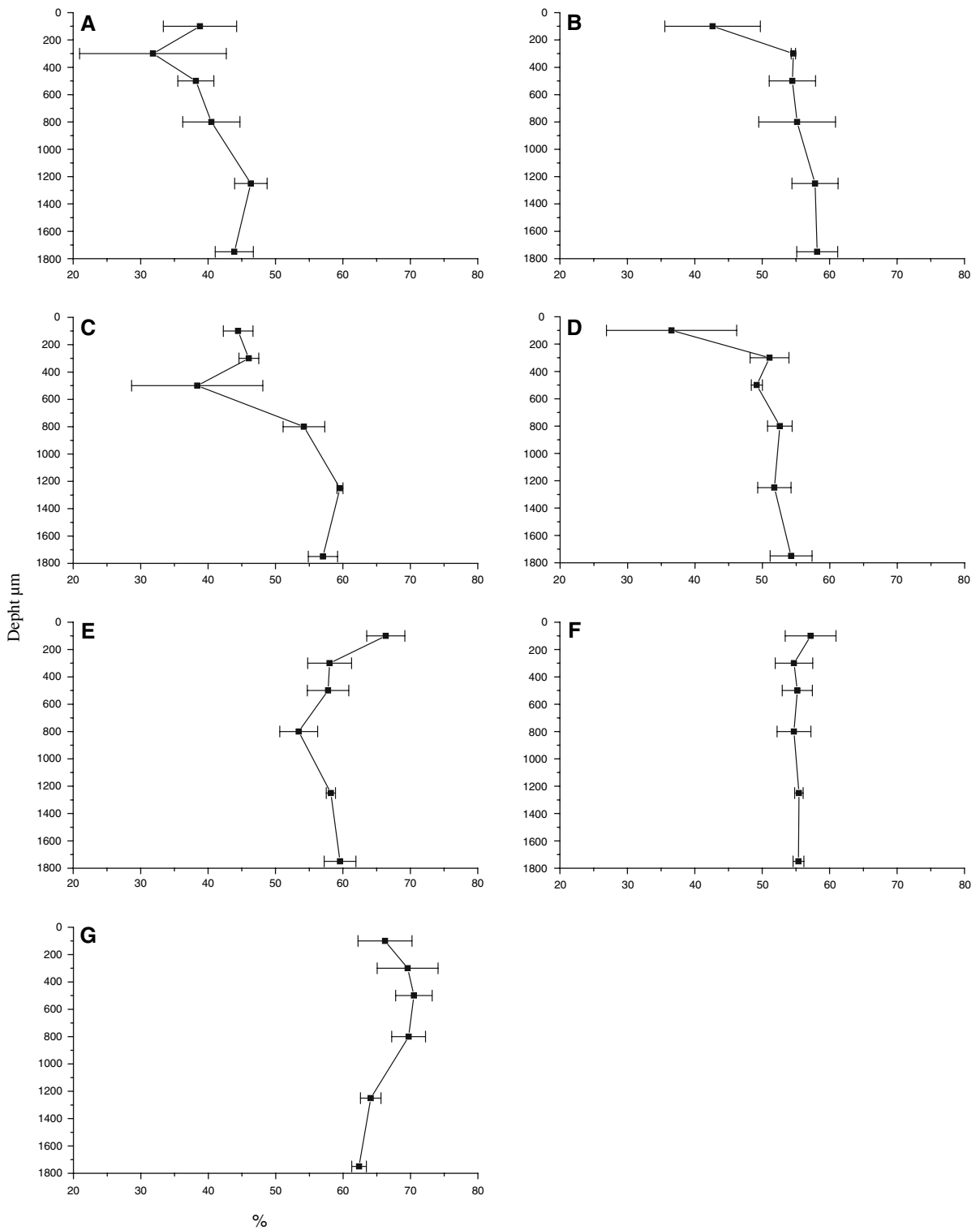
Fig. 3 Depth profile, showing the changes in water content with depth over time, (**A**) day 0, (**B**) day 1, (**C**) day 3, (**D**) day 5, (**E**) day 7, (**F**) day 13 and (**G**) day 45. Error bars are standard error, $n = 3$
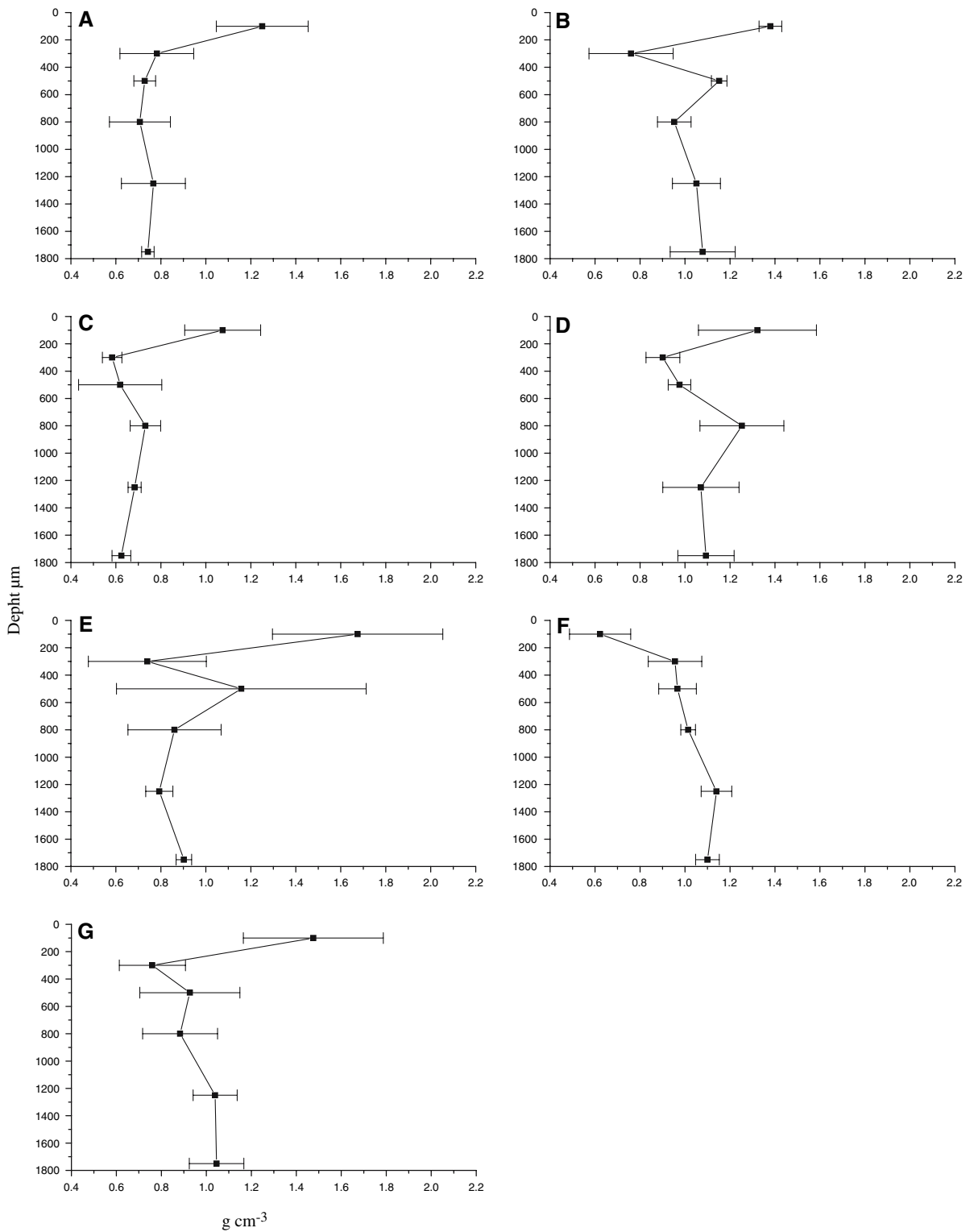
Fig. 4 Depth profile, showing the changes in bulk density with depth over time, (**A**) day 0, (**B**) day 1, (**C**) day 3, (**D**) day 5, (**E**) day 7, (**F**) day 13 and (**G**) day 45. Error bars are standard error, $n = 3$

Fig. 5 Depth profile, showing the changes in chlorophyll *a* concentration with depth over time, (**A**) day 0, (**B**) day 1, (**C**) day 3, (**D**) day 5, (**E**) day 7, (**F**) day 13 and (**G**) day 45. Error bars are standard error, *n* = 3

Fig. 6 Depth profile, showing the changes in colloidal carbohydrate concentration with depth over time, (A) day 0, (B) day 1, (C) day 3, (D) day 5, (E) day 7, (F) day 13 and (G) day 45. Error bars are standard error, $n = 3$
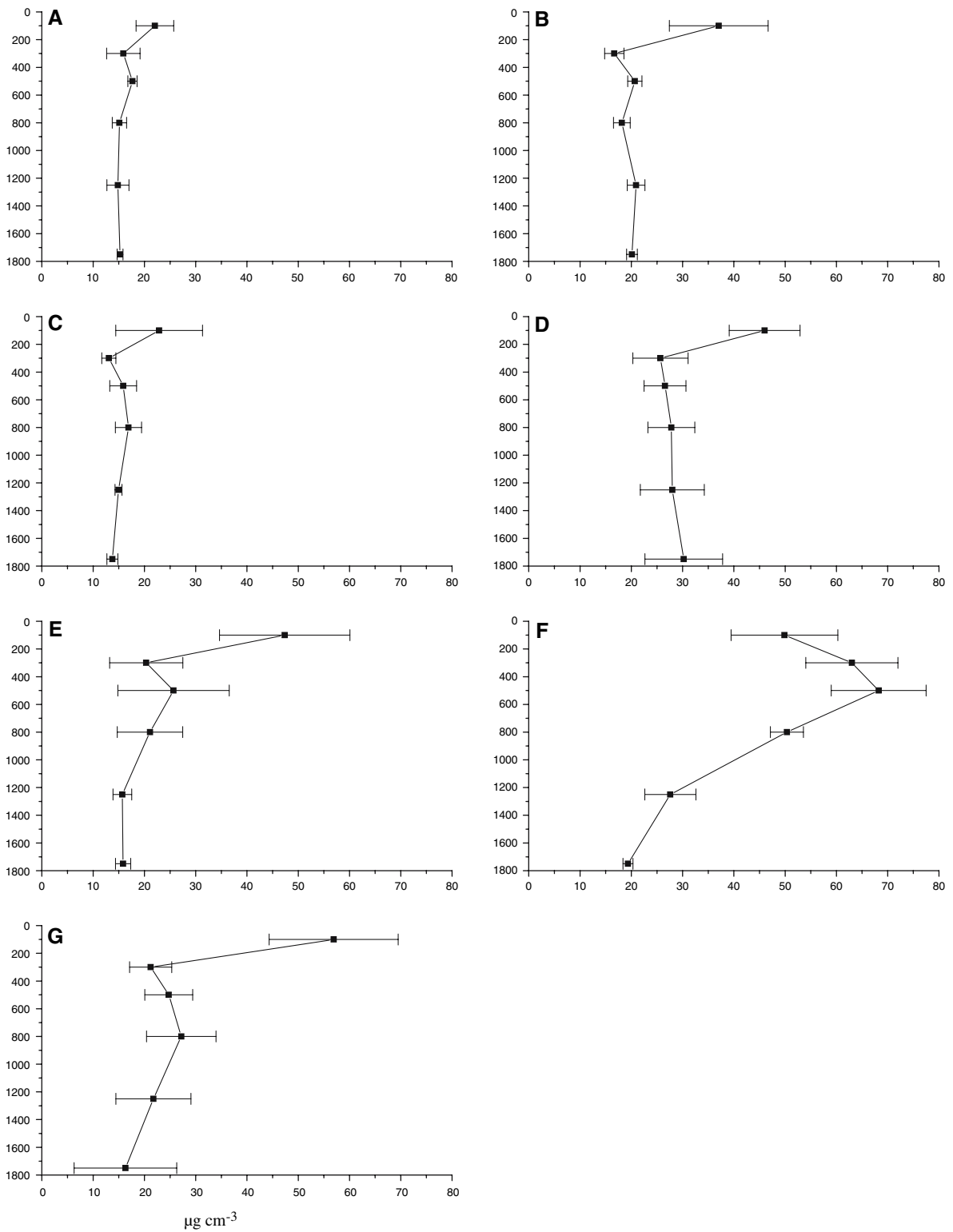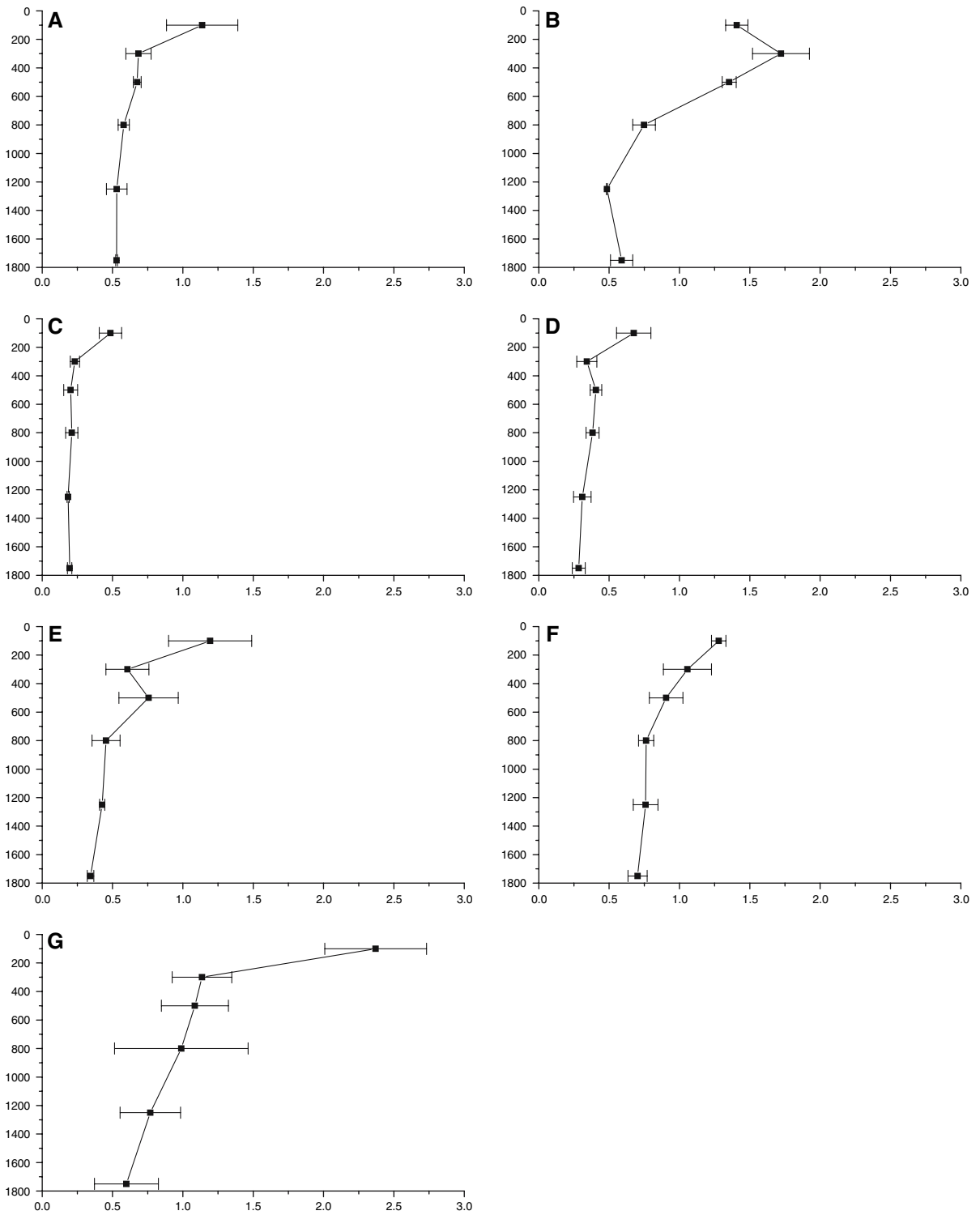
and development of a diatom biofilm. Visually the structure of the biofilm in the first few days is consistent with images from natural biofilms (see for example Stal & Défarge, 2005); however, the structure at the end of the experiment is less likely to be found naturally, because of disturbance by macrofauna and wave/tidal currents.

The depth profile showed that both bulk density and water content were highly variable with depth, showing no clear pattern. Blistering of the biofilm caused sections of biofilm to be lifted up, revealing fresh sediment that was then colonised by new biofilm. LTSEM images showed that as the biofilm grew, it became multilayered, with epipelic diatoms overlying chain forming species. This layering and mosaic of older and newer biofilm is the most likely cause of the variable bulk density and water content. Many field studies have shown that areas with a biofilm have higher water contents than areas without, but these data are often confounded by concomitant differences in sediment grain size. This study shows that significant differences in water content

between areas with and without a biofilm can occur simply through the growth of the biofilm.

Depth profiles showed that chlorophyll a and colloidal carbohydrate concentration was largest at the surface and decreased with depth, this pattern generally tended to strengthen with time (Figs. 5, 6), although the large variability in the data meant that for chlorophyll a these changes were not significant. This supports previous work showing the same pattern when data are expressed as contents (Taylor and Paterson, 1998; de Brouwer & Stal 2001; Kelly et al., 2001; Herlory et al., 2004) and refutes the contention that such profiles are entirely a function of density changes with depth (Flemming & Delafontaine, 2000). Flemming and Delafontaine's point remains valid however, if density does increase with depth the pattern of values decreasing with depth would be artificially enhanced if data were expressed as contents. The biogeochemical variables are most strongly correlated with each other in the top 200 μm section (Table 3). The LTSEM images show that this is also the section where the majority of the biofilm

Table 3 Pearson's correlation of sediment properties, bold values are significant at $P < 0.05$, $n = 7$

	Time	Erosion threshold	Density	Water content	Chlorophyll a	Carbohydrate
A						
Time	1.00					
Erosion threshold	**0.92**	1.00				
Density	0.20	0.15	1.00			
Water content	**0.73**	**0.73**	0.31	1.00		
Chlorophyll a	0.29	0.42	0.41	0.29	1.00	
Carbohydrate	**0.52**	**0.56**	0.33	**0.52**	0.37	1.00
B						
Time	1.00					
Erosion threshold	**0.92**	1.00				
Density	0.09	−0.04	1.00			
Water content	**0.57**	**0.57**	0.13	1.00		
Chlorophyll a	**0.47**	**0.52**	0.33	0.43	1.00	
Carbohydrate	**0.73**	**0.68**	0.39	**0.48**	**0.58**	1.00
C						
Time	1.00					
Erosion threshold	**0.92**	1.00				
Density	0.29	0.35	1.00			
Water content	**0.48**	**0.48**	0.27	1.00		
Chlorophyll a	−0.09	0.19	**0.47**	−0.15	1.00	
Carbohydrate	0.30	0.40	**0.48**	−0.01	0.31	1.00

A = 2 mm section, B = 0–200 μm section, C = 1500–2000 μm section

Fig. 7 LTSEM images showing changes in the sediment microstructure and biofilm growth with time in the laboratory. (**A**) Cross sectional fracture face of the sediment on day 1 showing structure with depth, arrow indicates the surface of the sediment, the sediment exhibits the typical open 'card-house' structure of cohesive sediments, scale bar = 100 μm, (**B**) the surface of the sediment on day 1 is bare, scale bar = 100 μm, (**C**) a close up image shows the surface to consist primarily of mineral grains with only the occasional small diatom, scale bar = 10 μm, (**D**) a fracture face of the sediment on day 3 shows that some structural changes in the arrangement of sediment particles has occurred (compare to 7a), scale bar = 100 μm
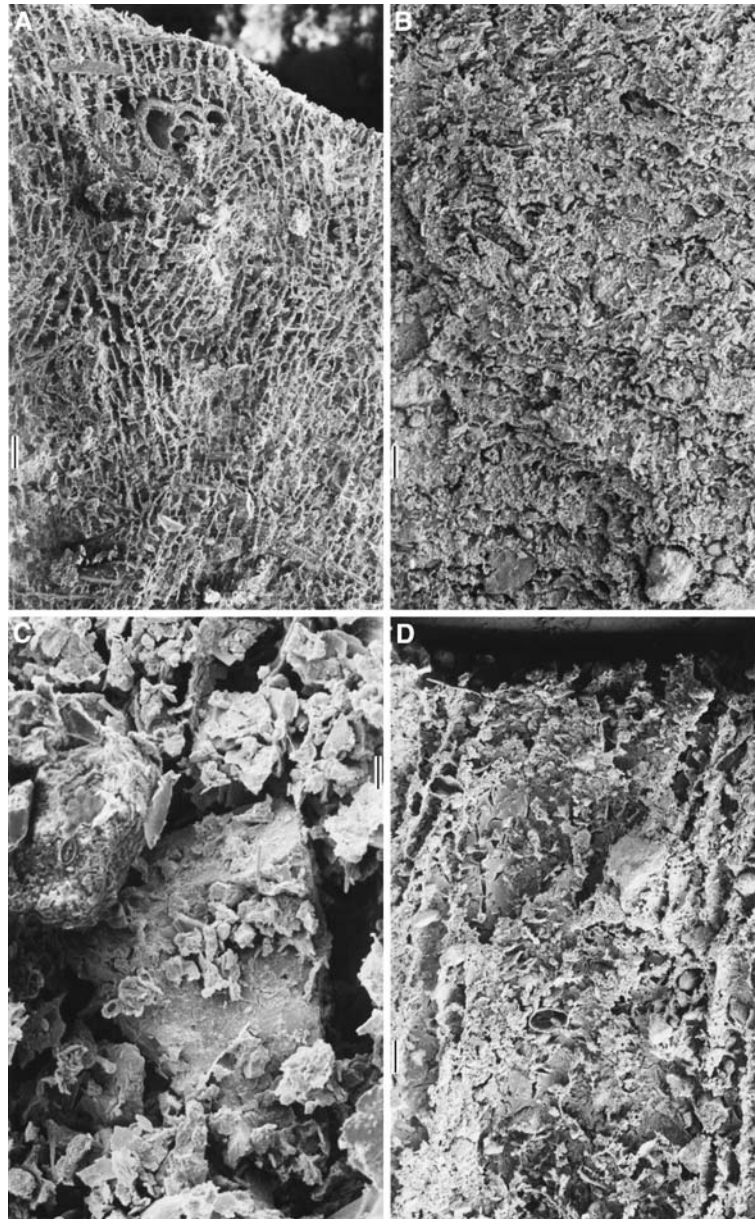

occurs; this is also true for natural biofilms (see for example Tolhurst, 2003; Herlory et al., 2004; Stal & Défarge, 2005). This suggests that in this case the changes that occur in these variables over the course of the experiment are due to the biofilm and its growth. Whilst correlations of water content and carbohydrate might be expected for water-soluble carbohydrates, there is no correlation between these variables in the 1750 μm section, indicating that the strong correlation at the surface is due to the presence

of the biofilm and not a causative relationship between the amount of water and amount of colloidal carbohydrate.

The rather bizarre pattern in the colloidal carbohydrate in the surface 2 mm, with large concentrations of carbohydrate on day 0 increasing on day 1 and a massive drop on day 3 followed by a steady increase over the rest of the experiment were unexpected. It is unclear what caused this pattern. It is possible that the increase at the beginning of the

Fig. 8 (**A**) by day 3, the
sediment surface is covered
in a loose organic layer,
scale bar = 100 μm, (**B**) a
close up image reveals this
to consist of mineral grains
and many small diatoms,
scale bar = 10 μm, (**C**) by
day 45 the biofilm (bottom)
has peeled away from the
sediment to reveal a
network of filaments just
below the biofilm(top),
scale bar = 100 μm, (**D**) a
close up image reveals these
filaments to be chain
forming species, as well as
the presence of
Harpacticoid copepods
(centre emerging from the
biofilm), scale bar = 100 μm
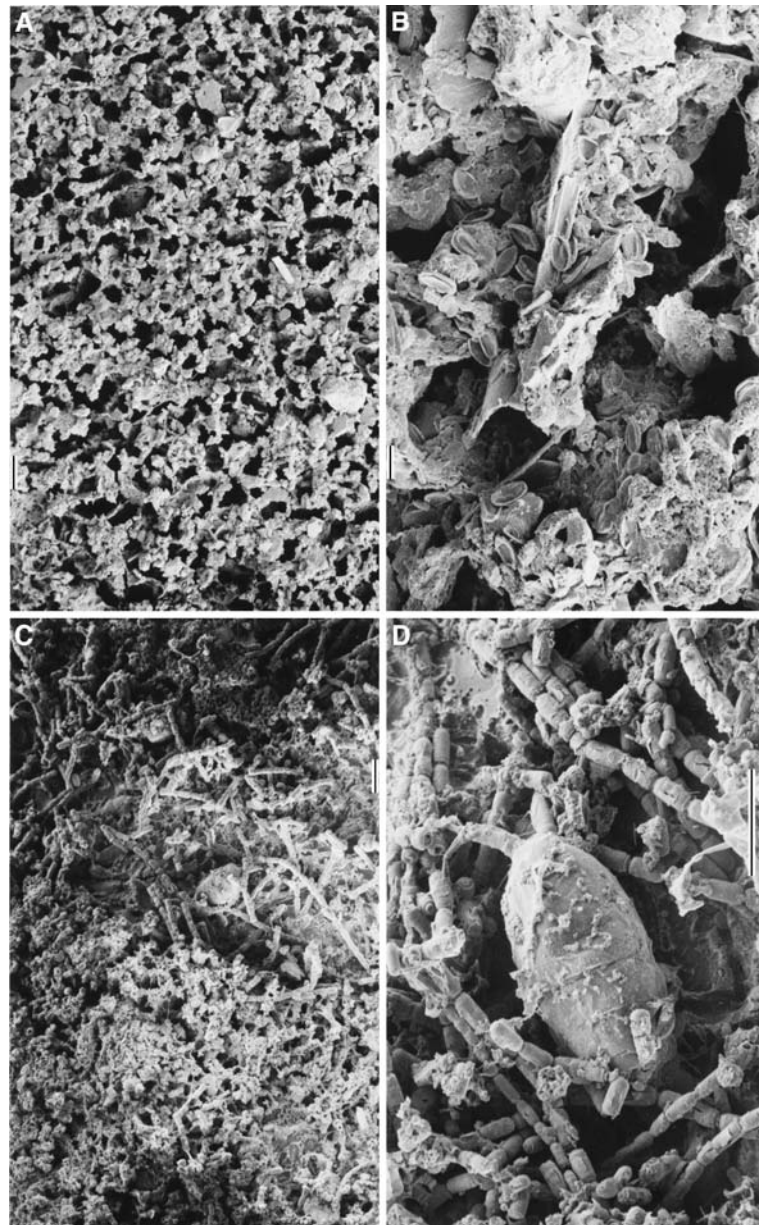
experiment was due to bacterial breakdown of
insoluble organic matter, such as organisms killed
by the freezing, into water soluble colloidal carbo-
hydrate, such as bacterial EPS. Unfortunately no
samples were taken for bacterial analysis, so this
remains conjecture. The decrease on day 3 would
then be explained by conversion of the water-
extractable fraction of carbohydrate into an insoluble
form or by respiration. Subsequently, the colloidal
carbohydrate exhibits a logarithmic increase, pre-

sumably reflecting the growth and development of the
biofilm and production of water-extractable carbohy-
drate due to photosynthesis (Stal & Défarge, 2005).

In abiotic muddy sediments there is an inverse
relationship between stability and water content, if
sediment stability were governed purely by the
abiotic properties there would be a positive correla-
tion with the bulk density and negative correlation
with water content. This was not the case. In this
study the biofilm had a primarily stabilising effect on

the sediments, although oxygen blistering did lead to localised surface destabilisation similar to that found by Sutherland et al. (1998). The increase in erosion threshold with time was not due to compaction, because there was no significant increase in bulk density with time. The positive correlation with colloidal carbohydrates and chlorophyll *a* supports various studies indicating that the stabilising effect of biofilms is due to secretion of EPS (Holland et al., 1974; Tolhurst et al., 2002) and armouring of the sediment surface by the diatom cells themselves (Tolhurst et al., 2003). However, it has been shown that the structuring of the EPS by the organisms is also important in stabilising the sediment (de Brouwer et al., 2002, 2005). The positive correlation with water content shows that this stabilisation is larger than any destabilisation due to increased water content supporting previous findings where large water contents have been associated with large erosion thresholds (e.g. Underwood & Paterson, 1993; Tolhurst et al., 2000) (Fig. 7, 8).

On day 0, as the sediments were submerged in water for the CSM measurements, the surface tension of the water was seen to remove sediment flocs. This did not occur on day 1, indicating some stabilisation of the sediment surface had occurred. By day 3 a biofilm was visible to the naked eye and by day 5, this had begun to blister as oxygen bubbles formed in the biofilm. On day 6, the biofilm was considerably blistered by oxygen bubbles and was being pulled away from the sediment by the small water currents in the tank from the flow through of seawater. On day 7, whilst removing the dishes from the culture tank pieces of biofilm, and associated sediment, were washed away from the sediment surface, despite great care being taken not to disturb the sediment. After day 7, the blistering appeared to have reached its peak and no new bubbles formed. By day 13, despite the sediment surface being highly blistered, none of the biofilm floated away. The blistering and lifting away of the biofilm is likely to increase spatial variability in the biogeochemical properties of the sediment.

This work demonstrates that newly exposed sediments can be rapidly colonised by microphytobenthos supporting an earlier in situ study by Consalvey et al. (2003). It also shows that the growth of a biofilm can significantly alter the properties of the sediment it is growing on, supporting field measurements that have shown significant differences in the properties of sediments with a biofilm to those without one (Tolhurst et al., 2000, 2006; de Brouwer et al., 2005). This data can be used during experimental design, for example in the timing of measurements after disturbance or biocide treatments. Changes in biogeochemical properties can be expected after just one day, especially in the absence of fauna. The data also show directional responses in biogeochemical variables due to biofilm growth and development, this information can be used to make predictions about the expected magnitude of changes within experiments and aid in the interpretation of data, for example in understanding interactions between diatoms and fauna.

References

Bellinger, B. J., A. S. Abdullahi, M. R. Gretz & Underwood G. J. C., 2005 Biofilm polymers: relationship between carbohydrate biopolymers from estuarine mudflats and uni-algal cultures of benthic diatoms. Aquatic Microbial Ecology 38(2): 169–180.

Black, K. S., T. J. Tolhurst, S. E. Hagerthey & D. M. Paterson, 2002. Working with natural cohesive sediments. Journal of Hydraulic Engineering 128(1): 2–7.

de Brouwer, J. F. C. & L. J. Stal, 2001. Short-term dynamics in microphytobenthos distribution and associated extracellular carbohydrates in surface sediments of an intertidal mudflat. Marine Ecology Progress Series 218: 33–44.

de Brouwer, J. F. C., G. K. Ruddy, T. E. R. Jones & L. J. Stal, 2002. Sorption of EPS to sediment particles and the effect on the rheology of sediment slurries. Biogeochemistry 61: 57–71.

de Brouwer J. F. C., K. Wolfstein, G. K. Ruddy, T. E. R. Jones & L. J. Stal, 2005. Biogenic stabilization of intertidal sediments: the importance of extracellular polymeric substances produced by benthic diatoms. Microbial Ecology 4: 501–512.

Chapman, M. G. & T. J. Tolhurst, 2004. The relationship between invertebrate assemblages and bio-dependant properties of sediment in urbanized temperate mangrove forests. Journal of Experimental Marine Biology and Ecology 304: 51–73.

Consalvey, M., Tolhurst, T. J. & Paterson, D. M. 2003. Intertidal Biofilm Recovery After a Simulated *In situ* Disturbance

Event. Coastal Zone Topics: Process, Ecology and Management Estuarine and Coastal Shelf Science Association.

Consalvey, M., D. M. Paterson & G. J. C. Underwood, 2004. The ups and downs of life in a benthic biofilm: migration of benthic diatoms. Diatom Research 19: 181–202.

Cyr, H. & K. E. Morton, 2006. Distribution of biofilm exopolymeric substances in littoral sediments of Canadian Shield lakes: the effects of light and substrate. Canadian Journal of Fisheries and Aquatic Sciences 63(8): 1763–1776.

Dade, W. B., J. D. Davis, P. D. Nichols, A. R. M. Nowell, D. Thistle, M. B. Trexler & D. C. White, 1990. Effects of bacterial exopolymer adhesion on the entrainment of sand. Geomicrobiology Journal 8: 1–16.

Decho, A. W., 1990. Microbial exopolymer secretions in ocean environments: their role(s) in food webs and marine processes. Oceanographic and Marine Biology Annual Review 28: 73–153.

Defew, E. C., T. J. Tolhurst & D. M. Paterson, 2002 Site-specific features influence sediment stability of intertidal flats. Hydrology and Earth System Science 6(5): 971–981.

Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers & F. Smith, 1956. Colorimetric method for determination of sugars and related substances. Annals of Chemistry 28: 350–356.

Flemming, B. W. & M. T. Delafontaine, 2000. Mass physical properties of muddy intertidal sediments: some applications, misapplications and non-applications. Continental Shelf Research 20: 1179–1197.

Ford, R. B., S. F. Thrush & P. K. Probert, 1999. Macrobenthic colonisation of disturbances on an intertidal sandflat: the influence of season and buried algae. Marine Ecology Progress Series 191: 163–174.

Hay, S. I., T. C. Maitland & D. M. Paterson, 1993. The speed of diatom migration through natural and artificial substrata. Diatom Research 8(2): 371–384.

Herlory, O., J. M. Guarini, P. Richard & G. F. Blanchard, 2004. Microstructure of microphytobenthic biofilm and its spatio-temporal dynamics in an intertidal mudflat (Aiguillon Bay, France). Marine Ecology Progress Series 282: 33–44.

Holland, A. F., R. G. Zingmark & J. M. Dean, 1974. Quantitive evidence concerning the stabilization of sediments by marine benthic diatoms. Marine Biology 27: 191–196.

de Jonge, V. N. & J. van den Bergs, 1987. Experiments on the resuspension of estuarine sediments containing benthic diatoms. Estuarine and Coastal Marine Science 24: 725–740.

Kelly, J. A., C. Honeywill & D. M. Paterson, 2001. Microscale analysis of chlorophyll a in cohesive intertidal sediments: the implication of microphytobenthoc distribution. Journal of the Marine Biological Association of the United Kingdom 81: 151–162.

Meleder, V., L. Barille, Y. Rince, M. Morancais, P. Rosa & P. Gaudin, 2005. Spatio-temporal changes in microphytobenthos structure analysed by pigment composition in a macrotidal flat (Bourgneuf Bay, France). Marine Ecology Progress Series 297: 83–99.

Paterson, D. M., R. M. Crawford & C. Little, 1986. The structure of benthic diatom assemblages: a preliminary account of the use and evaluation of low-temperature scanning electron microscopy. Journal of Experimental Marine Biology and Ecology 96: 279–289.

Paterson, D. M., 1995. Biogenic structure of early sediment fabric visualised by LTSEM. Journal of the Geological Society, London 152: 131–140.

Paterson, D. M., 1997. Biological Mediation of Sediment Erodibility: Ecology and Physical Dynamics. In Burt, N., R. Parker & J. Watts (eds), Cohesive Sediments. John Wiley & Sons Ltd.

Paterson, D. M., T. J. Tolhurst, J. A. Kelly, C. Honeywill, E. M. G. T. de Deckere, V. Huet, S. A. Shayler, K. S. Black, J. de Brouwer & I. Davidson, 2000. Variations in sediment stability and sediment properties across the Skeffling mudflat, Humber estuary, UK. Continental Shelf Research 20: 1373–1396.

Perkins, R. G., C. Honeywill, M. Consalvey, H. Austin, T. J. Tolhurst & D. M. Paterson, 2003. Changes in microphytobenthic chlorophyll a resulting from sediment compaction due to de-watering: opposing patterns in concentration and content. Continental Shelf Research 23: 575–586.

Stal, L. J. & C. Defarge, 2005. Structure and dynamics of exopolymers in an intertidal diatom Biofilm. Geomicrobiology Journal 22(7–8): 341–352.

Sutherland, T. F., C. L. Amos & J. Grant, 1998. The Erosion Threshold of Biotic Sediments: A Comparison of Methods. In Black, K. S., D. M. Paterson & A. Cramp (eds), Sedimentary Processes in the Intertidal Zone. Geological Society, Special Publications 139, London: 135–148.

Taylor, I. & D. M. Paterson, 1998. Microspatial variation in carbohydrate concentrations with depth in the upper millimetres of intertidal cohesive sediments. Estuarine, Coastal and Shelf Science 46: 359–370.

Tolhurst, T. J., K. S. Black, S. A. Shayler, S. Mather, I. Black, K. Baker & D. M. Paterson, 1999. Measuring the in situ erosion shear stress of intertidal sediments with the Cohesive Strength Meter (CSM). Estuarine, Coastal and Shelf Science 49: 281–294.

Tolhurst, T. J., R. Reithmüller & D. M. Paterson, 2000. In situ versus laboratory analysis of sediment stability from intertidal mudflats. Continental Shelf Research 20(10–11): 1317–1334.

Tolhurst, T. J., G. Gust & D. M. Paterson, 2002. The Influence of an Extracellular Polymeric Substance (EPS) on Cohesive Sediment Stability. In Winterwerp, J. C. & C. Kranenburg (eds), Fine Sediment Dynamics in the Marine Environment, Proceedings in Marine Science 5, 409–425.

Tolhurst, T. J., B. Jesus, V. Brotas & D. M. Paterson, 2003. Diatom migration and sediment armouring – an example from the Tagus Estuary, Portugal. Hydrobiologia 503: 183–193.

Tolhurst, T. J., A. J. Underwood, R. G. Perkins & M. G. Chapman, 2005. Content versus concentration: Effects of units on measuring the biogeochemical properties of soft sediments. Estuarine, Coastal and Shelf Science 63: 665–673.

Tolhurst, T. J., E. C. Defew, J. F. C. de Brouwer, K. Wolfstein, L. J. Stal & D. M. Paterson, 2006. Small-scale temporal and spatial variability in the erosion threshold of intertidal sediments. Continental Shelf Research 26(3): 351–362.

Underwood, G. J. C. & D. M. Paterson, 1993. Seasonal changes in diatom biomass, sediment stability and biogenic

stabilization in the Severn estuary. Journal of the Marine Biological Association of the UK 73(4): 871–887.

Underwood, G. J. C. & D. J. Smith, 1998. Predicting epipelic diatom exopolymer concentrations in intertidal sediments from sediment chlorophyll a. Microbial Ecology 35(2): 116–125.

Underwood, G. J. C. & Paterson, D. M. (2003). The importance of extracellular carbohydrate production by marine epipelic diatoms. Advances in Botanical Research 40: 183–240.

Wheatcroft, R. A. & C. A. Butman, 1997. Spatial and temporal variability in aggregated grain-size distributions, with implications for sediment dynamics. Continental Shelf Research 17(4): 367–390.

Wiltshire, K. H., T. Tolhurst, D. M. Paterson, I. Davidson & G. Gust, 1998. Pigment Fingerprints as Markers of Erosion. In Black, K. S., D. M. Paterson & A. Cramp (eds), Sedimentary Processes in the Intertidal Zone. Geological Society London Special Publications 139, 99–114.